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Super-hydrophilic silicone hydrogels with interpenetrating poly(2-methacryloyloxyethyl phosphorylcholine) networks

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ABSTRACT

We synthesized silicone hydrogels from 2-methacryloyloxyethyl phosphorylcholine (MPC) and bis(trimethylsilyloxy)methylsilylpropyl glycerol methacrylate (SiMA) using two methods: random copolymerization with a small amount of cross-linker (P(SiMA-*co*-MPC)) and construction of an interpenetration network (IPN) structure composed of cross-linked poly(MPC)(PMPC) chains and cross-linked poly(SiMA)(PSiMA) chains (PSiMA-*ipn*-PMPC). The polymerization was carried out by photoreaction. The surface hydrophilicity and water absorbability of P(SiMA-*co*-MPC) increased with an increase in the MPC unit composition. On the other hand, in the case of PSiMA-*ipn*-PMPC, a super-hydrophilic surface was obtained by the surface enrichment of MPC units. The optical and mechanical properties of PSiMA-*ipn*-PMPC are suitable for use as a material for preparing contact lenses. In addition, the oxygen permeability of PSiMA-*ipn*-PMPC remains high because of the PSiMA chains. The MPC units at the surface of the hydrogels reduce protein adsorption effectively. From these results for PSiMA-*ipn*-PMPC, we confirmed that it has the potential for application to silicone hydrogel contact lenses.

1. Introduction

Today, many people use soft contact lenses for vision correction. However, it has been reported that about 50% of soft contact lens wearers suffer from clinical symptoms such as eye infections and dry eyes [1]. It is recognized that these are caused by a shortage in the oxygen provided to cornea cells, mechanical stimulus to the eye induced by the surface of lenses, and protein adsorption by the lenses from tears [2]. Therefore, in addition to mechanical and optical properties, surface hydrophilicity, high oxygen permeability, and anti-biofouling properties are required for designing ideal lens materials.

Silicone hydrogel, which contains a silicone-based polymer segment, is one of the new materials being considered for manufacturing contact lenses [3]. The silicone-based polymer segments possess an affinity to oxygen. Thus, the oxygen permeability may be improved by a solution-diffusion mechanism. However, such silicone-based materials need surface treatment to improve their hydrophobicity. This involves plasma treatment and the addition of some surface-active agents [4]. Surface modification with phospholipid polymers has been recognized as an effective method to obtain materials with surface hydrophilicity and biocompatibility. Such phospholipid polymers, having various chemical structures and polymer architectures, were synthesized from 2-methacryloyloxyethyl phosphorylcholine (MPC) and its derivatives by conventional radical polymerization and living radical polymerization [5–8]. MPC polymers have been applied to many biomedical devices and used safely in clinical treatments, for example, they have been used in soft contact lenses [9–13], cardiovascular stents [14], implantable blood pumps [15], oxygenators [16], artificial hip joints [17–19], and diagnostic systems [20].

Recently, we synthesized cross-linked poly(MPC) hydrogels and characterized them for use as soft contact lens materials [21,22]. The poly(MPC) hydrogels showed reduced protein adsorption and good surface wettability. However, they had low oxygen permeability and mechanical weakness because of high water absorption ability. Therefore, in this study, we aimed to hybridize the properties of a silicone hydrogel and MPC polymer to realize an ideal soft contact lens material. There was a critical problem with the hybridization of these two organic materials because a methacrylate monomer bearing silicone moiety in the side chain is

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a hydrophobic monomer, while MPC is a hydrophilic monomer. Such incompatible compounds may cause microphase separation and form opaque materials. Thus, one of the significant issues to be solved was how to prevent microphase separation between the compounds. One solution to this problem—an interpenetrating polymer network (IPN) structure—may be a promising approach and is the proposed answer to this problem. An IPN can be defined as a combination of two or more polymer networks synthesized in juxtaposition. The entanglement of two cross-linked polymers leads to forced miscibility, compared with the usual incompatible blends, which results in good dimensional stability [23,24]. Furthermore, several studies have reported that IPN gels with MPC polymer networks have good surface hydrophilicity and protein adsorption resistance property based on the characteristics of the MPC polymer [25,26].

From the viewpoint of the polymerization process, we chose, bis(trimethylsilyloxy)methylsilylpropyl glycerol methacrylate (SiMA), which was used as the base compound of the silicone hydrogel. Two different processes for combining it with the MPC were examined. These were the simple random copolymerization of MPC and SiMA with a small amount of cross-linker, and the preparation of an IPN composed of cross-linked poly(MPC) (PMPC) and cross-linked poly(SiMA) (PSiMA). The relationships between the structures and properties of these hydrogels were investigated and are discussed from the viewpoint of silicone hydrogel contact lens materials.

2. Materials and methods

2.1. Materials

MPC was obtained from NOF Co., Ltd., Tokyo, Japan. It was synthesized by a previously reported method reported by Ishihara et al. [5]. SiMA was obtained from Vistakon, Ltd., USA, and was used as received. The chemical structure of SiMA and MPC is shown in Fig. 1. Triethyleneglycol dimethacrylate (TEGDMA), which was used as a cross-linker, was purchased from Tokyo Kasei, Tokyo, Japan. 2,2-Dimethoxy-2-phenylacetophenone (DMPA), which was used as a photoinitiator, and bovine serum albumin (BSA) were purchased from Sigma–Aldrich, MO, USA, and used as received. Isopropyl alcohol (IPA) and sodium dodecyl sulfate (SDS) were purchased from Kanto Chemical, Tokyo, Japan. Phosphate buffered saline was purchased from Immuno-Biological Laboratories Co., Ltd., and prepared under the recommended protocol.

2.2. Preparation of hydrogels

2.2.1. Synthesis of P(SiMA-co-MPC) by random copolymerization

Various proportions of SiMA and MPC (total monomer concentration: 2.0 M) were dissolved in IPA containing TEGDMA and DMPA (3.0 mol% to total monomer). After the elimination of oxygen by bubbling with argon gas, the mixture was poured into a mold made of poly(ethylene terephthalate) (PET) and the mold was sealed with a glass plate. Then, the mold was irradiated with UV light using an ultrahigh-pressure mercury lamp (UVL-400HA, Riko-kagaku Sangyo, Funabashi, Japan) for 30 min. The intensity of the UV light was 5 mW/cm^2 and the wavelength was in the range of 300–400 nm, which was determined with a filter. Next, polymerized specimens were taken from the mold and immersed in a mixture of IPA and water (50/50 by volume) for 24 h to ensure the complete removal of the unreacted compounds. The specimens thus prepared were cut into disks that were 15 mm in diameter and stored in pure water at 7 °C before use. The obtained P(SiMA-*co*-MPC) hydrogels were designated by their MPC unit compositions such as P(SiMA-*co*-MPC)(10), P(SiMA-*co*-MPC)(19), P(SiMA-*co*-MPC)(29), and P(SiMA-*co*-MPC)(39). The polymerization results are summarized in Table 1.

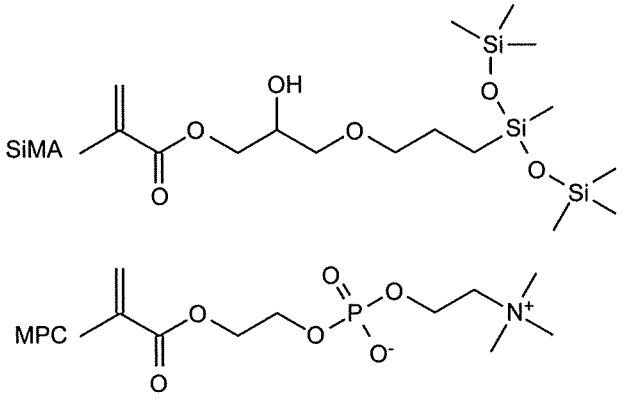

Fig. 1. Chemical structures of SiMA and MPC.

2.2.2. Synthesis of PSiMA-ipn-PMPC by double polymerization technique

Mixtures of SiMA, TEGDMA(1.0 mol % to SiMA), and DMPA(1.0 mol % to total monomers) were prepared. The primary gel was prepared from a mixture in the manner described previously and thoroughly dried at 60 °C for 24 h. Subsequently, the specimens were immersed in an IPA solution containing MPC 2.0 M), TEGDMA (3.0 mol% to MPC), and DMPA (3.0 mol% to monomers) for 24 h and allowed to swell to equilibrium at 25 °C. Each swollen specimen was placed on a PET plate and covered with a glass plate. Then, it was irradiated with UV light as previously described. The specimens were immersed in a mixture of IPA and water (50/50 by volume) in order to ensure the complete removal of the unreacted compounds. To eliminate the effect of oxygen, degassing of the solvent for polymerization and analysis was carried out by sonication for 15 min and then bubbling with argon gas for more than 5 min. The specimens thus prepared were cut into disks with a diameter of 10 mm and stored in pure water at 7 °C before use. The obtained IPN materials were designated by their MPC unit compositions such as PSiMA-*ipn*-PMPC(13), PSiMA-*ipn*-PMPC(19), PSiMA-*ipn*-PMPC(30), and PSiMA-*ipn*-PMPC(45). The preparation results are summarized in Table 1.

2.3. Chemical analysis

2.3.1. X-ray photoelectron spectroscopic analysis

X-ray photoelectron spectroscopy (XPS: AXIS-His 165, Shimadzu/Kratos, Kyoto, Japan) is a quantitative technique that reveals the elemental composition of the surface of a material. The phosphorus (P), nitrogen (N), and silicon (Si) contents were measured by XPS. The photoelectron take-off angle was 15°.

2.3.2. Microscopic infrared spectroscopic analysis

Microscopic FT-IR spectroscopy (IMV-4000 and FT/IR-6300, JASCO, Tokyo, Japan) was used to study the distribution of the MPC units in the hydrogels. The presence of the SiMA units and MPC units were confirmed from the absorbance of the Si-($O(CH_3)_3$) moiety at 842 cm^{-1} and the (–O–P(=O)O$^-$–O–) moiety at 966 cm^{-1}, respectively.

2.3.3. Thermal gravimetric analysis

Thermal gravimetric analysis (TGA: ETSAR6000, TG/DTA6200, Seiko, Chiba, Japan) is a classic technique to study the thermal stability of a polymer. TGA was conducted to confirm the IPN structure. Specimens swelled by water were cut into disks that were 4 mm in diameter. Measurements were conducted under the following conditions: 200 mL/min of N_2 flow and a temperature range of 25–700 °C with a 10 °C/min temperature change rate.

2.4. Characterization

2.4.1. Transparency measurement

The optical transparencies of the hydrogels swelled in distilled water were measured using a Vis/UV spectrophotometer (V-560, JASCO, Tokyo, Japan) in the wavelength range of 300–700 nm. Samples completely swelled by distilled water were slipped between two glass slides (1.2 mm thick) and transparencies were measured at 25 °C. For reference, the transparency of the commercially available soft contact lenses 1-DAY ACUVUE® (Vistakon) was also measured. The transparency of

Table 1
Characterization of hydrogels composed of MPC units and SiMA units.

Abbreviation	MPC unit composition (%)	EWC (%)	Young's modulus (MPa)	First decomposition temperature (°C)
PSiMA	0	2.4	57	344
P(MPC-*co*-SiMA)	10	16	10	ND[a]
	19	32	8	243
	29	52	8	240
	39	62	4	240
PMPC-*ipn*-PSiMA	13	19	ND	ND
	19	30	39	294
	30	40	41	291
	45	45	ND	ND

[a] ND means the data was not determined.

the hydrogels calculated using Lambert's equation was found to correspond to a thickness of 100 μm.

2.4.2. Equilibrium water content measurement

The equilibrium water content (EWC) of the hydrogels was determined by using the following equation:

$$EWC(\%) = \frac{W_{swell} - W_{dry}}{W_{swell}} \times 100$$

where W_{swell} is the weight of the hydrogel swelled by distilled water, and W_{dry} is the dry weight of the hydrogel.

2.4.3. Contact angle measurement by captive-bubble method

The captive-bubble method was used to determine the static contact angle (SCA). Each hydrogel was immersed in water to equilibrate and then fixed horizontally on a metal plate. A small air bubble was attached to the surface of the side of the hydrogel in the distilled water and the SCA in water was determined by the angle between the hydrogel and air bubble using a contact angle goniometer (CA-W, Kyowa, Saitama, Tokyo) at 25 °C. The measurement was repeated 5 times for each sample and the average was calculated.

2.4.4. Mechanical property evaluation

Young's modulus of materials was evaluated by means of a compression test (STA-1150, AND, Tokyo, Japan). Cylindrical samples (diameter: 10 mm; thickness: 5 mm) were compressed at a crosshead speed of 1 mm/min by a probe (diameter: 6 mm) at 25 °C.

2.4.5. Oxygen permeability measurement

The oxygen permeability of the hydrogels was defined as the product of the diffusion coefficient (D) and partition coefficient (k). It was determined by using the electrochemical method at 35 °C [27]. A rod-shaped electrode was inserted into a cylindrical cell filled with 1.0 mL of an electrolyte (a 0.50 mol/L aqueous KCl solution). The cathode was in contact with the water-swollen sample. The measured values of oxygen permeability were expressed in terms of barrers, which represent the volume of oxygen transmitted (10^{-11} cm^3(STP oxygen) cm^2/(cm^3(Material) s mmHg)).

2.4.6. Protein adsorption measurement

A single protein adsorption test was conducted to evaluate the anti-protein adsorption property of the hydrogels. Samples swelled by phosphate buffered saline (PBS, pH 7.4, ionic strength: 15 mM) were immersed in a PBS solution containing 4.5 mg/mL BSA at 37 °C for 15 min. After being rinsed in 30 mL PBS, the adsorbed protein was desorbed by sonication for 30 min in PBS containing 1 wt.% SDS [28]. The amount of protein adsorbed on the hydrogels was determined with a protein assay kit (Micro BCA™ protein assay reagent kit, #23235, Pierce, Rockford, IL, USA). For reference, the amount of protein adsorbed on the ACUVUE OASYS® lenses was also measured using the same protein adsorption test procedure.

3. Results and discussions

3.1. Surface properties of hydrogels composed of MPC unit and SiMA unit

The surface concentration of hydrophilic groups determines the wetting ability or lubrication of a material. It is also concerned with the biofouling property of a material. A problem with silicone hydrogel contact lenses is their hydrophobicity, which is a result of the silicone. Some surface treatments have been used to solve this problem. In our system, the MPC units are the key to improving the surface properties. We obtained an XPS signal from very near the surface (~3 nm) because the photoelectron angle detected was 15°. This allowed the composition of the MPC units at the surface to be investigated by comparing the ratios of nitrogen (N) or phosphorus (P) atoms to silicon atoms (Si) found with the XPS signals to those calculated on the basis of the initial concentrations of MPC and SiMA. Fig. 2 shows the relationship between the surface atomic P/Si ratios and total MPC unit compositions of the hydrogels. The existence of P is crucial evidence of MPC units. In Fig. 2, the theoretical line for the P/Si values also shows that the SiMA units and MPC units are incorporated homogeneously. The atomic composition, or P/Si value, at the surface of the P(SiMA-*co*-MPC) was in good agreement with the theoretical line. However, that of the PSiMA-*ipn*-PMPC was higher than the theoretical line. We observed almost

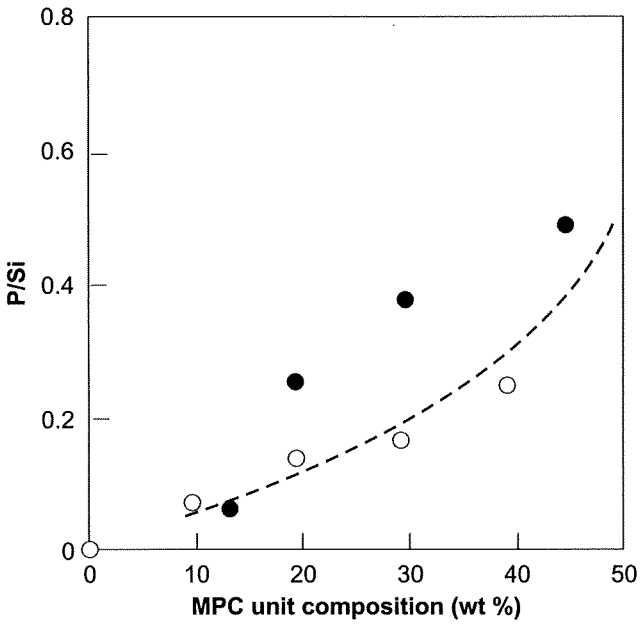

Fig. 2. MPC unit composition dependence of elemental ratio of phosphorus and silicon existing at the surface as measured by XPS. The open plots represent the P(MPC-*co*-SiMA) system and the closed plots represent the PMPC-*ipn*-PSiMA system.

the same MPC unit composition dependence for the N/Si values. To clarify the considerations, we analyzed a cross-section of the hydrogel using microscopic IR and made images of the MPC unit distribution near the surface. Fig. 3 shows images of the MPC unit distribution obtained by the microscopic FT-IR. The MPC units in the P(SiMA-*co*-MPC) hydrogel were located homogeneously. On the other hand, in the case of PSiMA-*ipn*-PMPC hydrogel, the density of the MPC units near the surface was higher than that in the bulk phase and gradient distribution was observed. These results indicated that while the SiMA and MPC units were homogeneously incorporated in the P(SiMA-*co*-MPC), the MPC units were enriched at the surface of the PSiMA-*ipn*-PMPC. The MPC units are hydrophilic and the poly(MPC) is a water-soluble polymer. Therefore, both P(SiMA-*co*-MPC) and PSiMA-*ipn*-PMPC contained water when they were immersed in water.

The surface contact angle by water and EWC were important for evaluating the surface and bulk hydrophilicity of the hydrogels. Fig. 4 shows the SCA and EWC of both hydrogels as functions of

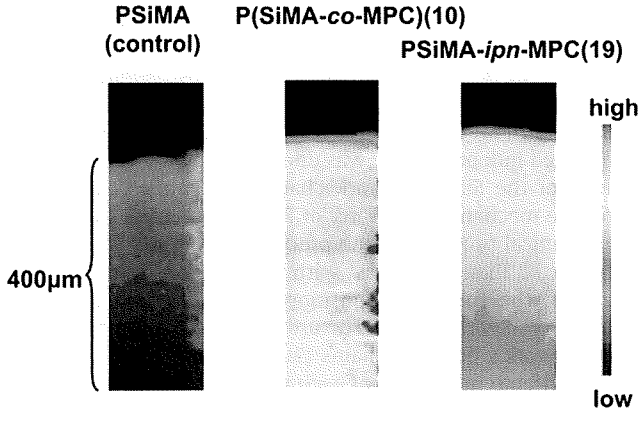

Fig. 3. Images of the MPC unit distribution at the near surface observed by microscopic FT-IR.

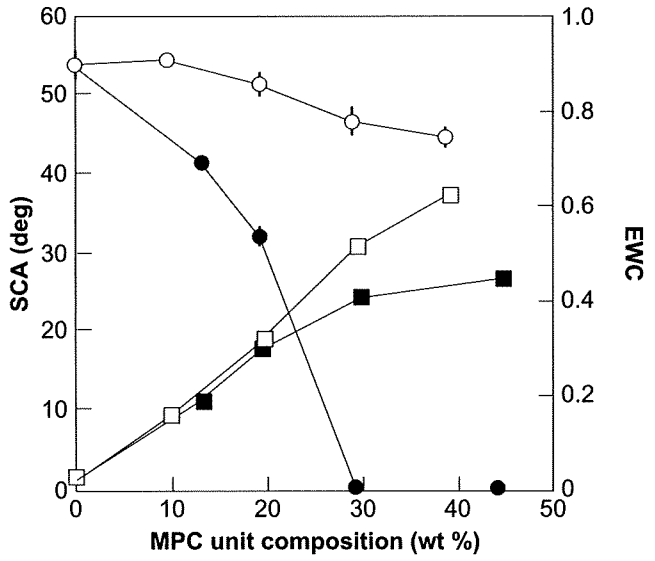

Fig. 4. MPC unit composition dependence of the SCA (circles) and EWC (squares) of the hydrogels. The open plots represent the P(MPC-CO-SiMA) system and the closed plots represent the PMPC-*ipn*-PSiMA system.

the MPC unit composition. The EWC increased with an increase in the MPC unit composition in both hydrogels. However, the SCA versus MPC unit composition showed a clear difference. While the SCA in the P(SiMA-*co*-MPC) system stayed between 45° and 55°, that of the PSiMA-*ipn*-PMPC system showed a drastic decrease with an increase in the MPC unit composition. Particularly, PSiMA-*ipn*-PMPC with an MPC unit composition of more than 30 wt.% indicated 0° of SCA. That is, the contact angle of the air bubble was 180° on the PSiMA-*ipn*-PMPC and the bubble could not adhere to the surface. Some studies have defined such a surface property as a super-hydrophilic surface [29]. This distinctive surface property of PSiMA-*ipn*-PMPC corresponds to its unique structure. Thus, surface enrichment by MPC units resulted in a super-hydrophilic surface.

3.2. Bulk properties of hydrogels

Specific mechanical and optical properties are other important factors that are required of materials for contact lenses. Table 1 lists the values of the Young's modulus and EWC for each hydrogel. The Young's modulus of P(SiMA-*co*-MPC) decreased with an increase in EWC. On the other hand, PSiMA-*ipn*-PMPC maintained a high modulus independent of the EWC. This difference in mechanical property is derived from the structural difference. For the P(SiMA-*co*-MPC), in which the SiMA and MPC units exist on the same polymer network chain, the elastic properties depended on the relatively low elasticity of the MPC units of the chain. The elasticity of PSiMA-*ipn*-PMPC, in which each polymer network chain is incorporated independently, was dominated by the relatively high elasticity of the SiMA network chain. This implies that we can control the mechanical property of PSiMA-*ipn*-PMPC by adjusting the mechanical property of the precursor SiMA gel. Some studies have reported that, in contrast with those synthesized by copolymerization, the first decomposition temperature of an organic hybrid material with an IPN structure arises from the entanglement of the network's polymer chains [30,31]. Table 1 shows the first decomposition temperature of each material as measured by TGA. While P(SiMA-*co*-MPC) degraded at around 245 °C, PSiMA-*ipn*-PMPC degraded at around 294 °C. This difference in the thermophysical property corresponds to a structural difference, confirming the formation of the IPN structure. Fig. 5 shows pictures of the obtained hydrogels, which were swelled by distilled water. In Fig. 6, the transmittances of the hydrogels at 550 nm are plotted against the MPC unit composition. As a control, transparency of commercially available soft contact lenses (1-DAY ACUVUE) was plotted in Fig. 6.

The optical tendencies were clearly different. While P(SiMA-*co*-MPC) increased in transparency with an increase in the MPC unit composition in the range from 10 wt.% to 30 wt.%, PSiMA-*ipn*-PMPC maintained a high transparency regardless of the MPC unit composition. This difference in the transparency tendencies may be explained by the structural difference. For PSiMA-*ipn*-PMPC, the introduced second polymer network entangled the first network and prevented microphase separation. Therefore, the PSiMA-*ipn*-PMPC could keep its excellent optical transparency regardless of the MPC unit composition.

3.3. Oxygen permeability of hydrogels based on the SiMA units

In general, oxygen permeation through silicone hydrogels has been recognized as a complex of two mechanisms: oxygen is transported as dissolved in water and directly dissolved in the silicone component. While the Dk value of water was evaluated as 80 barrer, the reported Dk value of silicone-based materials is much higher, for example, 600 barrer for polydimethylsiloxane [32].

Fig. 7 shows the relationship between the oxygen permeability and MPC unit composition of the hydrogel. As shown in Fig. 3, a change in the MPC unit composition induces a change in the EWC. The hydrogels showed decreases in the Dk value with an increase in the MPC unit composition, that is, an increase in the EWC of these materials. The oxygen permeability is depended on the surface concentration of SiMA units and formation of transfer pass way. In the case of P(SiMA-*co*-MPC) hydrogels, the oxygen permeability decreased dramatically with an increase in the MPC unit composition below 30 wt.%. This was due to lowering the SiMA units at the surface. The oxygen permeability became constant at higher MPC unit composition. This is due to the increase in the EWC and oxygen mainly permeates through water region in the hydrogel. This value is corresponded nicely to the Dk value of water (80 barrer). We could confirm that both MPC polymer materials had an oxygen permeation property based on the SiMA units independent of the synthesis method and structures.

For continuous wear contact lenses, the oxygen transmissibility (Dk/L) value, defined as the Dk value (10^{-11} cm^3(STP oxygen) cm^2/(cm^3(Material) s mmHg))(= 1 barrer) divided by the thickness L (cm), has been the typical index. A suitable Dk/L value for continuous wear contact lenses has been evaluated to be higher than 125.0 (10^{-9} cm^3(STP)/(cm^2 s mmHg)) [33]. We calculated the maximum hydrogel thicknesses that would still satisfy this requirement of a Dk/L value greater than 125.0 (10^{-9} cm^3(STP)/(cm^2 s mmHg)). These were 6.56×10^{-3} cm (65.6 μm) for SiMA-*co*-MPC(29), 6.72×10^{-3} cm (67.2 μm) for SiMA-*co*-MPC(39), 12.6×10^{-3} cm (126 μm) for PSiMA-*ipn*-PMPC(30), and 8.88×10^{-3} cm (88.8 μm) for PSiMA-*ipn*-PMPC(45). Since the thickness of commercial contact lenses is about 100 μm, these thickness values are sufficiently feasible.

3.4. Protein adsorption resistance of hydrogels based on MPC units

Fig. 8 shows the amount of protein adsorbed on various substrates. The amount of protein adsorbed on both P(MPC-*co*-SiMA) and PMPC-*ipn*-PSiMA was lower than that adsorbed on commercial soft contact lenses. Furthermore, an increase in the MPC unit composition induced a much lower protein adsorption. Thus, the MPC units played a significant role in this property. It is

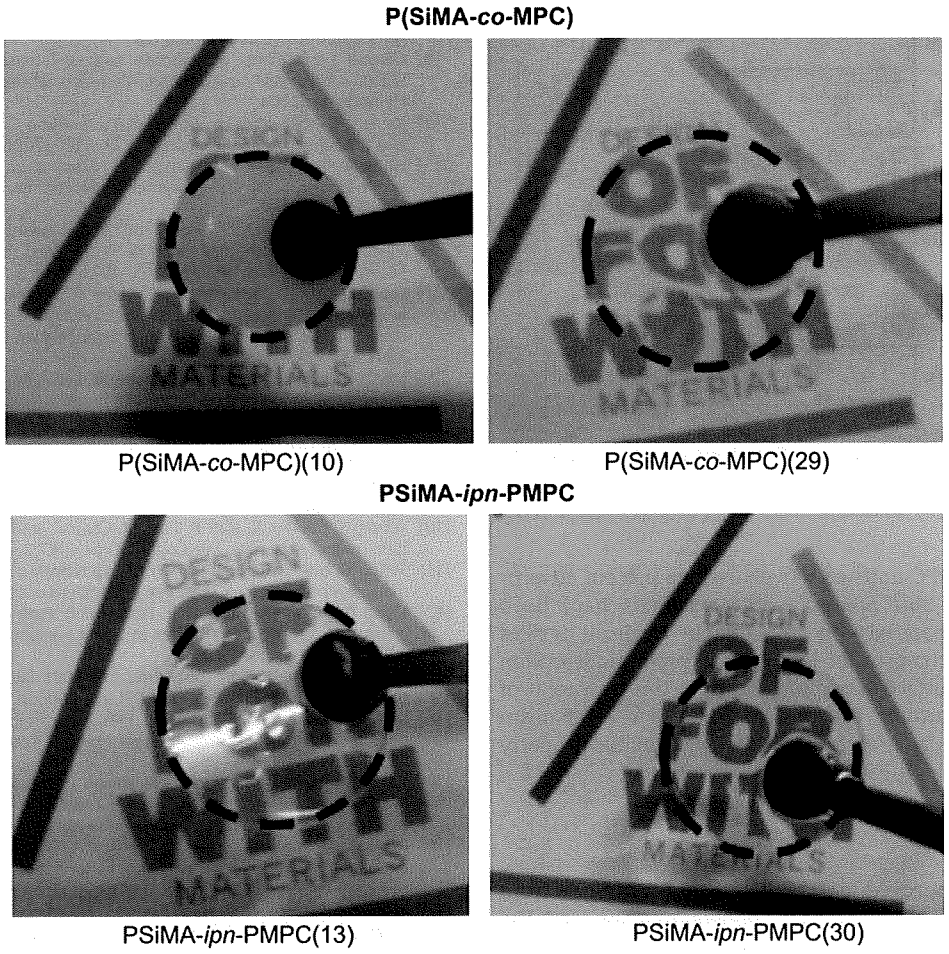

Fig. 5. Photographs of hydrogels.

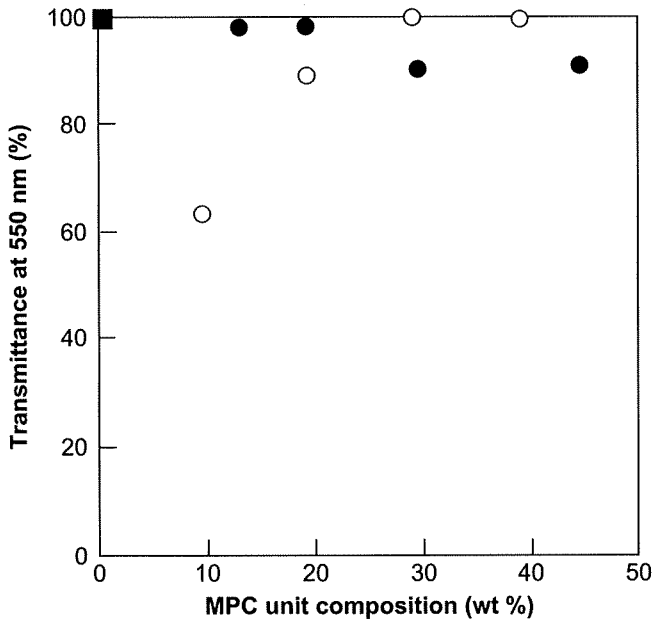

Fig. 6. Dependence of the transmittance at 550 nm on the MPC unit composition of the hydrogels. The open plots represent the P(MPC-*co*-SiMA) system and the closed plots represent the PMPC-*ipn*-PSiMA system. Closed square plot represents the data of 1-DAY ACUVUE.

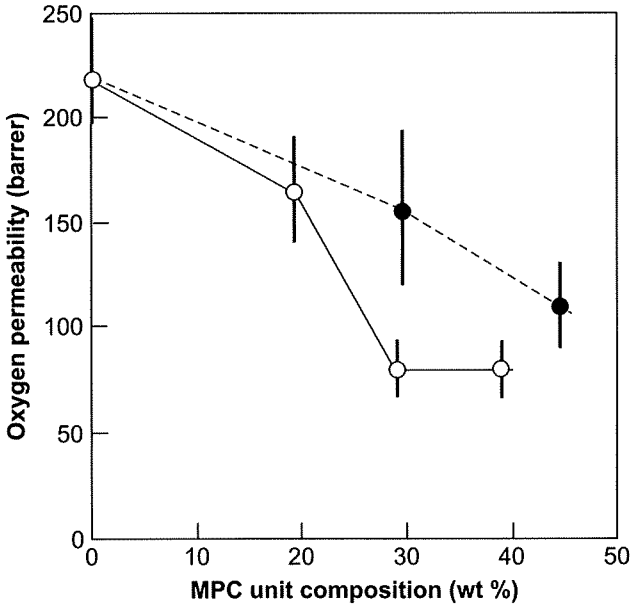

Fig. 7. MPC unit composition dependence of oxygen permeability as Dk value ($n = 3$). The open circle plots represent the P(MPC-*co*-SiMA) system and the closed circle plots represent the PMPC-*ipn*-PSiMA system.

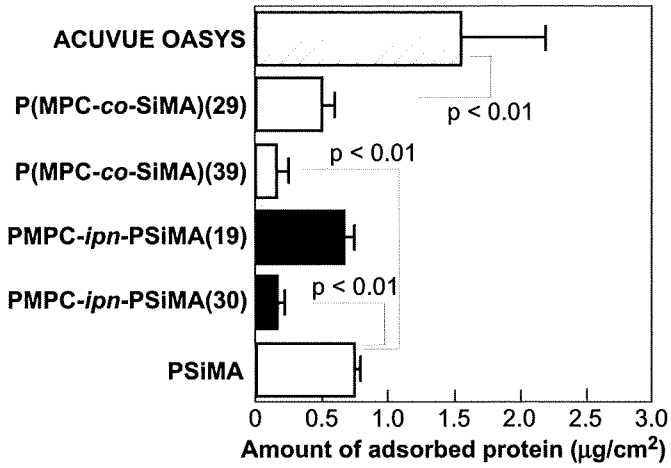

Fig. 8. Amount of protein adsorbed on the hydrogels ($n = 5$).

already known that MPC units effectively reduce protein adsorption [6,34–36]. Using this property, MPC polymers have been applied as coating materials on implantable medical devices [14,15,17–19]. The mechanism for the reduced protein adsorption on an MPC polymer surface was discussed from the viewpoint of the water structure around the surface [34]. This mechanism provides a suitable explanation for the super-hydrophilic property observed on the PMPC-*ipn*-PSiMA.

4. Conclusion

We could obtain improved hydrophilic silicone hydrogels from SiMA and MPC using two methods: copolymerization and the construction of an IPN structure using polymer chains. The PSiMA-*ipn*-PMPC showed good elasticity, optical transparency, high oxygen permeability, and a reduction in protein adsorption. The PSiMA-*ipn*-PMPC also possesses a super-hydrophilic surface achieved by the surface enrichment of MPC units. We confirmed that PSiMA-*ipn*-PMPC is an effective candidate material for silicone hydrogel contact lenses.

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Appendix

Figures with essential colour discrimination. Figs. 3 and 5 of this article are difficult to interpret in black and white. The full colour images can be found in the online version, at doi:10.1016/j.biomaterials.2010.01.026.

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Self-initiated surface grafting with poly(2-methacryloyloxyethyl phosphorylcholine) on poly(ether-ether-ketone)

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ABSTRACT

Poly(ether-ether-ketone) (PEEK)s are a group of polymeric biomaterials with excellent mechanical properties and chemical stability. In the present study, we demonstrate the fabrication of an antibiofouling and highly hydrophilic high-density nanometer-scaled layer on the surface of PEEK by photo-induced graft polymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC) without using any photo-initiators, i.e., "self-initiated surface graft polymerization." Our results indicated that the diphenylketone moiety in the polymer backbone acted as a photo-initiator similar to benzophenone. The density and thickness of the poly(MPC) (PMPC)-grafted layer were controlled by the photo-irradiation time and monomer concentration during polymerization, respectively. Since MPC is a highly hydrophilic compound, the water wettability (contact angle $<10°$) and lubricity (coefficient of dynamic friction <0.01) of the PMPC-grafted PEEK surface were considerably lower than those of the untreated PEEK surface (90° and 0.20, respectively) due to the formation of a PMPC nanometer-scale layer. In addition, the amount (0.05 $\mu g/cm^2$) of BSA adsorbed on the PMPC-grafted PEEK surface was considerably lower, that is more than 90% reduction, compared to that (0.55 $\mu g/cm^2$) for untreated PEEK. This photo-induced polymerization process occurs only on the surface of the PEEK substrate; therefore, the desirable mechanical properties of PEEK would be maintained irrespective of the treatment used.

1. Introduction

Poly(aryl-ether-ketone) (PAEK), including poly(ether-ether-ketone) (PEEK), is a new family of high performance thermoplastic polymers, consisting of an aromatic backbone molecular chain interconnected by ketone and ether functional groups, i.e., a benzophenone (BP) unit is included in its molecular structure. Polyaromatic ketones exhibit enhanced mechanical properties, and their chemical structure is stable, resistant to chemical and radiation damage, and compatible with several reinforcing agents (such as glass and carbon fibers); therefore, they are considered to be promising materials for not only industrial applications but also biomedical applications. In the 1980s, the *in vivo* stability of various PAEK materials and the tissue response to those were investigated [1]. Recently, PEEK has emerged as the leading high-performance super-engineering plastic candidate for replacing metal implant components, especially in the field of orthopedics and spinal surgery [2]. In recent studies, the tribological and bioactive properties of PEEK, which is used as a bearing material and flexible implant in orthopedic and spinal surgeries, has been investigated [3–5]. However, conventional single-component PEEK cannot satisfy these requirements (e.g., antibiofouling, wear resistance, and fixation to a bone) for use as an artificial joint or intervertebral body fusion cage [2]. For further improving the capabilities of PEEK as an implant biomaterial, various studies have focused upon the lubricity and antibiofouling of the polymer, either via reinforcing agents or surface modifications [6,7]. Therefore, multicomponent polymer systems have been designed in order to synthesize new multifunctional biomaterials. In order to use PEEK and related composites in the implant applications, they can be engineered to have a wide range of physical, mechanical, and surface properties.

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Surface modification is one of the most important technologies for the preparation of new multifunctional biomaterials for satisfying several requirements; surface modifications used today include coating, blending, and grafting. In general, graft polymerization is performed most frequently using either of the following methods that utilize chemical and/or physical processes: (a) surface-initiated graft polymerization or "grafting from" methods in which the monomers are polymerized from initiators or comonomers, and (b) adsorption of the polymer to the substrate or "grafting to" methods, such as reaction of the end groups of the ready-made polymers with the functional groups of the substrate. The "grafting from" method has an advantage over the "grafting to" method wherein it forms a high-density polymer brush interface with a multifunctional polymer; this advantage proves to be functionally effective.

It is well known that when BP is exposed to photo-irradiation such as ultraviolet-ray (UV)-irradiation, a pinacolization reaction is induced; this results in the formation of semi-benzopinacol radicals (i.e., ketyl radicals) that act as photo-initiators. Therefore, in this study, we have focused upon a BP unit in PEEK and formulated a self-initiated surface-graft polymerization method that utilizes the BP unit in graft-from polymerization (Fig. 1) [8]. This polymerization reaction involving free radicals is photo-induced by UV-irradiation. Under UV-irradiation, a BP unit in PEEK can undergo the following reactions in the monomer aqueous solutions [9–15]: the pinacolization reaction (photo-reduction by the H-abstraction of a BP unit in PEEK) results in the formation of a semi-benzopinacol radical, which can initiate the graft-from polymerization of the feed monomer as the main reaction, and the graft-to polymerization (the radical chain end of the active-polymer couples with the semi-benzopinacol radical of the PEEK surface) as a sub-reaction. In addition, a photo-scission reaction occurs as a sub-reaction, which may not need an hydrogen (H)-donor. The cleavage reaction induces recombination and the graft-from polymerization. When water polymerization is performed in the presence of an H-donor, a phenol unit may be sub-sequentially formed due to H-abstraction. This technique enables the direct grafting of the functional polymer onto the PEEK surface in the absence of a photo-initiator, thereby resulting in the formation of a C–C covalent bond between the functional polymer and PEEK substrate.

2-Methacryloyloxyethyl phosphorylcholine (MPC), a methacrylate monomer bearing a phosphorylcholine group, is used to synthesize polymer biomaterials having excellent biocompatibility [16–25]; the MPC polymers have potential applications in a variety of fields such as biomedical science, surface science, and bioengineering because they possess unique properties such as excellent antibiofouling, and low friction abilities. Thus, surface modification with the MPC polymer on medical devices is effective for obtaining biocompatibility. Several medical devices have already been developed by utilizing MPC polymers and have been used clinically; therefore, the efficacy and safety of MPC polymers as biomaterials are well-established [23–25].

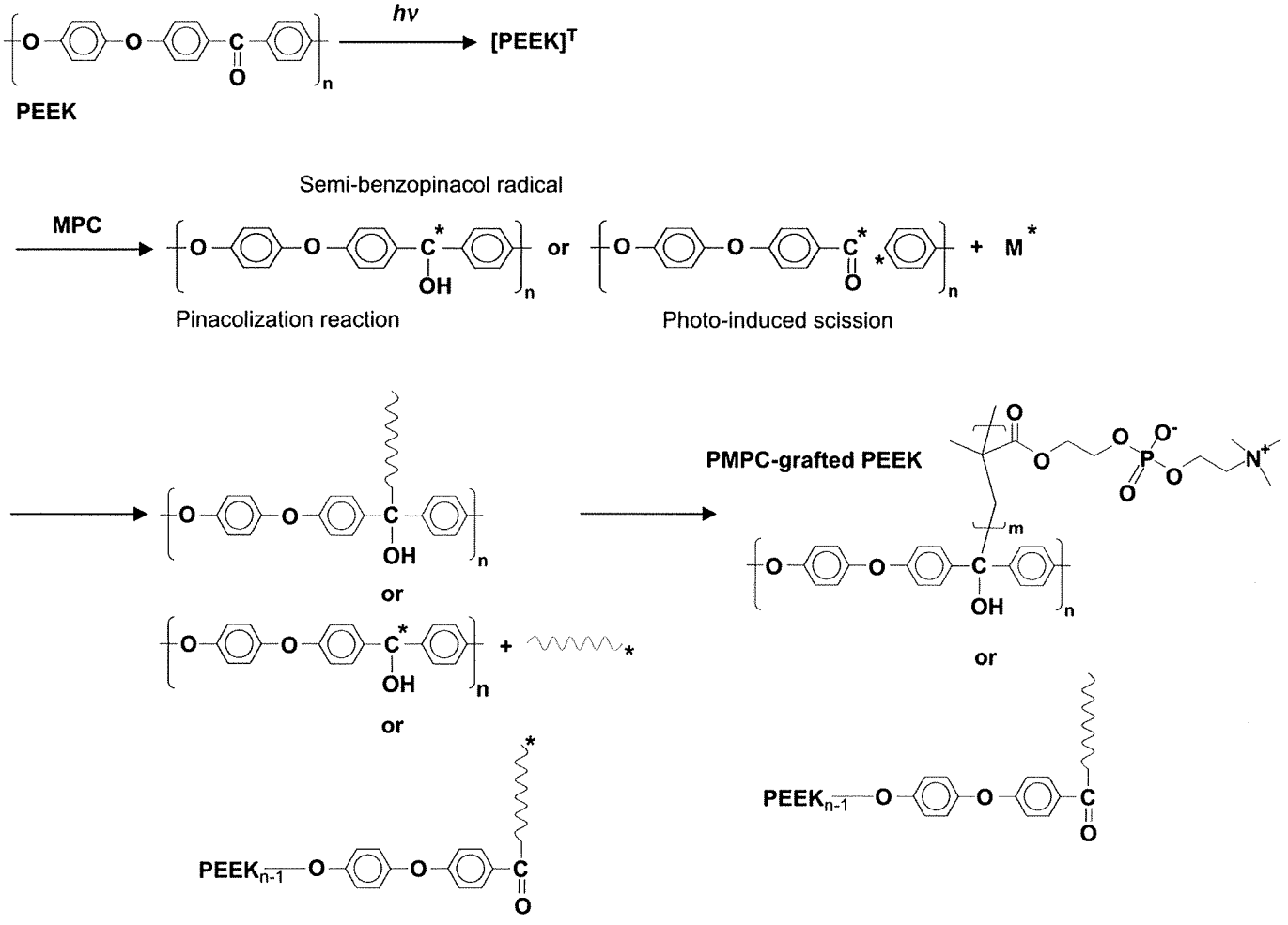

Fig. 1. Schematic illustration for the preparation of PMPC-grafted PEEK.

In this study, we have demonstrated the fabrication of a biocompatible and highly hydrophilic nanometer-scale-modified surface by poly(MPC) (PMPC)-grafting onto the self-initiated PEEK surface using a photo-induced pinacolization reaction; further, we also investigated the effects of photo-irradiation time and MPC concentration variability on PMPC-graft polymerization. The results revealed that it was possible to control the PMPC-graft layer in order to improve wettability, lubricity, and anti-protein adsorption for developing multifunctional PEEK biomaterials.

2. Materials and methods

2.1. Self-initiated graft polymerization of MPC

The preparation of PMPC-grafted PEEK is schematically illustrated in Fig. 1. PEEK specimens were machined from an extruded PEEK (450 G; Victrex PLC, Thornton-Cleveleys, UK) bar stock, which was fabricated without stabilizers or additives. The surfaces of the PEEK specimens were ultrasonically cleaned in ethanol for 20 min, and then dried in vacuum. MPC was industrially synthesized using the method reported by Ishihara et al. [26] and supplied by NOF Corp. (Tokyo, Japan). It was dissolved in degassed water to obtain 0.25- and 0.50-mol/L MPC aqueous solutions, and PEEK specimens were immersed in these solutions. Photo-induced graft polymerization was carried out at 60 °C for 5–90 min on the PEEK surface under UV-irradiation (UVL-400HA ultra-high pressure mercury lamp; Riko-Kagaku Sangyo Co. Ltd., Funabashi, Japan) with an intensity of 5 mW/cm^2; a filter (model D-35; Toshiba Corp., Tokyo, Japan) was used to restrict the passage of UV-light to wavelengths of 350 ± 50 nm. After polymerization, the PMPC-grafted PEEK specimens were removed from the reaction solution, washed with pure water and ethanol to remove non-reacted monomers and non-grafted polymers, and dried at room temperature.

2.2. Surface analysis of PMPC-grafted PEEK

The functional group vibrations of the PMPC-grafted PEEK surfaces were examined by Fourier-transform infrared (FT-IR) spectroscopy with attenuated total reflection (ATR) equipment. The FT-IR/ATR spectra were obtained using an FT-IR analyzer (FT/IR615; JASCO Co. Ltd., Tokyo, Japan) for 32 scans over the range of 1000–1800 cm^{-1} at a resolution of 4.0 cm^{-1}.

The surface elemental conditions of the PMPC-grafted PEEK surfaces were analyzed by X-ray photoelectron spectroscopy (XPS). The XPS spectra were obtained using an XPS spectrophotometer (AXIS-HSi165; Kratos/Shimadzu Co., Kyoto, Japan) equipped with a 15-kV Mg-K_α radiation source at the anode. The take-off angle of the photoelectrons was maintained at 90°. Five scans were taken for each sample.

The static-water contact angles on the PMPC-grafted PEEK surfaces were measured by the sessile drop method using an optical bench-type contact angle goniometer (Model DM300; Kyowa Interface Science Co. Ltd., Saitama, Japan). Drops of purified water (1.0 μL) were deposited on the PMPC-grafted PEEK surfaces, and the contact angles were directly measured with a microscope after 60 s of dropping. Measurements were repeated fifteen times for each sample, and the average values were regarded as the contact angles.

2.3. Cross-sectional observation by transmission electron microscopy

A cross-section of the PMPC layer on the PMPC-grafted PEEK surface was observed using a transmission electron microscope (TEM). The specimens were first embedded in epoxy resin, stained with ruthenium oxide vapor at room temperature, and then sliced into ultra-thin films (approximately 100-nm thick) by using a Leica Ultra Cut UC microtome (Leica Microsystems, Ltd., Wetzlar, Germany). A JEM-1010 electron microscope (JEOL, Ltd., Tokyo, Japan) was used for the TEM observation at an acceleration voltage of 100 kV. The thickness of the PMPC layer was determined by averaging ten points on the cross-sectional TEM image.

2.4. Gravimetric measurement of PMPC-grafted layer

The PMPC-grafted PEEK specimens were weighed on a microbalance (Sartorius Supermicro S4; Sartorius AG, Goettingen, Germany) to determine the physical properties of the PMPC-grafted layer. The physical properties were obtained by the following equations:

$$\text{PMPC} - \text{graftextent}(\text{g/cm}^2) = (W_g - W_0)/S \quad (1)$$

$$\text{PMPC} - \text{graftlayerdensity}(\text{g/cm}^3) = (W_g - W_0)/S \times T \quad (2)$$

where W_0 is the initial weight of the untreated PEEK substrate, W_g is the weight of the PMPC-grafted PEEK in dry condition, S is the grafted surface area of the PEEK substrate, and T is the thickness of the PMPC-graft layer determined by cross-sectional TEM observation. The weighing was repeated five times for each sample, and the average values were regarded as the weight of the samples.

2.5. Characterization of protein adsorption by micro-bicinchoninic acid method

The amount of protein adsorbed on the PMPC-grafted PEEK surfaces was measured by the micro-bicinchoninic acid (BCA) method. Each specimen was immersed in Dulbecco's phosphate-buffered saline (PBS, pH 7.4, ion strength = 0.15 M; Immuno-Biological Laboratories Co. Ltd., Takasaki, Japan) for 1 h to equilibrate the PMPC-grafted surface. The specimens were immersed in bovine serum albumin (BSA, $M_w = 6.7 \times 10^4$; Sigma-Aldrich Corp., MO, USA) solution at 37 °C for 1 h. The protein solution was prepared in a BSA concentration of 4.5 g/L, i.e., 10% of the concentration of human plasma levels. Then, the specimens were rinsed five times with fresh PBS and immersed in 10.0 g/L sodium dodecyl sulfate (SDS) aqueous solution and shaken at room temperature for 1 h to completely detach the adsorbed BSA on the PMPC-grafted surface. A protein analysis kit (micro BCA protein assay kit, #23235; Thermo Fisher Scientific Inc., IL, USA) based on the BCA method was used to determine the BSA concentration in the SDS solution, and the amount of BSA adsorbed on the PMPC-grafted PEEK surface was calculated.

2.6. Friction test

The friction test was performed using a pin-on-plate machine (Tribostation 32; Shinto Scientific Co. Ltd., Tokyo, Japan). Each of the PMPC-grafted PEEK surfaces was used to prepare five sample pieces. A 9-mm-diameter pin with Co-Cr-Mo alloy was prepared. The surface roughness (R_a) of the pin was <0.01, which was comparable to that of femoral head products. The friction tests were performed at room temperature with a load of 0.98 N, sliding distance of 25 mm, and a frequency of 1 Hz for a maximum of 100 cycles. Pure water was used as a lubricant. The mean coefficients of dynamic friction were determined by averaging five data points from the 100 (96–100) cycle measurements.

2.7. Mechanical test

The mechanical properties of untreated PEEK and PMPC-grafted PEEK with a 0.50-mol/L MPC concentration and 90-min photo-irradiation time were evaluated with tensile and flexural tests. Tensile testing was performed according to ISO527 standard using a type 1B tensile bar specimen and a crosshead speed of 50 mm/min. Flexural testing was performed according to ISO178 standard with a crosshead speed of 2 mm/min. The results derived from each measurement in the mechanical test were expressed as the mean values ± standard deviation. The statistical significance ($p < 0.05$) was estimated by Student's t-test.

3. Results

Fig. 2 shows the FT-IR/ATR and XPS spectra of untreated PEEK and PMPC-grafted PEEK with a 0.50-mol/L MPC concentration and 90-min photo-irradiation time. Transmission absorption peaks were observed at 1600, 1490, 1280, 1190, and 1160 cm^{-1} for both untreated PEEK and PMPC-grafted PEEK (Fig. 2(A)). These peaks are chiefly attributed to the diphenyl ether group, phenyl rings, or aromatic hydrogens in the PEEK substrate [8,27]. However, absorption peaks at 1720 and 1080 cm^{-1} (shoulder peak) were observed only for PMPC-grafted PEEK. These peaks corresponded to the carbonyl group (C=O) and phosphate group (P–O) in the MPC unit, respectively [8,25]. The XPS spectra of the binding energy region of the nitrogen (N) and phosphorous (P) electrons showed peaks for untreated PEEK and PMPC-grafted PEEK, whereas, peaks were not observed in the case of untreated PEEK (Fig. 2(B)). The peaks at 403 and 134 eV were attributed to the $-N^+(CH_3)_3$ and phosphate groups, respectively. These peaks indicate the presence of phosphorylcholine in the MPC units. After PMPC-grafting, the peaks attributed to the MPC unit were clearly observed in both the FT-IR/ATR and XPS spectra of the PMPC-grafted PEEK. These results indicate that PMPC was successfully grafted on the PEEK surface [8,25].

Fig. 3 shows the N and P concentrations of the PMPC-grafted PEEK as a function of the photo-irradiation time during polymerization at 0.25- and 0.50-mol/L MPC concentrations. The N and P concentrations increased with the photo-irradiation time. When the photo-irradiation time was shorter than 45 min, the N and P concentrations of the PMPC-grafted PEEK surface with a 0.50-mol/L MPC concentration were higher as compared with those with a 0.25-mol/L MPC concentration. The N and P concentration in the PMPC-grafted PEEK with both 0.25- and 0.50-mol/L MPC

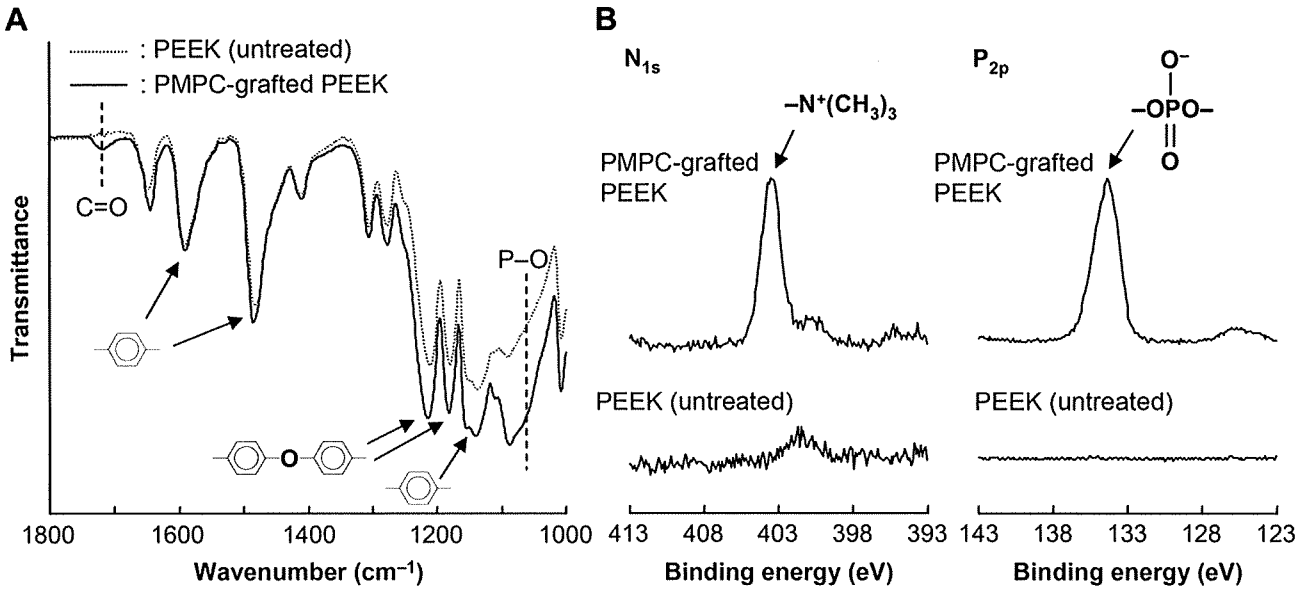

Fig. 2. FT-IR/ATR (A) and XPS (B) spectra of untreated and PMPC-grafted PEEK.

concentrations increased to 5.2 up to a photo-irradiation time of 90 min; those values were almost equivalent to the theoretical elemental composition (N = 5.3, P = 5.3) of PMPC. These results indicate that the PMPC layer fully covered the surface of the PEEK substrate.

Fig. 4 shows the cross-sectional TEM images of PMPC-grafted PEEK obtained with a 0.50-mol/L concentration and various photo-irradiation times as well as the PMPC-graft layer thickness determined by the TEM observations. With photo-irradiation times longer than 45 min, a uniform PMPC layer was clearly observed on the PEEK surface (Fig. 4(A)). The PMPC-graft layer thickness increased with the photo-irradiation time (Fig. 4(B)). In the case of both 0.25- and 0.50-mol/L MPC concentrations, when the photo-irradiation time was greater than 45 min, the PMPC-graft layer thicknesses became almost constant at 40 and 100, respectively.

Fig. 5 shows the physical properties of PMPC-graft layer on the PEEK surface as a function of the photo-irradiation time at MPC concentrations of 0.25 and 0.50 mol/L. The PMPC-graft extent of the PMPC-grafted PEEK with both 0.25- and 0.50-mol/L MPC concentration increased gradually with the photo-irradiation time (Fig. 5(A)). The PMPC-graft layer density of the PMPC-grafted PEEK with 0.25-mol/L MPC concentration increased proportionally to 2.3 g/cm^3 with the photo-irradiation time. In the case of PMPC-grafted PEEK obtained with 0.50-mol/L MPC concentration, the PMPC-graft layer density rapidly increased to 1.3 g/cm^3 up to a photo-irradiation time of 10 min; it then increased slowly to 2.2 g/cm^3 with an increase in the photo-irradiation time.

Fig. 6 shows the static-water contact angle of PMPC-grafted PEEK obtained with MPC concentrations of 0.25 and 0.50 mol/L as a function of the photo-irradiation time. The static-water contact angle of untreated PEEK was 90° and decreased markedly with an increase in the photo-irradiation time. When the photo-irradiation time was 90 min, the static-water contact angle of PMPC-grafted PEEK was the lowest value at <10°.

Fig. 7 shows the coefficient of dynamic friction of PMPC-grafted PEEK obtained with 0.25- and 0.50-mol/L MPC concentrations and various photo-irradiation times. For PMPC-grafted PEEK obtained with both 0.25- and 0.50-mol/L MPC concentrations, these coefficients of dynamic friction decreased markedly with an increase in photo-irradiation time. The PMPC-grafted PEEK obtained with 90 min photo-irradiation time was the lowest at <0.01, and exhibited approximately 95% reduction in their coefficients of dynamic friction when compared with the untreated PEEK.

Fig. 8 shows the amount of adsorbed BSA of PMPC-grafted PEEK as a function of the photo-irradiation time with MPC concentrations of 0.25 mol/L and 0.50 mol/L. The amount of adsorbed BSA of PMPC-grafted PEEK decreased remarkably with an increase in photo-irradiation time.

The mechanical properties of untreated PEEK and PMPC-grafted PEEK are summarized in Table 1. Tensile properties and flexural modulus did not differ significantly ($p > 0.05$) between untreated PEEK and PMPC-grafted PEEK. In contrast, there was a small but significant difference ($p < 0.05$) in the flexural strength and strain of untreated PEEK and PMPC-grafted PEEK examined in this study. However, both untreated PEEK and PMPC-grafted PEEK met the ASTM F2026 requirements [28].

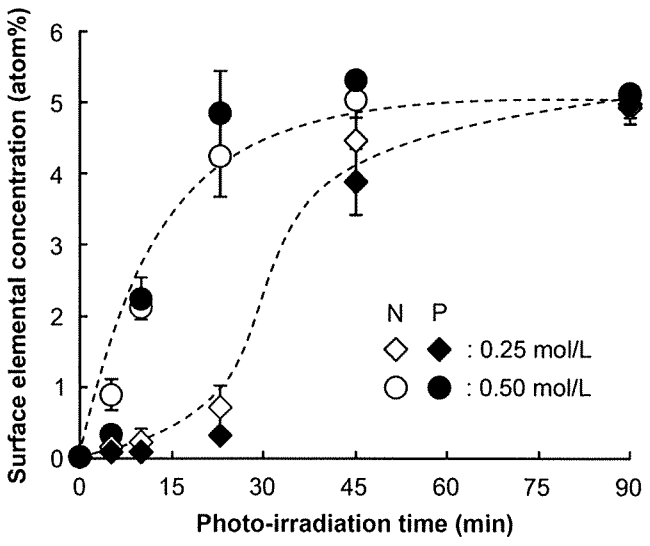

Fig. 3. Surface elemental concentration ($n = 5$) of PMPC-grafted PEEK as a function of the photo-irradiation time with MPC concentrations of 0.25 and 0.50 mol/L. Bar: Standard deviation.

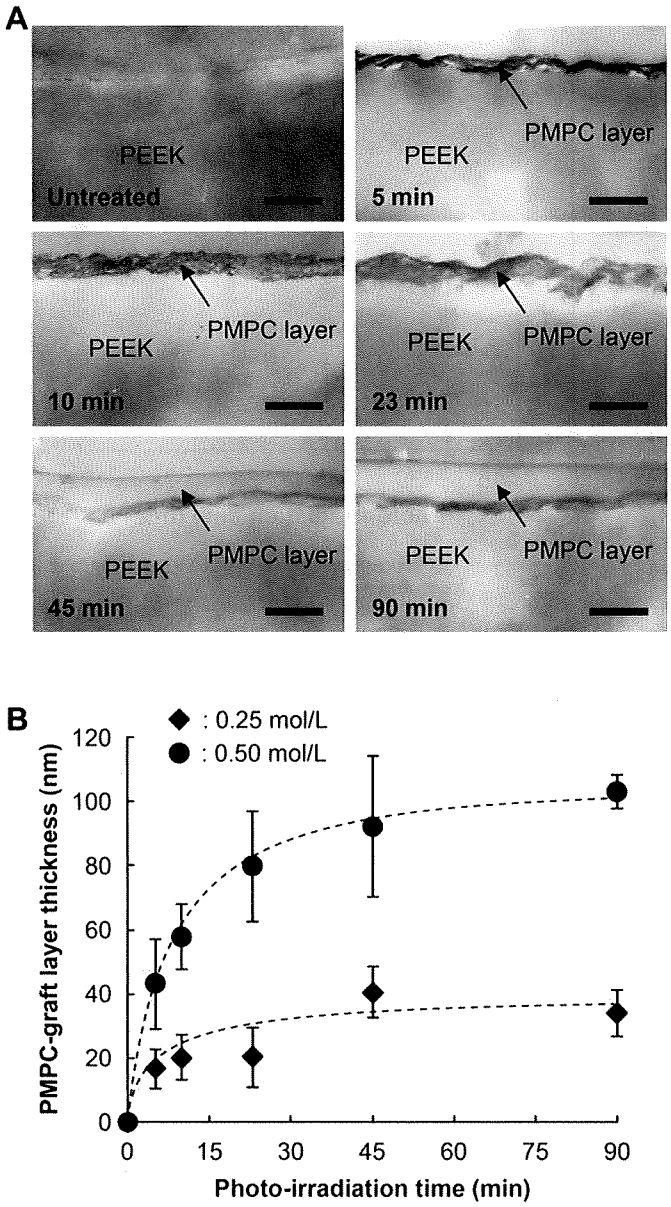

Fig. 4. Cross-section of the PMPC-grafted PEEK. (A) Cross-sectional TEM images of PMPC-grafted PEEK obtained with a MPC concentration of 0.5 mol/L and various photo-irradiation times. Bar: 100 nm. (B) PMPC-graft layer thickness ($n = 10$) determined by TEM observation. Bar: Standard deviation.

4. Discussion

In this study, we demonstrated the fabrication of a biocompatible and highly hydrophilic nanometer-scale-modified surface by PMPC-grafting onto the self-initiated PEEK surface using a photo-induced grafting-from polymerization reaction, i.e., "self-initiated surface graft polymerization." The following methods were employed in our study: (a) grafting from polymerization for the formation of a high-density graft polymer layer, (b) photo-induced polymerization in the absence of photo-initiators, and (c) using biocompatible hydrophilic macromolecules, which exhibited photo-reduction by H-abstraction of a BP unit in PEEK from an H-donor; this induced surface-initiated graft polymerization of the feed methacrylate-type monomer (i.e., MPC) on the PEEK surface, even in the absence of a photo-initiator such as BP. This report discusses the structure of the PMPC layer of PMPC-grafted PEEK in terms of the condition variability of self-initiated surface graft polymerization and its effects on antibiofouling and hydrophilicity.

It is important to control the graft layer thickness and density for optimizing several areas of applications, e.g., in adhesion, colloid stabilization, and lubrication. In Fig. 4, when the photo-irradiation time was greater than 45 min, the PMPC-graft layer thicknesses became almost constant. Moreover, at the same photo-irradiation time of 90 min, it was shown that the higher the monomer concentration, the thicker the graft layer obtained: the PMPC-graft layer thickness (approximately 100 nm) of the PMPC-grafted PEEK obtained with 0.50-mol/L MPC concentration was thicker than that (approximately 40 nm) with 0.25-mol/L MPC concentration. The phenomenon can be attributed to the fact that the graft layer thickness increases with monomer concentration. When the PMPC layer has a brush-like structure, the graft layer thickness may correlate with the molecular weight of the grafted PMPC. The high-density PMPC-graft layer on the PEEK surface is assumed to exhibit a brush-like structure [29,30]. It is generally well known that the reaction rate of radical polymerization is extremely high [31]. It was observed that the graft layer thickness (i.e., molecular weight of the graft polymer) was greatly dependent on the monomer concentration, but virtually independent of the photo-irradiation time. Thus, in this study, the length (molecular weight) of the PMPC-graft chains was assumed to be successfully controlled by the MPC concentration used for polymerization. This indicates that the length of the PMPC chain grafted on the PEEK surface increased with the MPC concentration during polymerization [32]. Additionally, a uniform PMPC layer was clearly observed on the surface of the PEEK substrate, and no cracks or delamination were observed at the PEEK substrate or the interface between the PMPC layer and PEEK substrate. These results indicate that the PMPC layer formed on the PEEK substrate is uniformly distributed over the substrate and is bound to the substrate by covalent C–C bonds.

On the other hand, the PMPC-graft layer density on the PEEK surface almost linearly increased to $>2.2\ g/cm^3$ with the photo-irradiation time, suggesting that the graft chain propagates steadily with increasing photo-irradiation time (Fig. 5(B)). In order to obtain the high-density PMPC-graft layer, the photo-irradiation time must be controlled. Further, interestingly, while using an MPC concentration of 0.50 mol/L, the rate of the increase in the PMPC-graft layer density was low with a photo-irradiation time above 10 min. The present self-initiated surface graft polymerization method is photo-induced by UV-irradiation onto the BP unit of PEEK surface. In this study, it is assumed that the UV-irradiation directly produces a high-concentration free radical, because this PEEK is a semi-crystalline structure (crystallinity, 30–40%) with a high-density BP unit in the surface. When the MPC concentration in a feed is also high, the self-initiated surface graft polymerization between the radicals on the PEEK surface and the MPC monomer occurs extremely rapidly in the reaction system, forming the high-density graft chain on the surface. Hence, diffusion of the MPC monomer onto the PEEK surface might be interfered by the high-density graft chain because of its high viscosity. When the monomers attached to the PEEK surface were subjected to UV-irradiation, radicals were freely formed on the PEEK surface in the early stage but not in the late stage of polymerization, probably because the high-density grafted polymer chains formed by then blocked the diffusion of the monomer to the PEEK surface. Therefore, it is supposed that the rate of increase in the PMPC-graft layer density changed due to the high concentration of free radicals and monomers.

The water-wettability of the PMPC-grafted PEEK surface is considerably greater than that of the untreated PEEK surface (Fig. 6), because of the presence of a nanometer-scaled PMPC layer (Fig. 4(A)): MPC is a highly hydrophilic compound, while PMPC is water-soluble. In Figs. 4–7, we observe that the dynamic coefficient

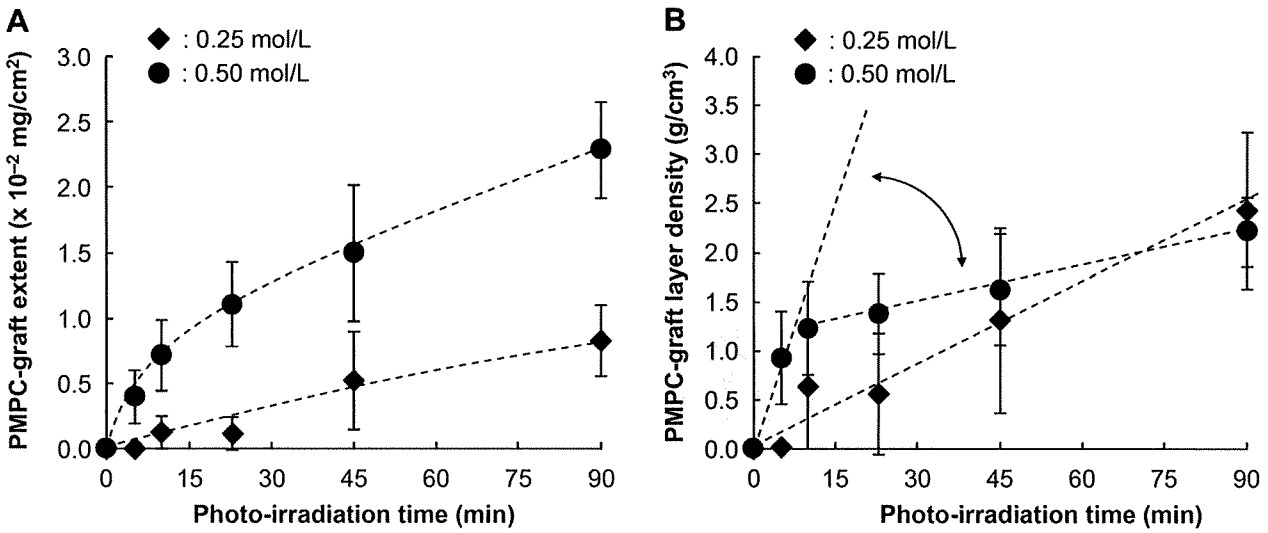

Fig. 5. Physical properties ($n = 5$) of PMPC-graft layer on PEEK surface as a function of the photo-irradiation time with MPC concentrations of 0.25 and 0.50 mol/L. (A) PMPC-graft extent by weighting, and (B) density calculated by (A) and mean PMPC-graft layer thickness of Fig. 4(B). Bar: Standard deviation.

of friction was greatly dependent on the water-wettability (static-water contact angle), but virtually independent of the structure (thickness and density) of the graft layer. A significant reduction in the static-water contact angle of the PMPC-grafted surface resulted in a substantial improvement in friction property. Fluid-film lubrication (or hydration lubrication) with the PMPC-grafted surface was achieved by the intermediate hydrated layer. It can be affirmed that this highly lubricated surface utilizing PMPC mimics the natural cartilage structure [33]. When the PEEK surface is modified by PMPC-grafting, the grafted PMPC causes a significant reduction in sliding friction between the graft surfaces because the thin water films that are formed act as extremely efficient lubricants. The water-lubrication systems utilizing PMPC suppress direct contact of the counter-bearing face with the PEEK substrate in order to reduce the frictional force. Thus, the PMPC-graft layer is expected to significantly increase the durability of the bearing biomaterials. On the other hand, when the photo-irradiation time was shorter than 23 min, the decrease in the static-water contact angle of the PMPC-grafted PEEK surface with a 0.25-mol/L MPC concentration was only slight. It was thought that the surface phenomenon was caused by the pinacolization reaction, because the static-water contact angle of the UV-irradiated PEEK surface without monomer was decreased to approximately 80° (data not shown).

The fabrication of surfaces that exhibit anti-protein adsorption and/or cell adhesion has been one of goals of surface engineering for medical devices. The adsorption of the BSA on the PMPC-grafted PEEK surface decreased to 10% ($p < 0.001$) with an increase in photo-irradiation time, as shown in Fig. 8, as compared to that in untreated PEEK. It is shown that the extent of protein rejection is related to the PMPC-graft extent (Figs. 5(A) and 8). Extensive grafting gives rise not only to an increase in the thickness of graft layer but also to an increase in the volume fraction (i.e., density) of graft segments in the layer. Therefore, it was thought that these characteristics of thickness and density of the PMPC-graft layer had a significant influence on protein adsorption. The mechanism of protein adsorption resistance on the PMPC-grafted surface is

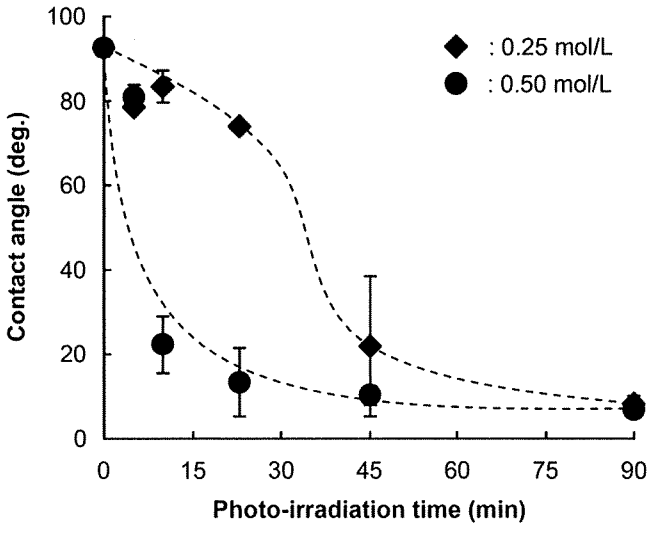

Fig. 6. Static-water contact angle ($n = 15$) of PMPC-grafted PEEK as a function of the photo-irradiation time with MPC concentrations of 0.25 and 0.50 mol/L. Bar: Standard deviation.

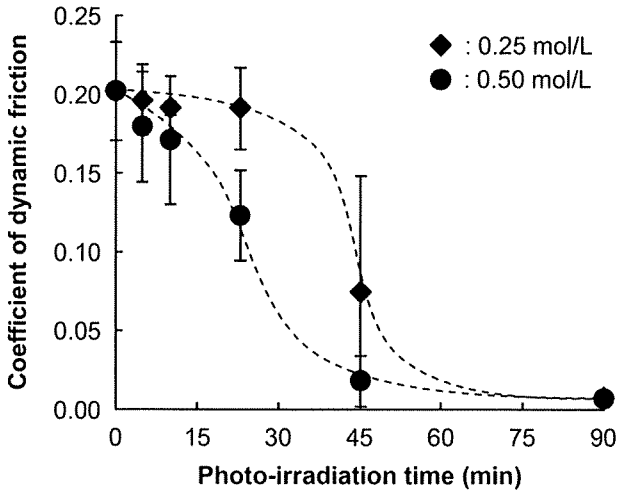

Fig. 7. Coefficient of dynamic friction ($n = 5$) of PMPC-grafted PEEK as a function of the photo-irradiation time with MPC concentrations of 0.25 and 0.50 mol/L. Bar: Standard deviation.

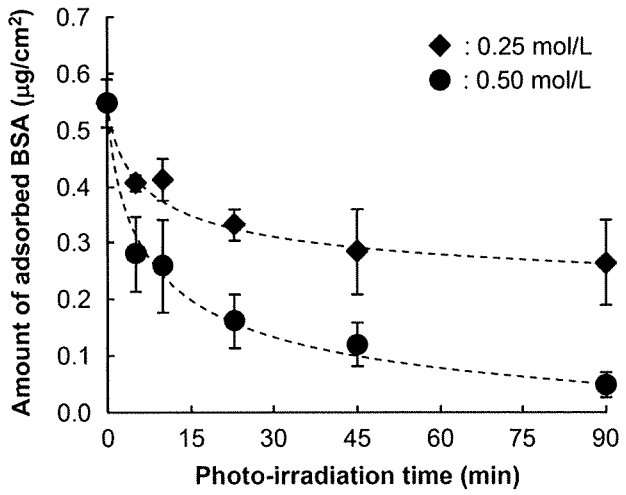

Fig. 8. Amount of adsorbed BSA ($n = 3$) of PMPC-grafted PEEK as a function of the photo-irradiation time with MPC concentrations of 0.25 and 0.50 mol/L. Bar: Standard deviation.

hypothesized as follows: the protein adsorption resistance attributed to the water-fluid film and hydration layer was due to water and hydrophilic macromolecules with volume exclusion effects. The presence of the water-fluid film and hydration layer is responsible for the easy detachment of proteins and the prevention of conformational changes in the adsorbed proteins [34]. These observations are consistent with the results of the static-water-contact angle measurements, cross-sectional TEM observations, and PMPC-graft layer weighting of the PEEK whose surface was modified by PMPC-grafting. These results imply that the PMPC-grafted PEEK surface is biocompatible in terms of tissue and blood compatibility, because MPC polymer-modified surfaces are known to exhibit *in vivo* antibiofouling [16,20,23,24].

As shown in Table 1, the mechanical properties of the PEEK are unchanged even after PMPC-grafting. This indicates that the photo-induced radical graft polymerization proceeds only on the surface of the PEEK substrate, while the properties of the substrate remain unchanged. Retention of the properties of the PEEK substrate is very important in clinical use, because the biomaterials used in implants act not only as surface-functional materials but also as structural materials *in vivo*.

The design of a well-characterized surface is a considerably important and difficult task. The photo-induced graft polymerization in the absence of photo-initiators of this study, i.e., "self-initiated surface graft polymerization" successfully prepared the surface with controlled graft layer thickness and density. This simple self-initiated surface graft polymerization would be highly suitable for industrial applications [35,36] as well as the development of medical devices [2–7]. The synthesis of a self-initiated biocompatible polymer having unique properties such as anti-protein adsorption and wettability by the photo-induced grafting-from polymerization reaction is a novel phenomenon in the field of biomaterials and bioengineering sciences, and the fabrication of the PMPC-grafted PEEK surface can result in the development of next-generation multifunctional biomaterials.

Table 1
Mechanical properties ($n = 10$) of untreated and PMPC-grafted PEEK.

Test method	Property	PEEK (untreated)	PMPC-grafted PEEK	*t*-Test
Tensile test	Yield strength (MPa)	109.4 (0.4)[a]	109.9 (0.6)	N.S.
	Ultimate strength (MPa)	71.5 (1.5)	71.7 (1.7)	N.S.
	Elongation (%)	24.0 (9.5)	24.4 (9.0)	N.S.
Flexural test	Ultimate strength (MPa)	168.9 (0.6)	173.7 (1.8)	<0.05
	Ultimate strain (%)	6.4 (0.1)	6.7 (0.2)	<0.05
	Modulus (GPa)	3.9 (0.0)	3.9 (0.0)	N.S.

[a] The standard deviations are shown in parenthesis.

5. Conclusions

A biocompatible and highly hydrophilic nanometer-scale-modified surface was successfully fabricated on the PEEK substrate by the photo-induced graft polymerization of PMPC in the absence of photo-initiators, i.e., "self-initiated surface graft polymerization." Since MPC is a highly hydrophilic compound, the water wettability and lubricity of the PMPC-grafted PEEK surface were greater than that of the untreated PEEK surface due to the formation of a PMPC nanometer-scale layer. In addition, the amount of BSA adsorbed on the PMPC-grafted PEEK surface considerably decreased compared to that in the case of untreated PEEK. The density and thickness of the grafting layer could be controlled by the photo-irradiation time and monomer concentration.

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Selection of highly osteogenic and chondrogenic cells from bone marrow stromal cells in biocompatible polymer-coated plates

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Abstract: To enrich the subpopulation that preserves self-renewal and multipotentiality from conventionally prepared bone marrow stromal cells (MSCs), we attempted to use 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer-coated plates that selected the MSCs with strong adhesion ability and evaluated the proliferation ability or osteogenic/chondrogenic potential of the MPC polymer-selected MSCs. The number of MSCs that were attached to the MPC polymer-coated plates decreased with an increase in the density of MPC unit (0–10%), whereas no significant difference in the proliferation ability was seen among these cells. The surface epitopes of CD29, CD44, CD105, and CD166, and not CD34 or CD45, were detectable in the cells of all MPC polymer-coated plates, implying that they belong to the MSC category. In the osteogenic and chondrogenic induction, the MSCs selected by the 2–5% MPC unit composition showed higher expression levels of osteoblastic and chondrocytic markers (COL1A1/ALP, or COL2A1/COL10A1/Sox9) at passage 2, compared with those of 0–1% or even 10% MPC unit composition, while the enhanced effects continued by passage 5. The selection based on the adequate cell adhesiveness by the MPC polymer-coated plates could improve the osteogenic and chondrogenic potential of MSCs, which would provide cell sources that can be used to treat the more severe and various bone/cartilage diseases. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res 92A: 1273–1282, 2010

Key words: bone marrow stromal cell (MSC); 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer; osteogenesis; chondrogenesis; cell adhesion

INTRODUCTION

Bone marrow mesenchymal stem cells or stromal cells (MSCs) retain the potential to differentiate into multiple cell lineages that include osteoblasts, chondrocytes, adipocytes, myoblasts, and early progenitors of neural cells.[1–3] Because MSCs can be easily obtained from a small aspirate of bone marrow and they rapidly proliferate during the early passages of the expansion culture, human MSCs are regarded as one of the attractive cell sources for regenerative medicine in bone, cartilage, heart, nerve, and other tissues. However, MSCs are principally collected from bone marrow aspirates only through their selection by adhesiveness onto the plastic culture dishes,[4] and therefore, they include various subpopulations of cells which possess different proliferation rates or differentiation potentials. During the long-term culture with repeated passages, the balance among the subpopulations in the MSCs changes as a result of the difference in the proliferation rates, which may cause a deterioration of the self-renewal property or multipotentiality after repeated passages.[5]

To isolate or enrich the subpopulation that preserves the self-renewal and the multipotentiality

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from the conventionally prepared MSCs, various kinds of efforts have been made in the past decade. It was reported that the sizes and structures of the cells could distinguish the cells possessing a great potential for multilineage differentiation, termed rapid self-renewal (RS) cells, from the heterogeneity of the MSCs.[6] The RS cells had a shaped round shape with approximately a 7-μm diameter, and could be purified by using a 10-μm filter.[6] However, some limitations had been pointed out in the paper that the filtration process could only provide a low yield of purified RS cells because the other-sized cells rapidly obstructed the filter pores. The RS cells were also characterized by the low forward scatter and low side scatter of light during a flow cytometric analysis.[7] During cell sorting with the criteria of a low forward scatter and low side scatter, the subpopulation was successfully enriched for the RS cells, which increased the differentiation potential for osteoblasts and adipocytes. Although the cell sorting technologies of flow cytometry have been highly anticipated for the effective isolation of a specific subpopulation, some issues including the acquisition rates of target cells, the prevention of pathogen contamination, or the mechanical and thermodynamic damage to cells by the cell sorter should be cautiously evaluated before clinical use.

The MSC isolation was also attempted, using some surface epitopes, including CD13, CD29 (integrin β1), CD44 (hyaluronan receptor), CD73(SH3), CD90 (Thy-1), CD105 (Endoglin), CD166 (activated leukocyte cell adhesion molecule/ALCAM), PDGF receptor or Stro-1, all of which are highly expressed in the MSCs.[6,8] The combination with CD34 and CD45 (leukocyte common antigen/LCA), either of which is a marker of hematopoietic stem cells, could exclude the hematopoietic lineage from the MSCs. However, as the expression level of the markers in the MSCs was quite similar to those of fibroblastic cells that are also contained in bone marrow aspirates and that decrease the multipotency and self-renewal,[8] specific selection of the MSCs from such heterogenetic cell populations could not be sufficiently obtained even by flow cytometry or a magnetic cell sorting system.

Serum deprivation is one of the possible methods to concentrate the subpopulation possessing a high proliferation and differentiation potential from the heterogeneity of the MSCs.[9] When early-passage human MSCs were cultured in serum-free medium without cytokines or other supplements, a subpopulation of the cells was attached to the plates and survived for 2–4 weeks. Afterward, such cells began to proliferate in serum-containing medium, and prominently showed stem cell properties including long telomeres or a high expression of the octomer-binding transcription factor 4 (OCT-4). The findings suggested that such cells that possess a strong adherent ability and survive despite the harsh environments may show a high quality of stem cell properties.

On the basis of this hypothesis, we attempted to select some subpopulations of MSCs showing a high adhesiveness on the culture plates. For selection, the cell adhesiveness was adjusted by the coating of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers. The MPC polymers are designed with inspiration from cell membrane surface and well-known biocompatible polymers that can reduce protein adsorption or subsequent cell adhesion significantly.[10–12] On the basis of this fundamental biocompatibility, the MPC polymers have been used for preparing medical devices, for example, the surfaces of intravascular stents, intravascular guide wires, soft contact lenses, an artificial lung or artificial hip joint.[13–16] Some of these are already clinically available.

We examined the selectivity of MSCs using MPC polymer-coated plates and evaluated the proliferation ability or differentiation potential of the MPC polymer-selected subpopulation. Especially, we focused on the osteogenic and chondrogenic ability, because bone and cartilage tissue engineering using MSCs are highly desired for clinical applications.

MATERIALS AND METHODS

Preparation of MPC polymer-coated plates

Coating of the MPC polymer onto the polystyrene (PS) surface of the culture plates was performed by a simple dip-coating using MPC polymer solutions. The composition of MPC units was controlled by mixing poly(*n*-butyl methacrylate) (Poly(BMA)) and poly(MPC-*co*-BMA)(PMB30). These polymers were synthesized by a conventional radical polymerization. Poly(BMA) was a homopolymer of BMA without MPC unit (molecular weight = 4.0×10^5), and PMB30 was a copolymer composed of 30% of MPC units and 70% of BMA units (molecular weight = 6.0×10^5). In this study, each polymer was dissolved in a mixture of tetrahydrofran (THF) and ethanol (1:9 by volume) as solvents, and then poly(BMA) and PMB30 solutions were prepared (0.25 wt %). To control the MPC unit composition in the range between 0, 1, 2, 5, and 10% of MPC unit composition, these polymer mixtures in the solution were prepared. The dip-coating was carried out in the clean bench as follows: (i) 200 μL of the solution was poured into the each culture plates (ϕ 2.2 cm), (ii) the polymer solution was removed after 5 s, (iii) the coating was repeated and the resulting culture plate was dried over night, and (iv) the MPC polymer-coated culture plate was sterilized by UV irradiation for an adequate time. Therefore, the resulting MPC unit density on the plate was 0, 1, 2, 5, and 10% MPC unit composition.

Surface elemental analysis of the MPC polymer-coated PS plate was carried out by X-ray photoelectron spectroscopy (XPS, AXIS-His, Shimadzu/KRATOS, Kyoto, Japan). The X-ray source used for XPS measurements was Mg Ka source. The take-off angle of the photoelectrons was fixed

as 90°. At least five points of the sample were measured by XPS and these intensities were averaged before the following calculation. The surface compositions of the MPC units were calculated as follows. The ratio of signal intensity at 133 eV based on the phosphorus atom attributed to the MPC units over that at 285 eV based on the carbon atoms attributed methyl groups and methylene groups in both BMA and MPC units was determined. The calibration was carried out using the ratio obtained from the XPS spectra of both poly(BMA) and poly(MPC)-coated PS plate as 0% and 100% of MPC unit, respectively.

MSC preparation and selection by MPC polymer-coated plates

All procedures for the present experiments were approved by the ethics committee or institutional committee for animal research of the University of Tokyo Hospital (ethics permission #622). Figure 1(a) indicates the experimental design. Human MSCs were obtained from the femur of osteoarthritic patients who underwent total hip replacement at the University of Tokyo Hospital, after informed consent. Cells in bone marrow aspirates (100 μL/φ 2.2 cm dish) were seeded on MPC polymer-coated culture plates with various MPC unit compositions as 0–10%, and cultured using the hMSC bullet kit (Cambrex, East Ruatherford, NJ) in a 37°C/5% CO_2 incubator. Rat MSCs were collected from 6-week-old male Sprague-Dawley rats (Nisseizai, Tokyo, Japan). After the epiphyses of the tibias were removed, the marrow was flushed out by using a syringe filled with medium and filtered through a 70-μm nylon mesh. The obtained bone marrow materials (100 μL/φ 2.2 cm dish) were plated and cultured in the same manner as human MSCs.

The cells were harvested by treatment using trypsin-EDTA solution. After the cell harvest of the primary culture from the MPC polymer-coated plates, the cells were reseeded onto the conventional PS culture plates at a density of 5.0×10^3 cells/cm^2. Passages were performed when the cells were approaching confluence. The medium was changed three times per week. The cell numbers were counted by a haematocytometer, while the viability of the cells was checked by trypan blue staining. Cell proliferation was also colorimetrically measured by cell counting kit-8 (Dojin, Kumamoto, Japan), a week after cell seeding.

Flow cytometric analysis

Cells were harvested using trypsin-EDTA solution, centrifuged at 1500*g* for 5 min, and resuspended at 5×10^6 cells/mL in phosphate-buffered saline (PBS) containing 3% fetal bovine serum. Aliquots containing 10^5 cells were incubated with individual primary antibodies or control IgG for 30 min at room temperature. The cells were washed in PBS containing 3% fetal bovine serum and incubated with a fluorescent conjugated secondary antibody for 30 min at room temperature. Samples were analyzed using a FACS LSL II (BD, Franklin Lakes, NJ). The following monoclonal antibodies were used: mouse monoclonal antibodies against human CD29 (integrin β1, BD), human CD34 (Chemicon, Victoria, Australia), human CD44 (hyaluronan receptor, Ancell, Bayport, MN), human CD45 (LCA, Cymbus, Chandlers Ford, UK), human CD105 (Endoglin, Ancell), CD166 (ALCAM, Ancell), normal mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA) and fluorescein isothiocyanate (FITC)-conjugated rabbit antibody against mouse IgG (Santa Cruz Biotechnology).

Osteogenic and chondrogenic induction for MSCs

The osteogenic[1] or chondrogenic[17,18] differentiation was induced in MSCs according to previously reported procedures with some modifications. For the osteogenic differentiation, cells were seeded at 4.0×10^4 cells per 2.2-cm plates and maintained for 21 days in DMEM supplemented with 10% fetal bovine serum, 10 m*M* β-glycerophosphate, 100 n*M* Dexamethasone, and 50 μg/mL ascorbic acid-2-phosphate. For the chondrogenic differentiation, cells were seeded at 2×10^5 cells per 15 mL plastic centrifuge tube and maintained in 2 mL of serum-free a-MEM supplemented with 3500 μg/mL glucose, 6.25 μg/mL insulin, 6.25 μg/mL transferrin, 6.25 ng/mL selenite, 5.33 μg/mL linolate, 1.25 mg/mL bovine serum albumin, 10 ng/mL transforming growth factor-β3, 100 n*M* dexamethasone and 50 μg/mL ascorbic acid-2-phosphate. The cells were cultured under the chondrogenic status for 21 days. The medium was changed three times per week.

Total RNA extraction and real-time RT-PCR

The total RNA was isolated from MSC using the chaotropic Trizol method (Nippon-gene, Tokyo, Japan). The total mRNA (1 μg) was reverse transcribed using the Super Script reverse transcriptase with a random hexamer (Takara Shuzo, Shiga, Japan). The full-length or partial-length cDNA of the target genes, including the PCR amplicon sequences, was amplified by PCR, cloned into pCR-TOPO Zero II or pCR-TOPO II vectors (Invitrogen, Carlsbad, CA), and used as standard templates after linearization. The QuantiTect SYBR Green PCR Master Mix (Qiagen, Hilden, Germany) was used, and the SYBR Green PCR amplification and real-time fluorescence detection were performed with an ABI 7700 Sequence Detection system (Foster City, CA). All reactions were run in quadruplicate. The sequences of the primers were 5′-CTCCT CGCTTTCCTTCCTCT-3′ and 5′-GTGCTAAAGGTGCCAA TGGT-3′ for COL1A1; 5′-GAGTCAAGGGTGATCGTGGT-3′ and 5′-CACCTTGGTCT CCAGAAGGA-3′ for COL2A1; 5′-AGGAATGCCT GTGTCTGCT T -3′ and 5′-ACAGGCC TACCCAAACATGA-3′ for COL10A1; 5′- GACCCTTGACC CCCACAAT-3′ and 5′- GCTCGTACTGCATGTCCCCT-3′ for ALP; 5′-CATG AGCGAGGG CACTCC-3′ and 5′-TCGCTTCAGGTCAGCCTTG-3′ for Sox9; 5′-GAAGGTGA AGGTCGGAGTCA-3′ and 5′-GAAGATGGTGATGGGAT TTC-3′ for GAPDH.

Enzyme activity for ALP

The enzyme activity was histochemically detected in the MSCs in which the osteogenic differentiation was induced.

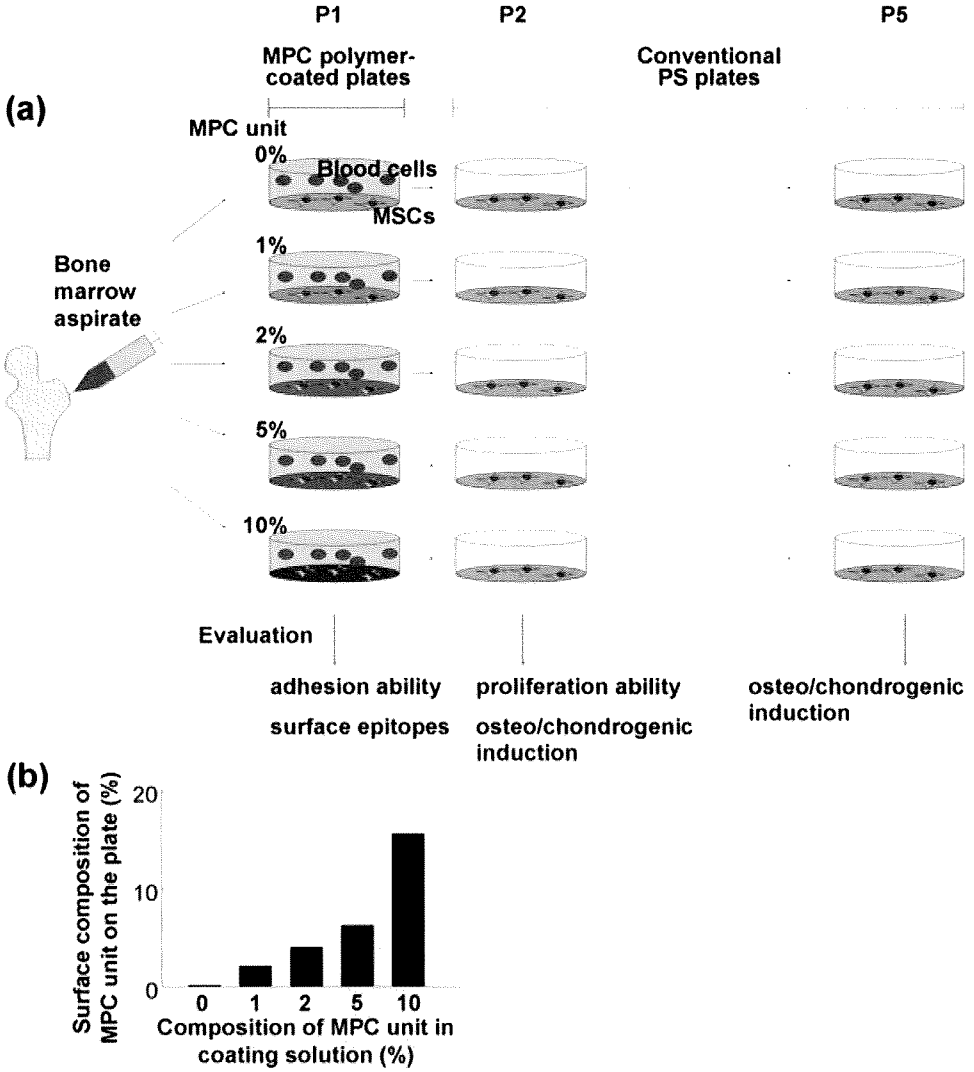

Figure 1. (a) The experimental design. Cells in bone marrow aspirates were seeded on MPC polymer-coated plates at the composition of 0–10% MPC unit, at passage 1, while the adhesion ability of MSCs to the MPC polymer-coated plates and the surface epitopes of MPC-selected cells were evaluated. Although cells were cultured on the MPC polymer-coated plates at passage 1, the cells were seeded onto the conventional PS plates thereafter. The proliferation of cells (passage 2) was measured by cell counting, while the differentiation potential for osteogenesis and chondrogenesis was examined at passages 2 and 5. (b) Relationship between MPC unit composition at the surface on PS plate after coating and that in polymer-coating solution. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

For ALP enzyme histochemistry, the cells were incubated with a mixture of 5 mg naphthol AS-BI phosphate (Sigma, St. Louis, MO) as a substrate and 18 mg of fast red violet LB salt (Sigma) diluted in 30 mL of 0.1 mol/L Tris-HCl buffer (pH 8.5). The images were taken by the digital camera, while the enzyme activity was quantitatively measured by histomorphometrical approaches using the software Scion Image alpha 4.0.3.2 (Scion, Frederick, MD).

RESULTS

Selection using MPC polymer-coated plates

Polymer coating of PS culture plate with the PMB30/poly(BMA) mixed solution was proceeded well, and the surface of the plate was covered with these polymers, completely. When the surface composition of MPC units on the plates was calculated from the XPS results, it was found that the MPC unit composition at the surface increased in parallel with that in the polymer mixed solution containing poly(BMA) and PMB30 used in a single dip coating as shown in Figure 1(b). We confirmed that the surface composition the MPC units could be controlled.

With these plates, we first selected some subpopulations of the MSCs according to the degrees of the adhesiveness on the culture plates coated with different compositions of the MPC unit. Human bone marrow aspirates (~0.1 mL) was seeded onto the culture plates with a 2.2 cm diameter coated with 0, 1, 2, 5, and 10%

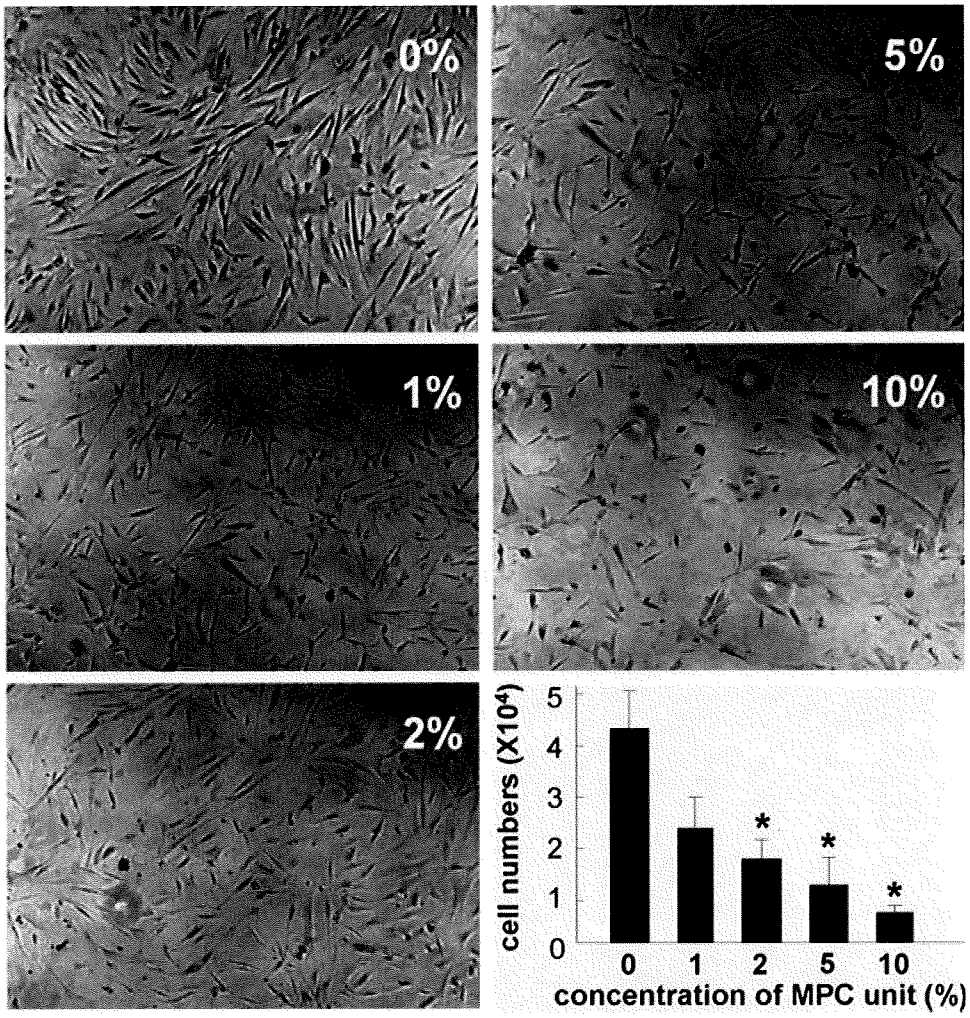

Figure 2. The adhesion of cells in human bone marrow aspirates onto the culture plates coated with MPC polymers with various compositions of MPC unit. The number of cells that were attached on the MPC polymer-coated plates at day 7 of the cell culture decreased according to the density of the MPC unit. All values are presented as mean plus standard deviation of five samples per group. Statistics were assessed using Dunnett's test (*$p < 0.01$ vs. 0% MPC unit composition).

MPC unit compositions. For 3 days, the number of adherent cells on the plate surface had plateaued on all plates. At 3 days, the medium was changed together with the floating cells and were replaced by another medium. The adherent cells continued to be cultured for four more days on the same MPC polymer-coated plates, and then were harvested for cell counting. The cells attached on the plate surface were observed to have a higher density on the dishes treated with a 0% MPC unit composition, compared with those of increasing the MPC unit composition, at 7 days (Fig. 2). The number of cells harvested from the plates had significantly decreased according to the increase in the density of the MPC polymer coating [Fig. 2 (graph)]. The cell numbers on the MPC polymer-coated dishes with 2% or 10% MPC units were approximately half or quarter of 0%, respectively.

To examine the proliferation ability of MPC polymer-selected MSCs, the cells harvested from each MPC polymer-coated plate were reseeded onto the conventional PS plates (φ 2.2 cm) with the same cell number of 1.9×10^4 in the second passage (passage 2), and then cultured for 7 days. The cells were equally proliferated during this period, while the total cell number after a 7 day-culture had not significantly changed among the cells derived from the different MPC polymer-coated plates [Fig. 3 (cell count)]. The result was represented by the experiment using the cell counting assay [Fig. 3 (cell counting assay)].

Surface epitopes of cells selected by MPC polymer-coated plates

We next examined the surface epitopes of the cells selected by the MPC polymer-coated plates (passage 1). It is known that CD29 (integrin β1), CD44 (hya-