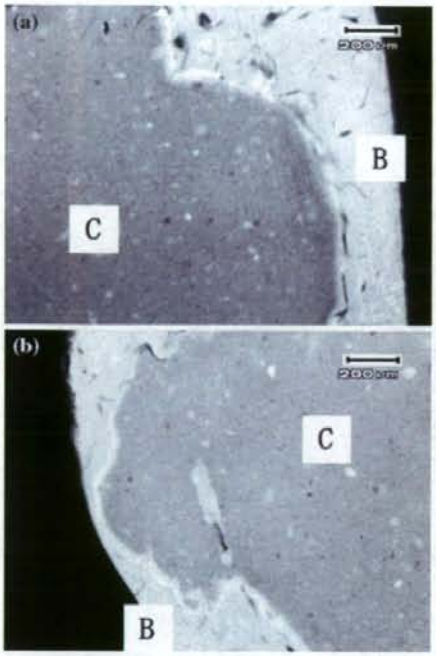

Fig. 7 Contact microradiography of (**a**) ST2-50c and (**b**) ST2-56c in rat tibiae 12 weeks after implantation. C, cement; B, bone. Bar = 200 μm

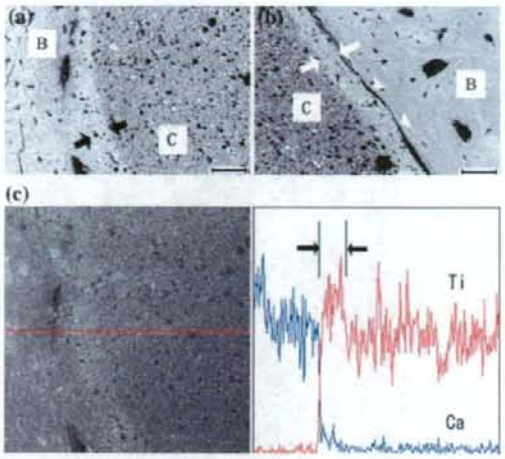

Fig. 8 Back-scattered scanning electron micrographs (SEM) of (**a**) ST2-56c and (**b**) ST2-50c in rat tibiae six weeks after implantation, and (**c**) SEM energy-dispersive X-ray (EDX) analysis of ST2-56c in the same site of (**a**). The white line is clearly visible in (**a**) and (**b**), and SEM–EDX analysis indicated that the white line is a Ti-rich layer. An increase of the intensity of the calcium peak was also detected along the outer margin of the white line. Arrowheads indicate thin intervening soft tissue. Between arrows = white line. C, cement; B, bone. Bar = 40 μm

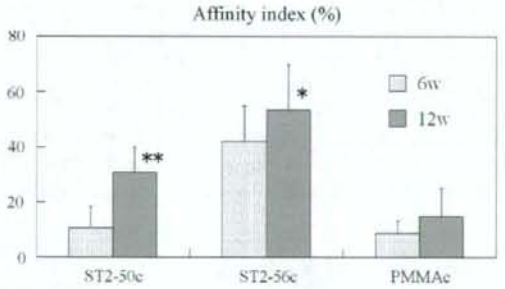

Fig. 9 Affinity indices (%) for ST2-50c, ST2-56c, and PMMAc in rat tibiae at six and 12 weeks after implantation (mean ± SD, $n = 12$). At six weeks, the value for ST2-50c was 10.6 ± 7.8 and 42.1 ± 12.9 for ST2-56c; and at 12 weeks, 30.8 ± 9.0 and 53.4 ± 16.6 respectively. The comparative affinity indices for PMMAc were 8.9 ± 4.4 and 14.9 ± 10.4 at six and 12 weeks, respectively [16]. *Significant compared with ST2-50c and PMMAc at six and 12 weeks. **Significant compared with PMMAc at 12 weeks

cement [10]. In contrast, micron-sized particles of mixed-phase anatase–rutile titania dispersed well in the cements, as was shown in this study. Indeed, it is difficult to simply compare the results of the experiments because there was little difference in the experimental conditions including the PMMA/MMA ratio and silanization of the powders. However, the difference in the particle size presumably influenced the degree of the particle dispersion.

The compressive and bending strengths of ST2-50c were much higher than those previously reported for PMMA-based composite cements containing nanosized spherical titania particles at 50 wt%, the compressive and bending strengths of which were 91.8 ± 7.7 and 25.5 ± 9.5 MPa, respectively [10]. Shinzato et al. developed PMMA-based composite cements containing glass beads and reported that a decreasing trend in the bending strength was observed as the glass bead size increased [16]. Their results suggest that cements containing smaller-sized titania could have higher strength. However, our findings were not consistent with theirs, and were presumably influenced mainly by the difference in filler dispersion in our studies, where the micron-sized titania dispersed well, whereas the nanosized titania used in the previous study formed some aggregates in the cement [10]. In this study, there was an increasing trend in compressive strength, bending strength, and bending modulus with increasing titania filler content. Juhasz et al. investigated the effect of filler content on the mechanical properties of AW-glass ceramic–polyethylene composites, and noted that a stiffening effect was observed as the AW-glass ceramic filler content increased from 10 to 50 vol% [17]. Their results were consistent with ours. In this study,

the mechanical strengths of the cements containing micron-sized titania particles met the criteria required by the ISO 5833 standard.

The setting times of the cements containing titania particles in this study were reduced, and the peak temperature decreased, as titania filler content increased. Only ST2-56c exhibited a lower peak temperature than PMMAc, and that of ST2-50c was almost the same as that of PMMAc. This was probably because the weight ratio of PMMA/MMA was 1/2 in ST2-50c, whereas the weight ratio of powder/liquid monomer was about 2/1 in PMMAc, and the content of MMA that polymerizes with an exothermic reaction was almost the same in ST2-50c and PMMAc. As less exothermic setting reactions for bone cement are desirable, both ST2-50c and ST2-56c would be acceptable, but ST2-56c appeared to have lower peak-setting temperature properties and is therefore recommended.

Animal experiments revealed that both ST2-50c and ST2-56c were more osteoconductive than cements containing nanosized titania particles. According to a previous study, the affinity indices of cements containing nanosized titania particles were 20.9 ± 7.3% for cement containing 50 wt% titania particles and 31.3 ± 8.7% for cement containing 60 wt% titania particles at 12 weeks [10]. In the previous study [10] and in this study, high molecular weight PMMA powder was used because it showed low solubility in the MMA monomer during the polymerizing reaction [14]. As a result, bioactive fillers could be exposed at the cement surface without being covered by a layer of polymerized MMA [14]. Therefore, the amount of bioactive particles exposed on the cement surface, which must be proportional to cement bioactivity, was greatly influenced by the particle size of bioactive fillers. In this study, the micron size of the titania particles presumably had a beneficial effect on the osteoconductivity of ST2-50c and ST2-56c. However, ST2-56c was in direct contact with bone over larger areas than ST2-50c, at both six and 12 weeks after implantation into rat tibiae. As well as the larger amount of titania filler, presumably the decrease in toxic monomer content contributed to the higher affinity indices of ST2-56c compared with those of ST2-50c. Further research on bone-bonding strength is necessary to demonstrate the bioactivity of ST2-50c and ST2-56c.

The marginal white line seen on SEM was a Ti-rich layer, as revealed by SEM–EDX analyses (Fig. 8c) and has been similarly observed in cements containing nanosized titania particles [10]. In contrast, it has been reported that PMMA-based composite cement containing glass beads, AW-GC, or hydroxyapatite fillers developed by Shinzato et al. exhibited no such marginal line, although they used a similar PMMA/MMA system and bioactive fillers silanized with γ-methacryloxypropyltrimethoxy silane [14]. Therefore, the properties of the titania filler itself are suggested to contribute to the formation of the white line. Because a Ti-rich layer indicated bioactive titania gathered at the cement surface, it presumably contributes to the osteoconductivity of cements containing titania particles. SEM–EDX analyses also revealed an increase in the concentration of calcium along the outer margin of the white line, which suggests calcium ion transfer or bony tissue invasion into the cement margin. Although a small amount of unpolymerized MMA might leak out of the cement surface, SEM observations revealed no such cement degradation and that leakage was minimal. No toxic effects of the monomer were detected by Giemsa surface staining, and excellent osteointegration of ST2-56c and ST2-50c was seen.

The overall results of this study indicate that PMMA bone cement containing micron-sized titania particles is a promising bone cement for prosthesis fixation as well as for vertebroplasty, but further research on long-term osteointegration and bone-bonding strength should be performed before clinical application.

Conclusions

Three types of PMMA-based composite cements containing 40–56 wt% micron-sized bioactive titania powder were prepared, and their mechanical, setting, and biological properties were evaluated. Compressive strength, bending strength, and bending modulus increased with increasing content of titania filler. The mechanical strengths met the criteria required in the ISO 5833 standard. The peak temperature during the setting reaction decreased as the amount of filler increased, and ST2-56c exhibited a lower peak temperature than commercial PMMA cement. Cements ST2-50c and ST2-56c were revealed to be biocompatible and osteoconductive. This was especially the case with ST2-56c, as it was in direct contact with bone over large areas within six weeks after implantation into rat tibiae, and showed significantly higher affinity indices than those of ST2-50c within 12 weeks. Overall, the data indicate that bone cement containing micron-sized titania particles can be applied to prosthesis fixation as well as vertebroplasty, and ST2-56c is a good candidate cement.

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Effects of mobility/immobility of surface modification by 2-methacryloyloxyethyl phosphorylcholine polymer on the durability of polyethylene for artificial joints

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Abstract: Surface modification is important for the improvement in medical device materials. 2-Methacryloyloxyethyl phosphorylcholine (MPC) polymers have attracted considerable attention as surface modifiable polymers for several medical devices. In this study, we hypothesize that the structure of the surface modification layers might affect the long-term stability, hydration kinetics, wear resistance, and so forth, of medical devices such as artificial joints, and the poly(MPC) (PMPC) grafted surface might assure the long-term performance of such devices. Therefore, we investigate the surface properties of various surface modifications by using dip coatings of MPC-*co*-*n*-butyl methacrylate (PMB30) and MPC-*co*-3-methacryloxypropyl trimethoxysilane (PMSi90) polymers, or photoinduced radical grafting of PMPC and also the effects of the surface properties on the durability of cross-linked polyethylene (CLPE) for artificial joints. The PMPC-grafted CLPE has an extremely low and stable coefficient of dynamic friction and volumetric wear as compared to the untreated CLPE, PMB30-coated CLPE, and PMSi90-coated CLPE. It is concluded that the photoinduced radical graft polymerization of MPC is the best method to retain the benefits of the MPC polymer used in artificial joints under variable and multidirectional loads for long periods with strong bonding between the MPC polymer and the CLPE surface, and also to retain the high mobility of the MPC polymer. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 00A: 000–000, 2008

Key words: joint replacement; polyethylene; phosphorylcholine; surface modification; wear mechanism

INTRODUCTION

Polymeric biomaterials are widely used in the biomedical field for manufacturing artificial organs, medical devices, and disposable clinical apparatus.[1,2] Advancements in the biomedical field also demand substantial improvements in polymeric biomaterials. Conventional single-component polymer biomaterials cannot satisfy these requirements. Multicomponent polymer systems have therefore been designed and prepared for new multifunctional biomaterials.

Surface modification is one of the important means of preparing new multifunctional biomaterials. 2-Methacryloyloxyethyl phosphorylcholine (MPC) polymers have attracted considerable attention as surface-modifiable polymers for several medical devices.[3–11] MPC, a methacrylate with a phospholipid polar group in the side chain, is a monomer for preparing novel polymer biomaterials. An excellent synthetic route for MPC has been developed by Ishihara et al.[12] MPC can undergo conventional radical copolymerization with other methacrylate and styrene derivatives such as *n*-butyl methacrylate (BMA), *n*-dodecyl methacrylate (DMA), and 3-methacryloxypropyl trimethoxysilane (MPSi) to form poly(MPC-*co*-BMA), poly(MPC-*co*-DMA), and poly(MPC-*co*-MPSi), respectively.[5–11] These MPC polymers are some of the most common biocompatible

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and hydrophilic polymers studied thus far. They have potential applications in a variety of fields such as biology, biomedical science, and surface chemistry, because they possess unique properties such as good biocompatibility, high lubricity and low friction, antiprotein adsorption, and cell membrane-like surfaces. Several medical devices have already been developed by utilizing the MPC polymers and used clinically; therefore, the efficacy and safety of the MPC polymers as biomaterials are well established.[9–11]

When a natural joint in the human body ceases to function, for example, due to disease, trauma, or overuse, an artificial joint replacement often becomes necessary. There is a substantial increase in the number of artificial hip and knee joints used worldwide each year for primary and revised hip and knee joint replacements.[13] This indicates that a higher quality and longer lifetime have been increasingly desired for artificial joint replacements. Normally, artificial joints allow the body to regain mechanical and biological functions. Medical implants must be adapted to the dynamic loads experienced during use, and they must have the desired long-term biological interaction with the surrounding tissue. A typical artificial joint replacement system used as a medical device comprises a metallic surface made of a cobalt-chromium-molybdenum (Co-Cr-Mo) alloy that articulates against an ultra-high molecular weight polyethylene (UHMWPE) polymeric component. However, the artificial joint replacements are subjected to adhesive and abrasive wear, and both metallic and polymeric debris. These are known to produce a variety of cytokines and tumor necrosis factors that progressively resorb the bone by osteolysis, leading to aseptic loosening of the artificial joint after a number of years, which is recognized as a serious problem.[14,15] Different combinations of bearing surfaces and improvements in bearing materials have been studied with the aim of reducing the number of UHMWPE wear debris that induce osteolysis.[16–18]

Recently, we have developed an artificial hip joint by using poly(MPC) (PMPC) grafted onto the surface of cross-linked polyethylene (CLPE; PMPC-grafted CLPE); this device is designed to reduce wear and suppress bone resorption.[19–24] MPC has also been directly grafted from biomaterial surfaces through photoinduced radical polymerization.[25,26] This photoinduced radical polymerization facilitates the direct grafting of MPC onto biomaterial surfaces. The following are the expected advantages of this technique: (1) controllable graft polymer density and length and grafting site,[21,24] (2) covalent bonding between the graft polymer and biomaterial surfaces (as high immobility), which assures the long-term stability of graft chains, (3) high mobility of the graft polymer chain and/or free end groups of the polymer, and (4) occurrence of grafting only on the surface, and no effect of grafting on the bulk properties.[22] In particular, strong bonding between the surface modification and the surface is an important issue, which is associated with the long-term retention of the benefits of the surface modification used in artificial joints under variable and multidirectional loads, for a promising long-term performance of artificial joints.

In this study, we hypothesize that the structure of surface modification layers might affect the surface density of the phosphorylcholine group, long-term stability and mobility of the polymer chain, hydration kinetics, and so forth, and the PMPC-grafted surface might assure the long-term performance of artificial joints. Therefore, we investigate the surface properties of various surface modification layers with the MPC polymer and the effects of the surface properties on the durability of the CLPE for artificial joints. The results reveal that the structure of the PMPC-grafted layer on the CLPE surface plays an important role in reducing the wear of the orthopedic bearing surface in the long term.

MATERIALS AND METHODS

Materials

MPC was industrially synthesized using a previously reported method.[12] Poly(MPC-*co*-BMA) (PMB30; MPC:BMA unit mole fraction = 0.3:0.7)[12] and poly(MPC-*co*-MPSi) (PMSi90; MPC:MPSi unit mole fraction = 0.9:0.1)[8] were synthesized in ethanol using 2,2′-azobisisobutyronitrile as initiator by a conventional radical copolymerization method. A compression-molded UHMWPE (GUR1020 resin; Poly Hi Solidur, IN) sheet stock was irradiated with 50 kGy γ-rays in N_2 gas and annealed at 120°C for 7.5 h in N_2 gas in order to achieve cross-linking. The CLPE specimens were machined from this sheet stock after cooling.

MPC polymer coating

The preparation of the MPC polymer coated CLPE is schematically illustrated in Figure 1. The physical coating of PMB30 was carried out by the solvent evaporation method, where the CLPE specimens were dipped into ethanol solution containing 0.2 mass % PMB30 for 10 s for coating, and then placed in an ethanol vapor atmosphere at room temperature for 1 h. The coated CLPE specimens were again dipped for 10 s and placed in the ethanol vapor atmosphere at room temperature for 1 h (PMB30-coated CLPE). F1

The chemical coating of PMSi90 was also carried out by the solvent evaporation method. Before the PMSi90 coating, the CLPE specimens were irradiated with O_2 plasma at a 200 W high-frequency output and 150 mL/min O_2 gas flow for 2 min by using an O_2 plasma etcher (PR500,

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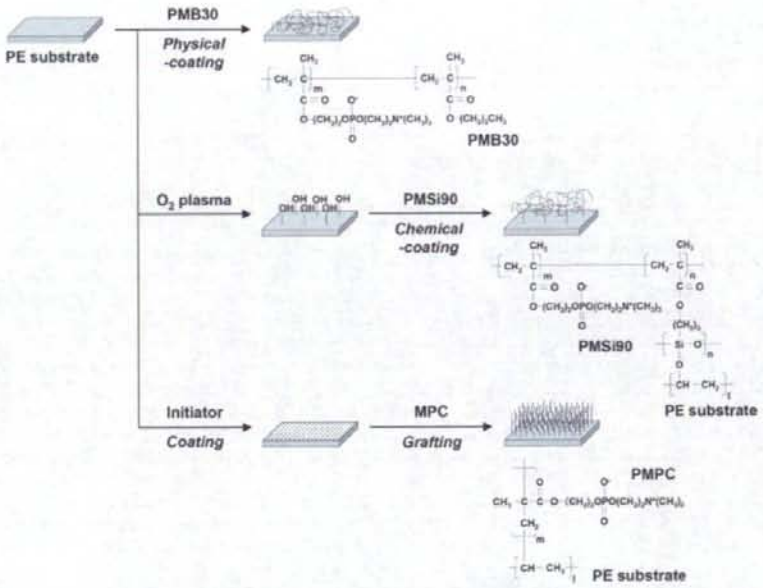

Figure 1. Scheme for the preparation of MPC polymer-coated CLPE and PMPC-grafted CLPE.

Yamato Scientific Co., Tokyo, Japan). The O_2 plasma irradiation formed the surface hydroxide layer. The CLPE specimens were dipped into ethanol solution containing 0.5 mass % PMSi90 and 0.063 mg/mL succinic acid (Kanto Chemical Co., Tokyo, Japan) for 10 s for the silanization of trimethoxysilane group of PMSi90 and placed in the ethanol vapor atmosphere at room temperature for 1 h. The coated CLPE specimens were annealed in air at 70°C for 3 h for dehydration (PMSi90-coated CLPE). These PMB30- and PMSi90-coated CLPE specimens were then sterilized by 25 kGy γ-rays in N_2 gas.

MPC graft polymerization

The preparation of the PMPC-grafted CLPE is schematically illustrated in Figure 1. The CLPE specimens were immersed in acetone (Wako Pure Chemical Industries, Osaka, Japan) solution containing 10 mg/mL benzophenone (Wako Pure Chemical Industries) for 30 s, and then dried in the dark at room temperature in order to remove the acetone. In previous studies, using ultraviolet spectroscopy, the amount of benzophenone adsorbed on the surface was reported to be 3.5×10^{-11} mol/cm^2.[3] MPC was dissolved in degassed pure water to obtain a concentration of 0.5 mol/L. Subsequently, the benzophenone-coated CLPE specimens were immersed in the aqueous MPC solutions. Photoinduced graft polymerization was carried out on the CLPE surface using ultraviolet irradiation (UVL-400HA ultra-high pressure mercury lamp; Riko-Kagaku Sangyo Co., Funabashi, Japan) with an intensity of 5 mW/cm^2 at 60°C for 90 min; a filter (model D-35; Toshiba Corp., Tokyo, Japan) was used to restrict the passage of ultraviolet light to wavelengths of 350 ± 50 nm. After the polymerization, the PMPC-grafted CLPE specimens were removed, washed with pure water and ethanol, and dried at room temperature. These specimens were then sterilized by 25 kGy γ-rays in N_2 gas.

Surface analysis

The functional group vibrations of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE surfaces were examined using attenuated total reflection (ATR) by Fourier transform infrared (FTIR) spectroscopy. The FTIR/ATR spectra were obtained in 32 scans over a range of 800–2000 cm^{-1} by using an FTIR analyzer (FT/IR615; Jasco International Co., Tokyo, Japan) at a resolution of 4.0 cm^{-1}.

The surface elemental contents of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE were analyzed using X-ray photoelectron spectroscopy (XPS). The XPS spectra were obtained using an XPS spectrophotometer (AXIS Hsi 165; Kratos Analytical Ltd., Manchester, UK) equipped with an Mg-Kα radiation source by applying a voltage of 15 kV at the anode. The take-off angle of the photoelectrons was maintained at 90°. Each measurement was scanned five times; five replicate measurements were performed for each sample, and the average values were considered for the surface elemental contents.

The static water-contact angles of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE were measured using an optical bench-type contact angle goniometer (model DM300; Kyowa Interface

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Science Co., Saitama, Japan) by the sessile drop method. Drops of purified water (1 μL) were deposited onto the surface modified CLPE with MPC polymer, and the contact angles were directly measured after 60 s by using a microscope according to the ISO 15989 standard.[27] Subsequently, 15 replicate measurements were performed for each sample, and the average values were considered as the contact angles.

Cross-sectional observation by transmission electron microscopy

A cross-section of the PMB30, PMSi90, and PMPC layers on the CLPE 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 (~100-nm thick) by using a Leica Ultra Cut UC microtome (Leica Microsystems, Wetzlar, Germany). A JEM-1010 electron microscope (JEOL, Tokyo, Japan) was used for the TEM observation at an acceleration voltage of 100 kV.

Characterization of protein adsorption by micro bicinchoninic acid method

The amount of protein adsorbed on the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE 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., Takasaki, Japan) for 1 h to equilibrate the surface modified by the MPC polymer. The specimens were immersed in bovine serum albumin (BSA, $M_w = 6.7 \times 10^4$; Sigma-Aldrich Corp., MO) solution at 37°C for 1 h. The protein solution was prepared in a BSA concentration of 4.5 g/L, that is, 10% of the concentration of the human plasma levels. Then, the specimens were rinsed five times with fresh PBS and immersed in 1 mass % sodium dodecyl sulfate (SDS) aqueous solution, and shaken at room temperature for 1 h to completely detach the adsorbed BSA on the surface modified by the MPC polymer. A protein analysis kit (micro BCA protein assay kit, No. 23235; Thermo Fisher Scientific, IL) based on the BCA method was used to determine the BSA concentration in the SDS solution, and the amount of BSA adsorbed on the surface modified by the MPC polymer was calculated.

Friction test

A friction test was performed using a ball-on-plate machine (Tribostation 32; Shinto Scientific Co., Tokyo, Japan). Each of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE surfaces was used to prepare six sample pieces. A Co-Cr-Mo alloy ball with a diameter of 9 mm was prepared. The surface roughness of the ball was Ra $\geq$ 0.01, which was comparable to that of femoral ball products. The friction tests were performed at room temperature with various loads in the range of 0.49–9.80 N, sliding distance of 25 mm, and frequency of 1 Hz for a maximum of 100 cycles.[28] Pure water was used as a lubricant medium. The mean coefficients of dynamic friction were determined by averaging five data points from the 100 (96–100) cycle measurements.

Hip joint simulator wear test

A 12-station hip joint simulator (MTS Systems Corp., MN) with the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE cups (n = 2), both having inner and outer diameters of 26 and 52 mm, respectively, was used for the hip joint simulator wear test. A Co-Cr-Mo alloy femoral ball component with a size of 26 mm (Japan Medical Materials Corp., Osaka, Japan) was used as the femoral component. A mixture of 25 vol % bovine serum, 20 mM/L of ethylene diamine tetraacetic acid, and 0.1 mass % sodium azide was used as a lubricant, according to the ISO 14242-1 standard.[29] The lubricant was replaced every 0.5×10^6 cycles. Walks that simulated a physiologic loading curve (Paul-type), with double peaks at 1793 and 2744 N loads with a multidirectional (biaxial and orbital) motion of 1 Hz frequency, were applied. The wear was determined by weighing the cups at intervals of 0.5×10^6 cycles. Load-soak controls (n = 2) were used to compensate the fluid absorption by the specimens.[30] The testing was continued until a total of 3.0×10^6 cycles were completed.

Statistical analysis

The results derived from each measurement in the water-contact angle estimation, friction test, and protein adsorption test were expressed as mean values and standard deviation. The statistical significance ($p < 0.05$) was estimated by Student's t-test.

RESULTS

Figure 2 shows the FTIR/ATR spectra of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE. An absorption peak was observed at 1460 cm^{-1} for all test specimens. This peak is mainly attributed to the methylene (CH_2) chain in the CLPE substrate and the MPC polymer chain. However, absorption peaks at 1240, 1080, and 970 cm^{-1} were observed only for the CLPE, whose surface was modified by the MPC polymer. These peaks corresponded to the phosphate group (P—O) in the MPC unit. Similarly, an absorption peak at 1720 cm^{-1} observed in the surface modified CLPE corresponded only to the carbonyl group (C=O) in the MPC unit. The absorption peak intensity of the P—O group of the PMPC-grafted CLPE was the highest in the CLPE, whose surface was modified by the MPC polymer. F2

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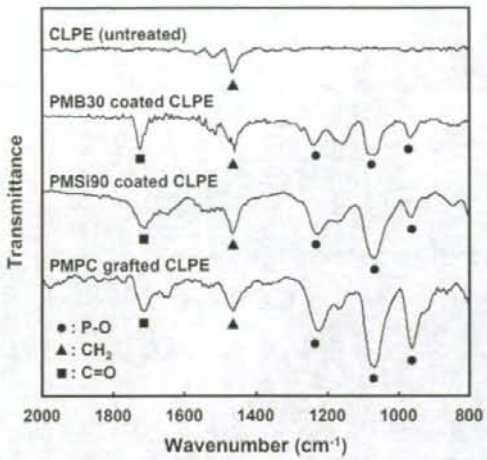

Figure 2. FTIR/ATR spectra of untreated CLPE, MPC polymer-coated CLPE, and PMPC-grafted CLPE.

T1 Table I summarizes the surface elemental compositions of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE. The nitrogen (N) and phosphorous (P) contents in all the CLPE specimens, whose surface were modified by the MPC polymer, were observed. The surface elemental compositions of both N and P in the surface modified CLPE increased with an increase in the MPC composition in the polymer for surface modification. In particular, the elemental compositions of N and P in the PMPC-grafted CLPE surface were 5.2 and 5.3 atom %, respectively. The elemental composition of the PMPC-grafted CLPE surface was almost equivalent to the theoretical elemental composition ($N = 5.3$, $P = 5.3$ atom %) of PMPC.

F3 Figure 3 shows the cross-sectional TEM images of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE. For the PMB30 and PMSi90 coatings, and PMPC grafting, a 100-nm thick MPC polymer layer was clearly observed on the surface of the CLPE substrate. No crack for poor adhesion and/or delamination was observed at the interface between MPC polymer layer and CLPE substrate. These results indicate that each surface modification layer on the CLPE substrate is uniform and cover closely, regardless of the binding conditions: the surface modification layers by the PMB30 and PMSi90 coatings, and PMPC grafting are combined with the substrate by physical adsorption and covalent bonds of Si—O—C and C—C, respectively. In the PMB30-coated CLPE, a bilayer structure attributed to dipping twice was clearly observed on the surface modification layer.

F4 Figure 4 shows the static water-contact angles of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE. The static water-contact angles of the untreated CLPE and PMB30-coated CLPE were 90° and 100°, respectively, and they decreased markedly to ~10° (i.e., 8°–13°, $p < 0.001$) by the PMSi90 coating and PMPC grafting.

F5 Figure 5 shows the amount of BSA adsorbed on the surfaces of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE. The amount of BSA adsorbed on the CLPE surface modified by the MPC polymer was considerably lesser ($p < 0.001$) than that of the untreated CLPE, that is, 0.05–0.10 μg/cm^2. These results imply that the surface modification by the MPC polymer results in good biocompatibility.

F6 Figure 6 shows the coefficients of dynamic friction of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE. As compared to the untreated specimens, the PMB30-coated and PMSi90-coated CLPE specimens showed a reduction of ~30% (i.e., 25–30%, not significant) in their coefficients of dynamic friction. Further, as compared to the untreated CLPE specimens, the PMPC-grafted CLPE specimens showed a reduction of ~84% ($p < 0.005$) in their coefficients of dynamic friction.

F7 Figure 7 shows the coefficients of dynamic friction of the untreated CLPE, PMB30-coated CLPE, PMSi90-coated CLPE, and PMPC-grafted CLPE as a function of the loads in the ball-on-plate friction test. The untreated CLPE showed the highest coefficient of dynamic friction, that is, ~0.075. This value was almost constant throughout the experiment. The coefficients of dynamic friction of the PMB30-coated

TABLE I
Surface Elemental Composition (atom %) of CLPE, MPC Polymer Coated CLPE, and PMPC Grafted CLPE ($n = 5$) AQ4

Sample	C	O	N	P	Si
CLPE (untreated)	99.8 (0.3)[a]	0.2 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PMB30 coated CLPE	69.9 (1.0)	25.5 (0.6)	2.1 (0.2)	2.5 (0.3)	0.0 (0.0)
PMSi90 coated CLPE	60.5 (0.7)	30.4 (0.4)	4.1 (0.2)	4.0 (0.2)	1.0 (0.0)
PMPC grafted CLPE	58.0 (0.2)	31.5 (0.2)	5.2 (0.1)	5.3 (0.1)	0.0 (0.0)
PMPC[b]	57.9	31.6	5.3	5.3	0.0

[a] The standard deviation is in parentheses.
[b] Theoretical elemental composition of PMPC.

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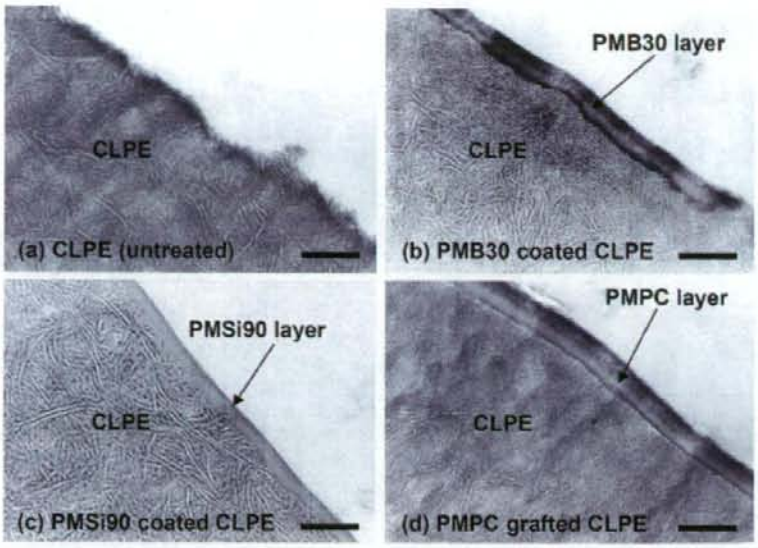

Figure 3. Cross-sectional TEM images of untreated CLPE, MPC polymer-coated CLPE, and PMPC-grafted CLPE. Bar: 200 nm.

CLPE and PMSi90-coated CLPE were smaller (~0.055) than those of the untreated CLPE at loads of up to 0.98 N; then, these coefficients increased to the level of the coefficients of the untreated CLPE at loads above 1.96 N. For both PMB30-coated CLPE and PMSi90-coated CLPE, the MPC polymer layer coating showed almost the same coefficients of dynamic friction. The PMPC-grafted CLPE showed a remarkably low friction coefficient of ~0.026 at a load of 0.49 N; this value decreased gradually and reached ~0.005 at a load of 9.80 N.

Figure 8 shows the gravimetric wear of the untreated CLPE, PMB30-coated CLPE and PMSi90-coated CLPE, and PMPC-grafted CLPE cups in the hip simulator wear test. It was observed that the wear in the PMPC-grafted CLPE cups was significantly lower than that in the untreated CLPE cups. There was no significant difference in the wear of the untreated CLPE and PMB30-coated CLPE cups. The PMSi90-coated CLPE cups showed slightly lower wear than the untreated CLPE cups; however, the weight change varied for each cup (standard F8

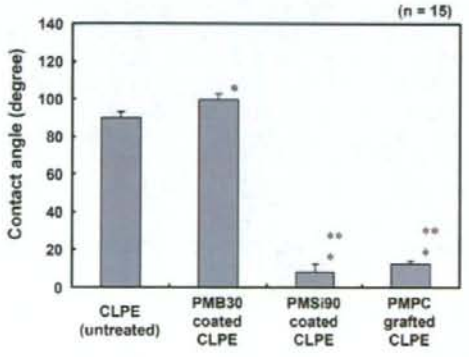

Figure 4. Static-water contact angle of untreated CLPE, MPC polymer-coated CLPE, and PMPC-grafted CLPE. Bar: Standard deviations. *: Significant difference ($p < 0.001$) as compared to the untreated CLPE, **: significant difference ($p < 0.001$) as compared to the PMB30-coated CLPE.

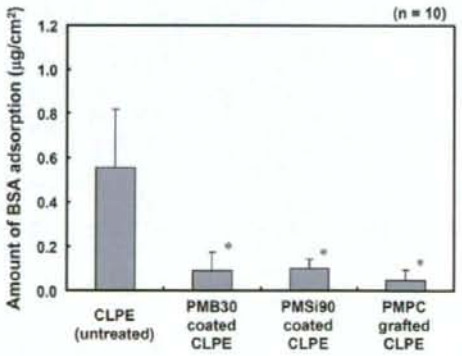

Figure 5. Amount of BSA adsorbed on the surfaces of the untreated CLPE, MPC polymer-coated CLPE, and PMPC-grafted CLPE. Bar: Standard deviations. *: Significant difference ($p < 0.001$) as compared to the untreated CLPE.

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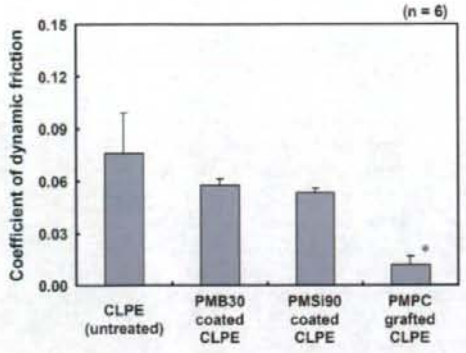

Figure 6. Coefficients of dynamic friction of untreated CLPE, MPC polymer-coated CLPE, and PMPC-grafted CLPE. Bar: Standard deviations. *: Significant difference ($p < 0.005$) as compared to the untreated CLPE.

deviation = ±9.0 mg). The PMPC-grafted CLPE cups showed a slight increase in weight. This was partially attributable to the enhanced fluid absorption in the tested cups as compared to that in the load-soak controls. While applying the gravimetric method, the weight loss in the tested cups is corrected by subtracting the weight gain in the load-soak controls; however, this correction cannot be achieved perfectly because only the tested cups are continuously subjected to motion and load. Usually, the fluid absorption in the tested cups is generally slightly higher than that in the load-soak controls. Consequently, the correction of the fluid absorption by using the load-soak data as the correction factor leads to a slight underestimation of the actual weight loss.[22,29] In this study, a steady wear rate was calculated using data from 2.0×10^6 to 3.0×10^6 cycles; in the untreated CLPE, PMB30-coated CLPE, and PMSi90-coated CLPE cups, these rates were 6.1, 5.9, and 4.5 mg/10^6 cycles, respectively. In contrast, the wear rate of the PMPC-grafted CLPE cups was markedly lower, that is, −1.5 mg/10^6 cycles.

DISCUSSION

In this study, we have investigated the surface properties of various surface modification layers formed on the CLPE surface by the physical and chemical coating of the MPC polymers or photoinduced radical grafting of MPC. Here, we discuss the durability of the CLPE whose surface is modified by the MPC polymer in terms of the characteristics of the nanometer-scale layer of the MPC polymer.

The layer, whose surface contains the physically coated PMB30, is combined with the substrate by physical adsorption.[11,12] The layer, whose surface contains the chemically coated PMSi90, is combined with the substrate by physical adsorption and/or slight Si—O—C covalent bonding ascribed to the 10% MPSi in the PMSi90 composition; a hydrolyzed silane molecule of PMSi90 has three —OH groups that react with the —OH groups of the surface oxide layer of the CLPE substrate induced by the O_2 plasma irradiation, and they form covalently siloxane bonds.[27] On the other hand, the surface modification layer obtained by PMPC grafting is combined with the substrate by strong C—C covalent bonding.[19–24]

In Figure 7, the coefficients of dynamic friction of the PMB30-coated CLPE and PMSi90-coated CLPE increased to the level of the coefficient of dynamic

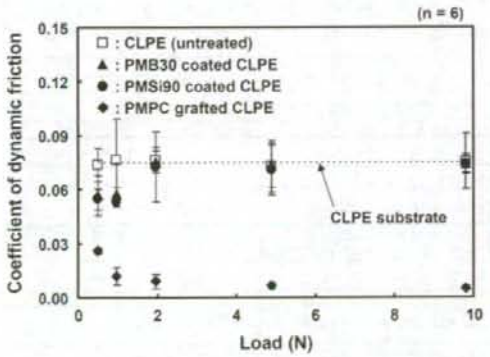

Figure 7. Coefficients of dynamic friction of the untreated CLPE, MPC polymer-coated CLPE, and PMPC-grafted CLPE as a function of loads in the ball-on-plate friction test. Bar: Standard deviations.

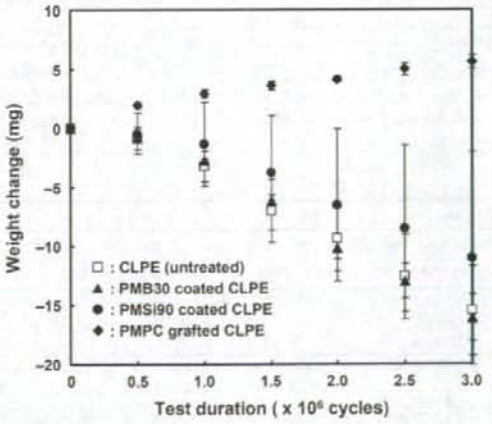

Figure 8. Weight change (volumetric wear) of the untreated CLPE, MPC polymer-coated CLPE, and PMPC-grafted CLPE in the hip joint simulator wear test. Bar: Standard deviations.

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friction of the untreated CLPE at loads above 1.96 N. In addition, as shown in Figure 8, there was no significant difference in the wear of the untreated CLPE and the MPC polymer coated CLPE cups in the hip joint simulator tests. In contrast, the PMPC-grafted CLPE showed an extremely low and stable coefficient of dynamic friction and volumetric wear as compared to the untreated CLPE and MPC polymer coated CLPE, as shown in Figures 7 and 8. These results indicate that the PMB30 and PMSi90 surface modification layers are removed from the CLPE surface. The surface modification layers by the PMB30 and PMSi90 coatings are combined with the substrate by physical adsorption and chemical bonds of Si—O—C, respectively. Therefore, the physical adsorption and chemical bonds of Si—O—C are ineffective in the case of large and multidirectional loads. The chemical bonds of Si—O—C are probably insufficient, because the MPSi composition in the PMSi90 polymer is 10%. Therefore, it is thought that a sufficient number of strong bonds between the surface modification layer and the CLPE surface are essential for the long-term retention of the benefits of the MPC polymer used in artificial joints under variable and multidirectional loads.

Since MPC is a highly hydrophilic compound, the MPC polymers PMSi90 and PMPC are water-soluble. The water-wettability of the PMSi90-coated and PMPC-grafted CLPE surfaces with a high MPC unit mole fraction (90 and 100%) was considerably greater than that of the untreated CLPE surface, as shown in Figure 4. Kobayashi et al. reported that the water molecules adsorbed on the surface of the highly hydrophilic PMPC act as lubricants and reduce the interaction between the PMPC and the counter-bearing face.[31] Therefore, it is thought that the artificial hip joint bearing with the PMSi90-coated and PMPC-grafted surfaces exhibited considerably higher lubricity than that with the untreated CLPE. The amount of water molecules absorbed on the surface of the CLPE (water thin-film) is expected to play an important role with regard to the property of low friction.

However, in reality fact as shown in Figure 6, the PMSi90-coated CLPE specimens exhibited a maximum reduction of ~30% in their coefficients of dynamic friction as compared to the untreated CLPE specimens. In contrast, the PMPC-grafted CLPE specimens exhibited a reduction of ~84%. It was previously reported that the polymer concentration (i.e., viscosity) increased with the friction coefficient in the mixed lubrication regime.[32] Therefore, It is assumed that an ultra-low friction of PMPC-grafted CLPE that occurs during sliding is related to the effective viscosity of the PMPC in the mixed lubrication of the intermediate hydrated layer. The high-density PMPC graft chains in the PMPC-grafted CLPE are assumed to exhibit a brush-like structure.[25,33] The viscosity of the PMPC layer reflects the mobility of the free end groups of the MPC polymer or MPC polymer chains themselves.[34,35] In contrast, the mobility of PMSi 90 is limited by the cross-linking and network entanglement of the gel structure of PMSi90. Hence, we think that the polymer brush-like structure of the PMPC-grafted CLPE with high mobility of polymer chains can function as a considerably better surface hydration lubrication system of artificial joints than the gel structure of the PMSi90-coated CLPE. These considerations are based on the previous studies on charged polymers (polyelectrolytes) by Klein and coworkers.[34,35]

The significant reduction in the coefficient of friction of the grafted PMPC resulted in a substantial improvement in wear resistance. A previous study reported that the hydrogel cartilage surface was assumed to have a brush-like structure: a part of the proteoglycan aggregate brush bonds with the collagen network in the cartilage surface.[36] It is thought that the bearing surface with high-density PMPC in artificial hip joints has a brush-like structure similar to an articular cartilage. We assume that the bearing surface of the artificial hip joint combined with a 100-nm thick MPC polymer layer results in a fluid film lubrication (or mixed lubrication) of the intermediate hydrated layer; which suggests that this novel artificial hip joint mimics the natural joint cartilage.

As shown in Figure 7, the coefficients of dynamic friction of the untreated CLPE surface were constant for all load values. In contrast, those of the PMPC-grafted CLPE surface decreased with increasing load. These results show that the PMPC-grafted layer does not follow Amonton's law of $F = \mu N$. The maximum contact stress of ~62 MPa at a load of 9.8 N is higher than the yield tensile strength of the CLPE (~23 MPa). The maximum contact stress is roughly calculated by the Hertzian theory. The elastic CLPE substrate is deformed slightly by the loads, the low friction coefficient may have been shown in order to get much more amount of water thin-film with the larger contact area of concave surface. We also think that these results imply that the lubrication of the PMPC-grafted CLPE is dominated by the hydrodynamic lubrication mechanism.

As shown in Figure 4, the static water-contact angle of the PMB30-coated CLPE was ~100°. Sibarani et al. reported that the PMB30-coated polymer surfaces showed high advancing (~100°) and low receding (~20°) contact angles.[5] Moreover, Yamasaki et al. reported that the PMB30-coated polymer surfaces required more than 30–300 min to achieve complete equilibrium.[37] This indicates that the PMB30 cannot be hydrated easily, due to the low MPC unit composition of the copolymer and low mobility of

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the polymer chain. However, as shown in Figure 5, the PMB30-coated CLPE surface, which could form a phosphorylcholine-enriched surface after equilibrating for 1 h, showed excellent biocompatibility as an antiadsorption surface for BSA.

The adsorption of the representative plasma protein, BSA, on the CLPE surface decreased to 9–18% due to the surface modification by the MPC polymer, as shown in Figure 5. It is hypothesized that the mechanism of protein adsorption resistivity on the surface modified by the MPC polymer is based on the water structure resulting from the interactions between water molecules and phosphorylcholine groups.[26,37,38] The large amount of free water around the phosphorylcholine group is considered to detach proteins easily and even prevent conformational changes in the adsorbed proteins.[26,38] Hence, we expect that the protein adsorption will decrease with increasing MPC unit composition in the MPC copolymer. However, in this study, there was no significant difference in the protein adsorption of the CLPE, whose surfaces was modified by the MPC polymer (the MPC unit compositions were 30, 90, and 100%). The reduction in protein adsorption is also considered to be caused by the presence of a hydrated layer around the phosphorylcholine group.[26,39] The latter consideration is consistent with the results of the water contact angle measurement, friction test, and cross-sectional TEM observations of the CLPE, whose surface is modified CLPE by the MPC polymer (Figs. 3, 4, and 6). The previous studies reported that the protein concentration of lubricants such as bovine serum considerably affected the wear rate of the UHMWPE cups in the joint simulator test: the protein concentrations in the synovial fluids of both normal and diseased joints (~20–40 mg/mL), including joints after total arthroplasty, were associated with the highest wear rates.[40,41] We think that the antiprotein adsorption surface on the CLPE prepared by the MPC polymer will prevent the highest adhesive wear rates *in vivo* caused by the protein adsorption. Moreover, the CLPE whose surface is modified by the MPC polymer is expected to exhibit tissue and blood compatibility as biocompatibility, because previous studies have reported that the MPC polymer modified surfaces exhibit *in vivo* biocompatibility.[3–11]

CONCLUSION

In this study, we systematically investigated the surface properties of the various surface modification layers formed on the CLPE surface by the MPC polymer by dip coating or photoinduced radical grafting. It is concluded that several important issues are involved in the long-term retention of the benefits of the MPC polymer used in artificial joints under variable and multidirectional loads, for example, strong bonding between the MPC polymer and the CLPE surface and high mobility of the free end groups of the MPC polymer. We should employ the photoinduced radical graft polymerization to create strong covalent bonding between the CLPE substrate and the surface modification layer, and also to retain the high mobility of polymer chains of that layer.

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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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Keywords:	bone marrow stromal cell (MSC), 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer , osteogenesis, chondrogenesis, cell adhesion

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%), while 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.

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]

In order to isolate or enrich the subpopulation that preserves the self-renewal and the multipotentiality 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 to 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 in spite of the harsh environments may show a high quality of stem cell properties.

Based on 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] Based on these 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, the 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 (0.25wt%). 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 2.2cm), (ii) the polymer solution was removed after 5 seconds, (iii) the coating was repeated and the resulting culture