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Figure legends

Figure 1. The experimental design. Cells in bone marrow aspirates were seeded on MPC polymer-coated plates at the concentration of 0-10%, 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.

Figure 2. The adhesion of cells in bone marrow aspirates onto the culture plates coated with different concentrations of the MPC polymer. 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 polymer. All values are presented as mean plus standard deviation of 5 samples per group. Statistics were assessed using Dunnett's test (*: $P<0.01$ vs 0% MPC).

Figure 3. Proliferation of the cells that had been selected by the plate coated with different concentrations of the MPC polymer. The cells cultured on the MPC polymer-coated plates were harvested and then re-seeded onto the conventional PS plates. The cell numbers were counted at 7 days of culture (graph). No significant difference was seen among the proliferation of the cells harvested from each MPC polymer-coated plate (0-10%). The dashed line indicats the number of cells originally seeded on the plate (19,000 cells).

Figure 4. Gene expression of COL1A1 and ALP in the osteogenic induction. Signficant expression of COL1A1 gene was found in the MSCs selected by the MPC polymer-coated plates (2-5%) at

passage 2, while the high expression level in the 5% MPC continued by passage 5. Also, in the ALP expression, the promotion effect was observed in 2-5% MPC, expecially at passage 5. All values are presented as mean plus standard deviation of 5 samples per group. Statistics were assessed using Dunnett's test (*: $P<0.01$ vs 0% MPC).

Figure 5. Gene expression of COL2A, COL10A1 and Sox9 during the chondrogenic induction. The expressions of COL2A1, COL10A1 and Sox9 genes peaked at 2-5% MPC not only at passage 2, but also at passage 5. All values are presented as mean plus standard deviation of 5 samples per group. Statistics were assessed using Dunnett's test (*: $P<0.01$ vs 0% MPC).

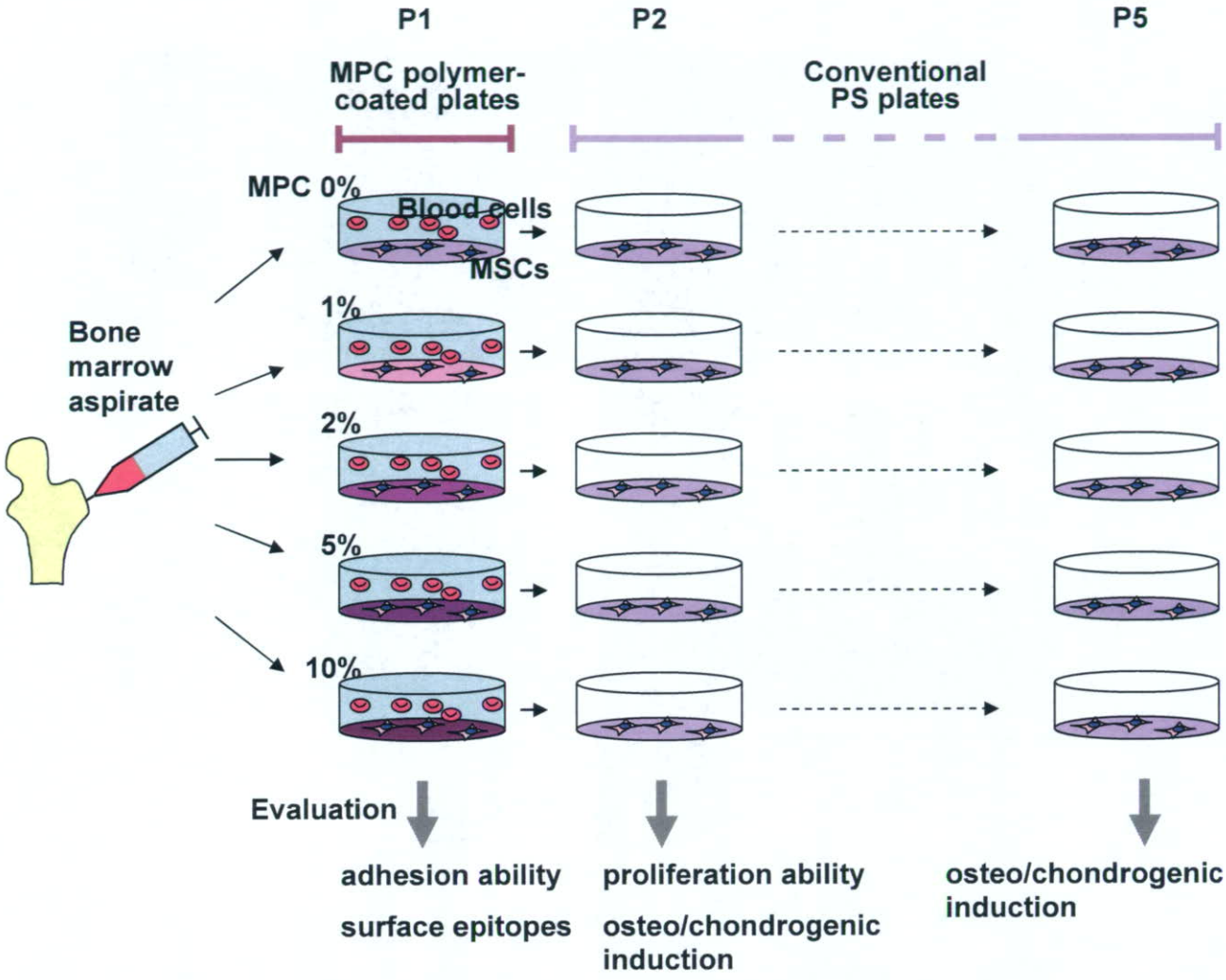
P1
P2
P5
MPC polymer-coated plates
Conventional PS plates
MPC 0%
Blood cells
MSCs
1%
2%
5%
10%
Bone marrow aspirate
Evaluation
adhesion ability
surface epitopes
proliferation ability
osteo/chondrogenic induction
osteo/chondrogenic induction

Figure 1

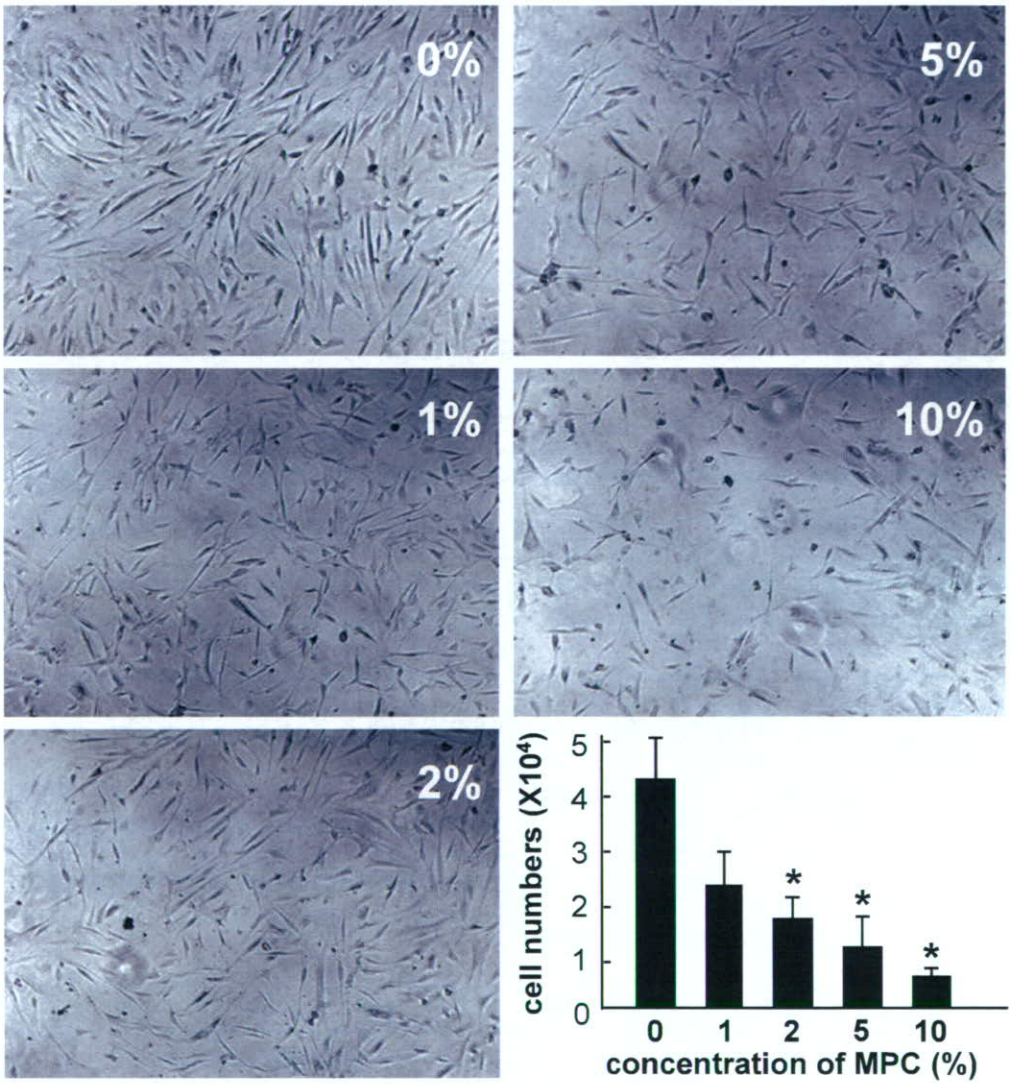
0%
5%
1%
10%
2%
cell numbers (X10⁴)
0
1
2
3
4
5
*
*
*
0
1
2
5
10
concentration of MPC (%)

Figure 2

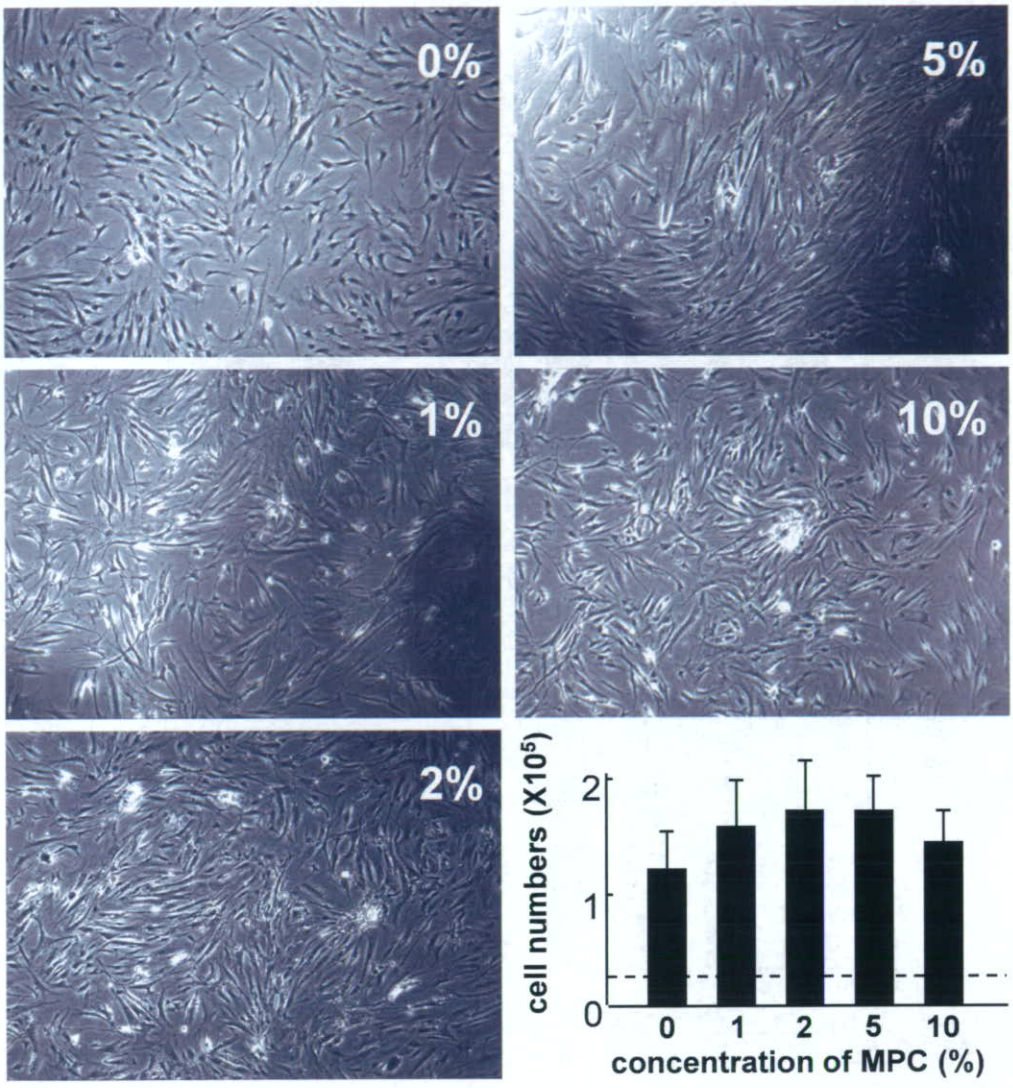

Figure 3

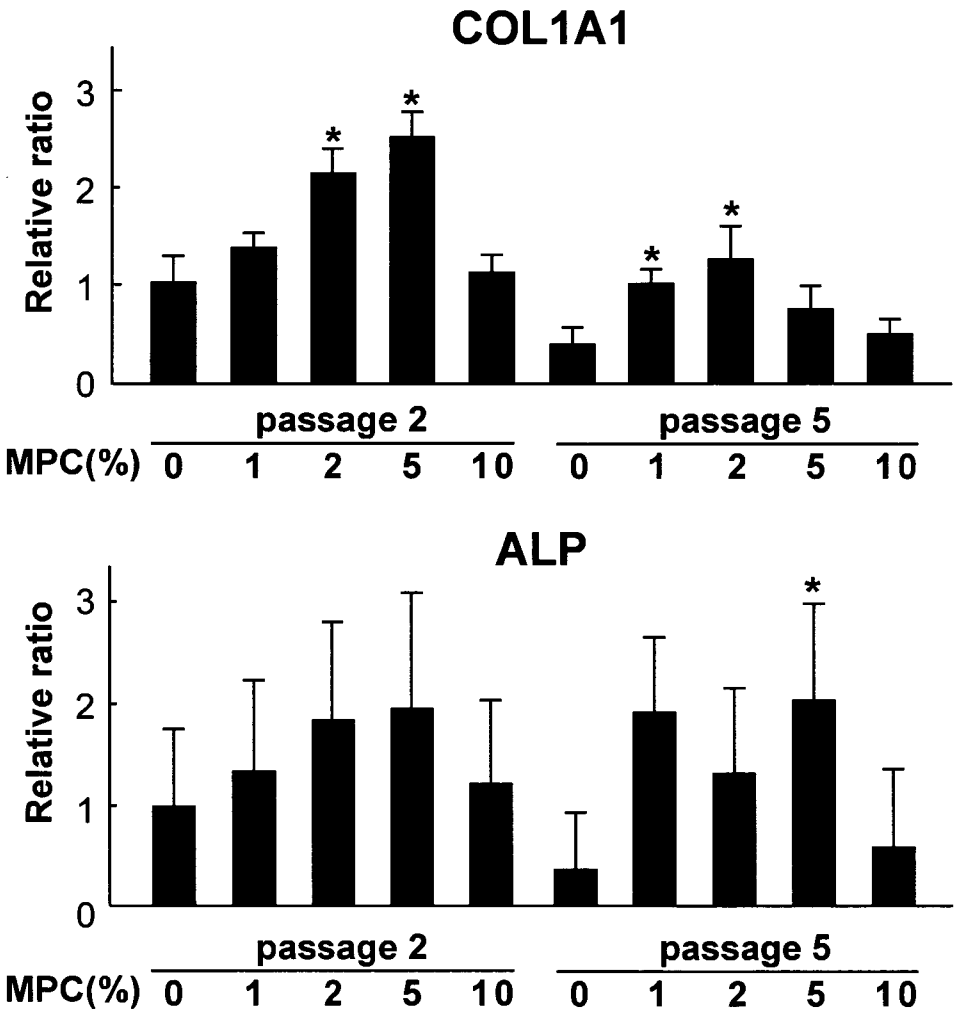
COL1A1
Relative ratio
3
2
1
0
*
*
*
*
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10
ALP
Relative ratio
3
2
1
0
*
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10

Figure 4

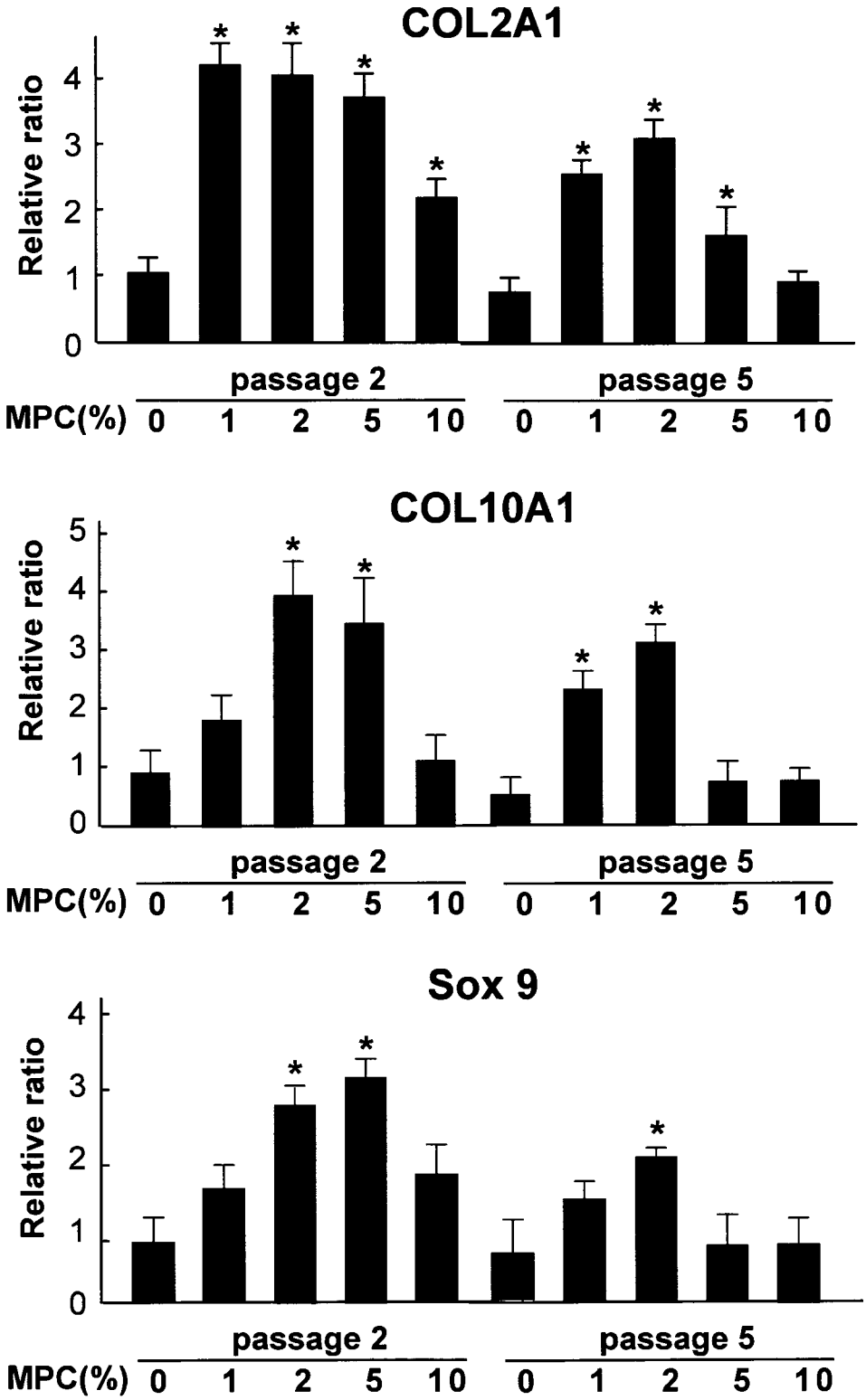
COL2A1
Relative ratio
0
1
2
3
4
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10
COL10A1
Relative ratio
0
1
2
3
4
5
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10
Sox 9
Relative ratio
0
1
2
3
4
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10

Figure 5

Table 1. Expression of surface epitopes in MPC-selected cells

Surface epitoptes	MPC 0%	MPC 1%	MPC 2%	MPC 5%	MPC 10%
CD29 (Integrin β1)	++	++	+++	++	++
CD44 (Hyaluronan receptor)	++	++	++	++	++
CD105 (Endoglin)	+	+	+	+	+
CD166 (ALCAM)	+	+	+	+	+
CD34	-	-	-	-	-
CD45 (LCA)	-	-	-	-	-

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

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

Running title: Poly(MPC) grafted Co-Cr-Mo

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ABSTRACT

Osteolysis, caused by wear particles from polyethylene cups in artificial hip joints, is a topic of great concern. To reduce this wear and develop a novel artificial hip joint system, we produced a super-lubricious metal-bearing material: for this, we grafted a 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer onto the surface of a cobalt-chromium-molybdenum (Co-Cr-Mo) alloy. For ensuring long-term benefit retention of poly(MPC) on the Co-Cr-Mo alloy for application as a novel artificial hip joint system, several issues must be considered: strong bonding between poly(MPC) and Co-Cr-Mo surface, high mobility of free end groups of the poly(MPC) layer, and high density of the introduced poly(MPC). Considering these issues, we introduced a 3-methacryloxypropyl trimethoxysilane (MPSi) intermediate layer and a photoinduced graft polymerization technique to create a strong covalent bond between the Co-Cr-Mo substrate and the poly(MPC) chain via the MPSi layer. The thickness and density of the poly(MPC) layer on the surface increased with the MPC concentration and photoirradiation time. The grafted poly(MPC) layer successfully provided super-lubricity to the Co-Cr-Mo surface. The poly(MPC)-grafted cross-linked polyethylene/poly(MPC)-grafted Co-Cr-Mo or cartilage/poly(MPC)-grafted Co-Cr-Mo bearing interface mimicking natural joints showed an extremely low friction coefficient of 0.01, which is as low as that of natural cartilage interface. A super-lubricious metal-bearing surface would enable the development of a novel biocompatible artificial hip joint system—artificial femoral head for partial hemi-arthroplasty and metal-on-polymer/metal type for total hip arthroplasty.

INTRODUCTION

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

In total hip arthroplasty (THA), osteolysis caused by the wear particles from UHMWPE has been recognized as a serious issue.[2–4] Efforts to decrease these particles have focused on bearing material improvement and the use of combinations other than metal-on-UHMWPE.[5–7] Recently, a metal-on-metal type artificial hip joint system consisting of Co-Cr-Mo acetabular and femoral components has been studied.[8] The advantages of the Co-Cr-Mo/Co-Cr-Mo bearings are that they do not generate UHMWPE wear debris and they exhibit decreased wear as compared to Co-Cr-Mo/UHMWPE bearings.[9,10] However, even in Co-Cr-Mo/Co-Cr-Mo bearings, aseptic loosening induced by wear particles and metallosis remains as serious an issue in revision surgeries.[11,12] In addition to metallosis, electrochemical corrosion and carcinogenesis occurring

due to the dissemination of wear particles to the other parts of the body have been reported.[13]

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

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

been well verified.[28–30]

In general, there are two methods for modifying the polymer surface. The first method involves surface absorption or reaction with small molecules[31,32] and the second, grafting polymeric molecules onto the substrate through covalent bonding.[33] Most frequently, grafting polymerization is performed using either of the following methods: (1) surface-initiated graft polymerization, termed as the "grafting from" method, in which monomers are polymerized from initiators or comonomers; and (2) adsorption of the polymer to the substrate, termed as the "grafting to" method (i.e., dipping, cross-linking, and ready-made polymers with reactive end groups reacting with the functional groups of the substrate).[34,35] The "grafting from" method has an advantage over the "grafting to" method in that it synthesizes a high-density polymer brush. The novel artificial joint developed in this study is super-lubricious surface with nanometer-scale poly(MPC) modification. This surface modification was accomplished by using a photo-induced radical polymerization technique that was similar to the one used in the "grafting from" method.

To ensure *in vivo* long-term retention of this poly(MPC) graft on the Co-Cr-Mo alloy, it is necessary to create strong covalent bonding between the Co-Cr-Mo alloy substrate and the poly(MPC) graft chain. Organosilanes have already been known as surface coupling agents to enhance bonding between a metal or a metal oxide surface and an organic resin such as dental resin, and they can strongly bind metals to resins in dental implants.[36] Organic silanes or silane coupling agents comprise at least a hydrolyzable alkoxysilyl or chlorosilyl group and an organofunctional group.[37] The agents are effective to introduce organofunctional groups into the siloxane network

polymer. The organofunctional group in the silane could be useful to improve bonding with the organic overlayer. 3-Methacryloxypropyl trimethoxysilane (MPSi) is a simple surface coupling agent consisting of three methoxy groups, a propyl chain, and a functional methacrylate, and the structure of its main chain is equivalent to that of MPC.

In this study, based on the advantages of biocompatibility and hydrophilicity of poly(MPC), the "grafting from" method and the polymer strongly bound to the metal of silanization, a super-lubricious metal bearing material in which the poly(MPC) was grafted onto the surface of the Co-Cr-Mo alloy (Co-Cr-Mo-*g*-MPC) has been introduced for developing a novel artificial hip joint system, i.e., artificial femoral head and metal-on-metal (Co-Cr-Mo/Co-Cr-Mo) type for THA. The surface structure and tribological properties of Co-Cr-Mo-*g*-MPC were also investigated.

MATERIALS AND METHODS

Chemicals

MPC was synthesized industrially by using the method developed by Ishihara et al.[22] and it was supplied by AI Bio-Chips Co., Ltd. (Tokyo, Japan). MPSi was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Succinic acid and ethanol were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). 2-Hydroxy-l-[4-(hydroxyethoxy)phenyl]-2-methyl-lpropanone (DAROCUR® 2959; D2959) was purchased from Ciba Specialty Chemicals Holding Inc. (Basel, Switzerland). D2959 is a highly efficient radical photoinitiator for ultraviolet (UV) curing of the systems containing unsaturated monomers and prepolymers, and it is particularly well known as a

cytocompatible UV photoinitiator with UV intensities of <6 mW/cm^2 that can perform polymerization for up to 10 min with a UV light of 365 nm.[38]

Co-Cr-Mo alloy substrate and pretreatments

The Co-Cr-Mo alloy was supplied by Yoneda Advanced Casting Co., Ltd (Takaoka, Japan). The chemical composition of the Co-Cr-Mo alloy used in this study is described in Table 1. This alloy was manufactured according to the ASTM F75 standard specification for Co-28Cr-6Mo alloy.[39] The Co-Cr-Mo samples were polished so that the average surface roughness ranged between 0.01–0.02 μm.

The polished Co-Cr-Mo samples were washed with acetone, and then immersed in 35 vol% nitric acid at room temperature for 35 min according to the ASTM F86-04 standard.[40,41] This treatment results in passivation by surface oxidation and it could lead to the dissolution of certain foreign materials that may remain from the previous procedure. Moreover, a previous study reported that the surface of as-polished Co-Cr-Mo alloy might lack the Cr content that the bulk possesses, and that surface etching by nitric acid treatment would have produced a Cr-rich surface layer.[41] We therefore treated the surface with nitric acid with the aim of increasing the Cr concentration by "re-surfacing."

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

surface oxide layer.[42]

MPSi silanization and MPC graft polymerization

The synthesis of Co-Cr-Mo-*g*-MPC is schematically illustrated in Fig. 1. The pretreated Co-Cr-Mo samples were immersed in an ethanol solution containing 5 mass% MPSi, 1 mass% succinic acid, and 0.1 mass% D2959 at room temperature for 12 h for silanization of the trimethoxysilane group. They were then annealed at 70°C for 3 h in air for dehydration. The MPC was dissolved in degassed pure water to attain concentrations ranging from 0.25 to 1.00 mol/L. Subsequently, the MPSi (containing D2959)-coated Co-Cr-Mo samples were immersed in aqueous MPC solutions. Photoinduced graft polymerization on the Co-Cr-Mo surface was performed using ultraviolet irradiation (UVL-400HA ultra-high pressure mercury lamp; Riko-Kagaku Sangyo Co., Ltd., Funabashi, Japan) with an intensity of 5 mW/cm^2 at 60°C for 23 to 180 min; a filter (Model D-35; Toshiba Corp., Tokyo, Japan) was used restrict the passage of ultraviolet light to wavelengths of 350 ± 50 nm. After the polymerization, the Co-Cr-Mo-*g*-MPC samples were removed from the solution, washed with pure water and ethanol, and dried at room temperature. For purification, washing with pure water and ethanol enables the removal of the free poly(MPC) and/or poly(MPC-*co*-MPSi) adsorbed on the Co-Cr-Mo surface.[43]

MPC graft polymerization on cross-linked polyethylene

Compression-molded UHMWPE (GUR1020 resin, Poly Hi Solidur Inc., IN, USA) bar stock was