The cells attached on the plate surface were observed to have a higher density on the dishes treated with a 0% MPC solution, compared with those of increasing concentration of MPC, 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 were approximately half or quarter in 0%, respectively.

In order to examine the proliferation ability of MPC-selected MSCs, the cells harvested from each MPC polymer-coated plate were re-seeded 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].

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 (hyaluronan receptor), CD105 (Endoglin) and CD166 (ALCAM) were expressed in MSC, but that CD34 and CD45 (LCA) were markers specific for heamatopoietic stem cells. Although the hematopoietic stem cell markers were negative in all cells selected by the plates coated with the 0, 1, 2, 5 or 10% of MPC solution, CD29, CD44, CD105 and CD166 were detectable in the cells of all MPC concentrations. The levels of the MSC markers in the cells selected by the 1-10% MPC were almost similar to those in cells of 0% that corresponds to the control MSC, implying that the MPC polymer-selected cells belong to the category of MSC on the surface epitopes [Table 1].

Osteogenic and chondrogenic potential of MPC-selected cells

After the culture on the MPC polymer-coated plates (passage 1), the cells were cultured on the conventional PS culture plates for a long term with repeated passages. By passage 5, the cell numbers had expanded by approximately 1000-fold in the cells of each MPC concentration (0-10%). Under the osteogenic condition, the cells selected by the MPC plates and cultured in the conventional PS ones for a single time (passage 2) more highly expressed the COL1A1 mRNA in the 2-5% MPC than in the 0%, but those by the 1 or 10% MPC plates did not show any significant increase in the COL1A1 expression. The promotion effects of the COL1A1 expression in 2% MPC continued even at passage 5, although the cells at passage 2 were more sensitive for the osteogenic differentiation than those at passage 5. ALP also tended to peaks at 2-5% MPC for both passages, although no statistical difference of the ALP expression was detected in passage 2 [Fig. 4].

The expression of the chondrocyte markers in the MPC-selected cells under the chondrogenic conditions was also enhanced in the 2-5% MPC, as observed during osteogenesis. Responding to the chondrogenic induction, the cells began to express COL2A1, COL10A1 and Sox 9, and especially cells selected by the 2% MPC showed a prominent expression of all chondrocyte markers not only at passage 2, but even at passage 5 [Fig. 5].

DISCUSSION

The adhesion capacity seems to have some association with the cellular activities and functions. Specific adhesion to the laminin and type IV collagen coated on the surface of the culture dishes could select the myogenic cells of the embryonic mouse thigh from fibroblastic cells. Over a brief time period (10-20 min), myoblasts from the embryonic mouse thigh muscle had adhered faster to the laminin than did the fibroblasts from the same tissue, while the latter adhered faster to the

fibronectin than the former.[18] Laminin-1 also enriched the osteoblast progenitor cells from rat calvarial cells when they were seeded on the culture wells coated with it. The laminin-1 inhibited cell attachment of the rat calvarial cells, but could select the highly osteogenic lineage according to the difference in the cell adhesiveness to that of the molecule.[19] Thus, through the selection of the cell adhesion to some molecules, a specific cell subpopulation that possesses a high differentiation potency would be concentrated from heterogeneity of the cell sources.

MSC expresses many adhesion-related molecules, like the integrin subunits α4, 5, 6, 8, 9, v/beta1, 3, 5, ICAM-1, ALCAM, VCAM-1, SCF, fibronection, E-cadherin and hyaluronan receptor[20-22] and can be bound to various ligands including limanin and E-cadherin to play biological roles through the cell-to-cell or cell-to-matrix contacts. As examples of the cell-to-cell contact with MSCs in vivo, homing functions for the hematopoietic cells of MSCs should be discussed. Through the cell-to-cell contacts with hematopoietic stem cells mediated by VCAM-1, fibronectin, SCF, E-cadherin, or ICAM-1, MSCs secrete extracellular matrix proteins, produce secreted/membrane-bound cytokines and regulate hematopoiesis.[21] MSCs are also recruited and adhered to the damaged tissues in order to participate in tissue repair. These cells can provide cell sources for tissue repair in bone, cartilage, and even skeletal muscle or myocardium that do not directly make contact with bone marrow. Once muscles are injured, the MSCs are delivered to the degenerative muscles from the circulation, are adhered to the lesion, take part in the regenerative process, and provide fully differentiated muscle fibers.[23] In the murine model of cardiac repair following ischemic injury, MSCs were mobilized from bone marrow, homed and generated cardiac myocytes. Among the adhesion molecules of the MSC such as integrin α4, 6, 8, 9, and β1, blockade of the integrin β1 by the neutralizing antibody reduced the total number of MSCs in the infracted myocardium, suggesting that MSCs utilized integrin β1 for cell adhesion to the myocardium and its regeneration.[22]

Thus, MSCs can be bound to various partners via many kinds of adhesion molecules to exert

physiological and pathological functions. Although the adhesiveness to some ligands likely selects a cell subpopulation with a high differentiation potency of a certain lineage, [18, 19] such a specific selection may have the risk to reduce the multipotency in MSCs. Therefore, we applied the selection system based not on the adhesiveness to specific molecules, but the general adhesion ability to the MPC polymer-coated plates. As a result, we could enrich the cells to have a high potency of both osteogenesis and chondrogenesis from the crude MSCs.

It has yet remained unknown why the strength of the adhesion ability in MSCs could enhance not the proliferation rate of the cells, but the differential potential for both osteogenesis and chondrogenesis. Speculating that such multipotent cells may show a stronger adhesion than fibroblastic cells in bone marrow, the MPC selection due to cell attachment could exclude the fibroblastic ones that possess a lower differentiation potential. However, as we do not currently possess the methods to exactly distinguish MSCs from fibroblastic cells using cell surface epitopes, it may be hard to prove that the MPC selection could concentrate the multipotent MSCs from a mixture of the MSCs with fibroblast, by flow cytometry that can exactly exclude the hematopoietic lineage from the MSCs.

MSC can be differentiated into a variety of tissues including bone, cartilage, tendon, fat, heart, muscle or brain, in vitro and in vivo.[1, 8] Autologous MSCs have advantages over embryonic stem cells, regarding the teratocarcinoma formation, immune rejection, or ethical problems. The cell sources have already been used for the treatment of osteogenesis imperfecta, bone/cartilage defects, myocardiac infarction, or skin ulcer.[24-27] On the other hand, MPC material has also been already applied in the clinical field for the surfaces of intravascular stents, intravascular guide wires, soft contact lenses, and the artificial lung, all of which were authorized by the United States Food and Drug Administration.[13, 14] Thus, the biocompatible polymer is regarded to be approved for safe clinical use.

The MPC selection is as simple as to culture MSCs with MPC polymer-coated plates in the first passage, which would reduce the risks of contamination or mismanagement during the culture procedure. The improvement of the MSCs in purity and multipotency by the MPC selection would provide promising technologies for the next generation-cell therapy that can be applied for more severe and other various diseases. The clinical application of the MPC-selected MSCs is now underway.

References

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-147.
2. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H and others. Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest 1999;103:697-705.
3. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A 1999;96:10711-10716.
4. Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luria EA, Ruadkow IA. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Exp Hematol 1974;2:83-92.
5. Sekiya I, Larson BL, Vuoristo JT, Cui JG, Prockop DJ. Adipogenic differentiation of human adult stem cells from bone marrow stroma (MSCs). J Bone Miner Res 2004;19:256-264.
6. Colter DC, Sekiya I, Prockop DJ. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. Proc Natl Acad Sci U S A 2001;98:7841-7845.

7. Smith JR, Pochampally R, Perry A, Hsu SC, Prockop DJ. Isolation of a highly clonogenic and multipotential subfraction of adult stem cells from bone marrow stroma. Stem Cells 2004;22:823-831.
8. Ishii M, Koike C, Igarashi A, Yamanaka K, Pan H, Higashi Y, Kawaguchi H, Sugiyama M, Kamata N, Iwata T and others. Molecular markers distinguish bone marrow mesenchymal stem cells from fibroblasts. Biochem Biophys Res Commun 2005;332:297-303.
9. Pochampally RR, Smith JR, Ylostalo J, Prockop DJ. Serum deprivation of human marrow stromal cells (hMSCs) selects for a subpopulation of early progenitor cells with enhanced expression of OCT-4 and other embryonic genes. Blood 2004;103:1647-1652.
10. Ueda H, Watanabe J, Konno T, Takai M, Saito A, Ishihara K. Asymmetrically functional surface properties on biocompatible phospholipid polymer membrane for bioartificial kidney. J Biomed Mater Res A 2006;77:19-27.
11. Sawada S, Iwasaki Y, Nakabayashi N, Ishihara K. Stress response of adherent cells on a polymer blend surface composed of a segmented polyurethane and MPC copolymers. J Biomed Mater Res A 2006;79:476-484.
12. Sibarani J, Takai M, Ishihara K. Surface modification on microfluidic devices with 2-methacryloyloxyethyl phosphorylcholine polymers for reducing unfavorable protein adsorption. Colloids Surf B Biointerfaces 2007;54:88-93.
13. Lewis AL, Tolhurst LA, Stratford PW. Analysis of a phosphorylcholine-based polymer coating on a coronary stent pre- and post-implantation. Biomaterials 2002;23:1697-1706.
14. Kihara S, Yamazaki K, Litwak KN, Litwak P, Kameneva MV, Ushiyama H, Tokuno T, Borzelleca DC, Umezu M, Tomioka J and others. In vivo evaluation of a MPC polymer coated continuous flow left ventricular assist system. Artif Organs 2003;27:188-192.

15. Moro T, Takatori Y, Ishihara K, Konno T, Takigawa Y, Matsushita T, Chung UI, Nakamura K, Kawaguchi H. Surface grafting of artificial joints with a biocompatible polymer for preventing periprosthetic osteolysis. Nat Mater 2004;3:829-836.
16. Goda T, Ishihara K. Soft contact lens biomaterials from bioinspired phospholipid polymers. Expert Rev Med Devices 2006;3:167-174.
17. Ishihara K, Iwasaki Y, Ebihara S, Shindo Y, Nakabayashi N. Photoinduced graft polymerization of 2-methacryloyloxyethyl phosphorylcholine on polyethylene membrane surface for obtaining blood cell adhesion resistance. Colloids Surf B Biointerfaces 2000;18:325-335.
18. Kuhl U, Ocalan M, Timpl R, von der Mark K. Role of laminin and fibronectin in selecting myogenic versus fibrogenic cells from skeletal muscle cells in vitro. Dev Biol 1986;117:628-635.
19. Roche P, Rousselle P, Lissitzky JC, Delmas PD, Malaval L. Isoform-specific attachment of osteoprogenitors to laminins: mapping to the short arms of laminin-1. Exp Cell Res 1999;250:465-474.
20. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. J Cell Physiol 1999;181:67-73.
21. Hall BM, Gibson LF. Regulation of lymphoid and myeloid leukemic cell survival: role of stromal cell adhesion molecules. Leuk Lymphoma 2004;45:35-48.
22. Ip JE, Wu Y, Huang J, Zhang L, Pratt RE, Dzau VJ. Mesenchymal stem cells utilize integrin beta 1 not CXC chemokine receptor 4 for myocardial migration and engraftment. Mol Biol Cell 2007;18:2873-2882.
23. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998;279:1528-1530.

24. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE and others. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 1999;5:309-313.

25. Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E, Marcacci M. Repair of large bone defects with the use of autologous bone marrow stromal cells. N Engl J Med 2001;344:385-386.

26. Vojtassak J, Danisovic L, Kubes M, Bakos D, Jarabek L, Ulicna M, Blasko M. Autologous biograft and mesenchymal stem cells in treatment of the diabetic foot. Neuro Endocrinol Lett 2006;27 Suppl 2:134-137.

27. Fox JM, Chamberlain G, Ashton BA, Middleton J. Recent advances into the understanding of mesenchymal stem cell trafficking. Br J Haematol 2007;137:491-502.

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).

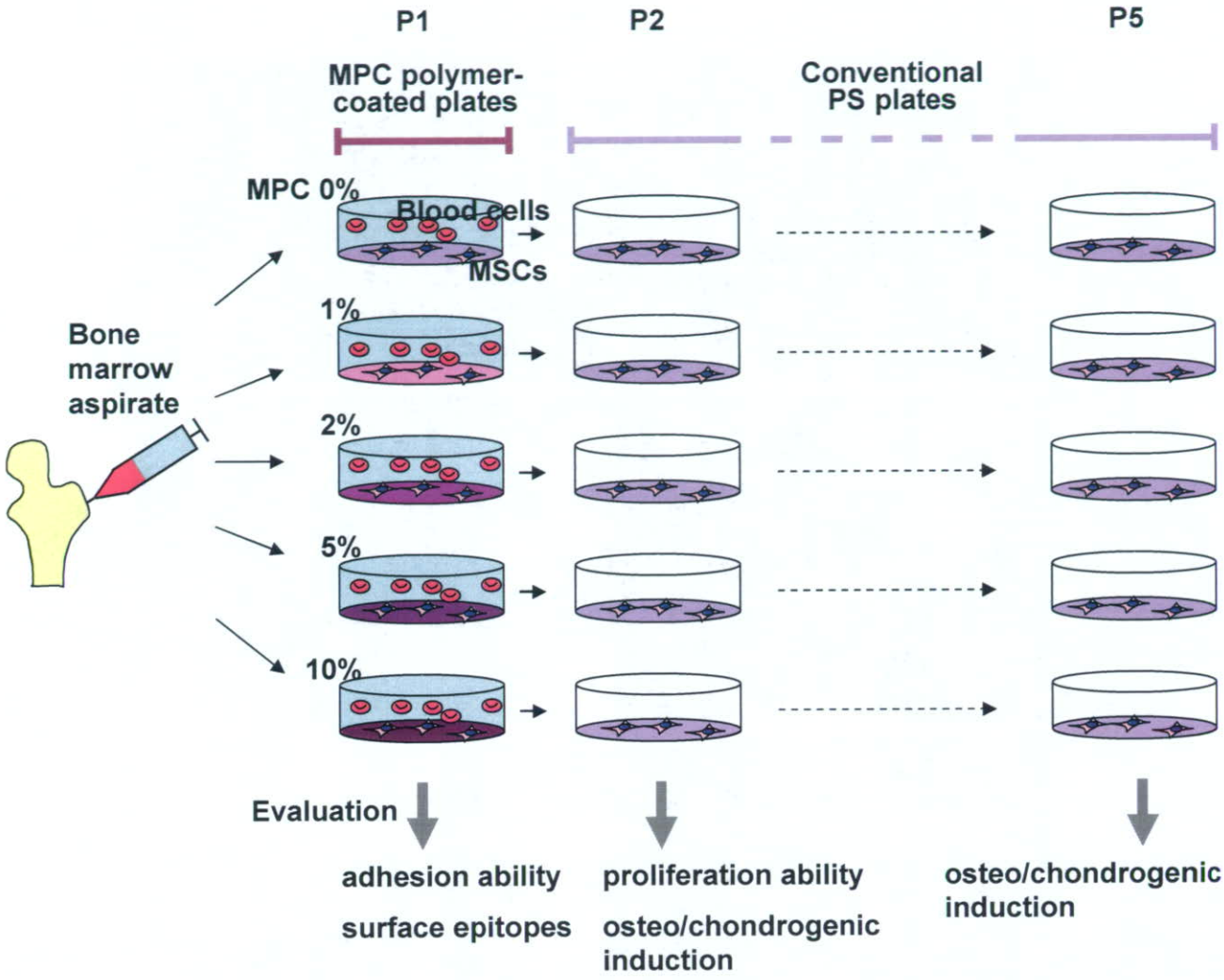
P1
P2
P5
MPC polymer-coated plates
Conventional PS plates
MPC 0%
Blood cells
MSCs
1%
2%
5%
10%
Bone marrow aspirate
Evaluation
adhesion ability
surface epitopes
proliferation ability
osteo/chondrogenic induction
osteo/chondrogenic induction

Figure 1

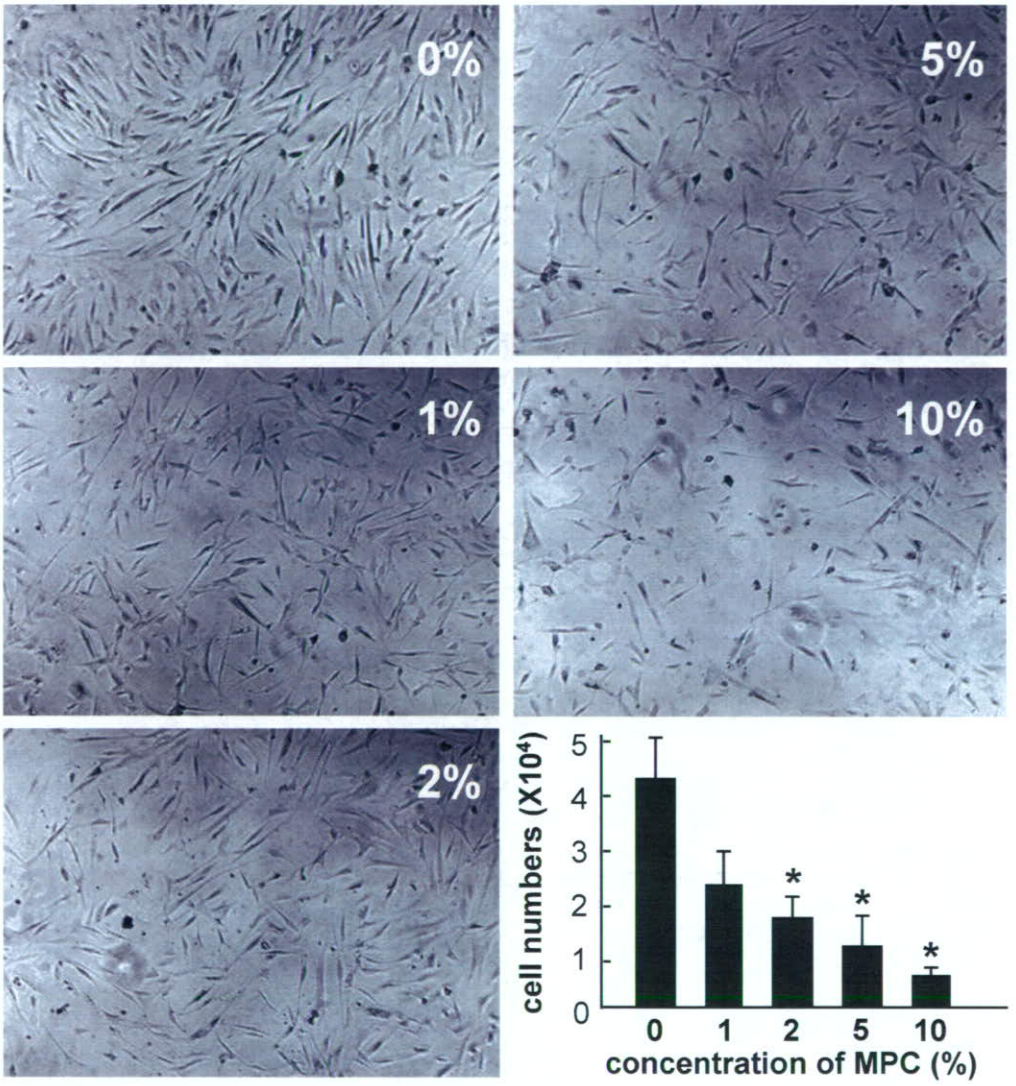
0%
5%
1%
10%
2%
cell numbers (X10⁴)
0
1
2
3
4
5
*
*
*
0
1
2
5
10
concentration of MPC (%)

Figure 2

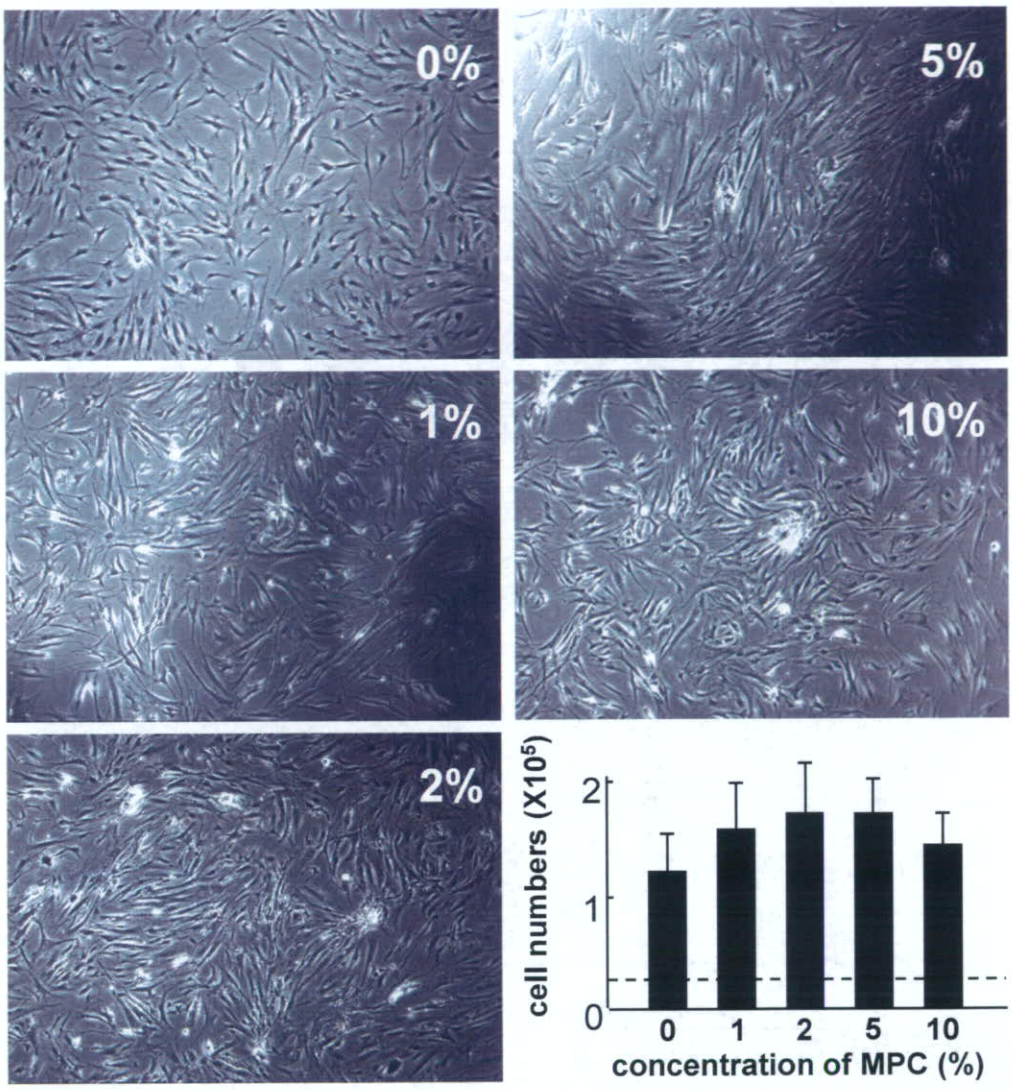

Figure 3

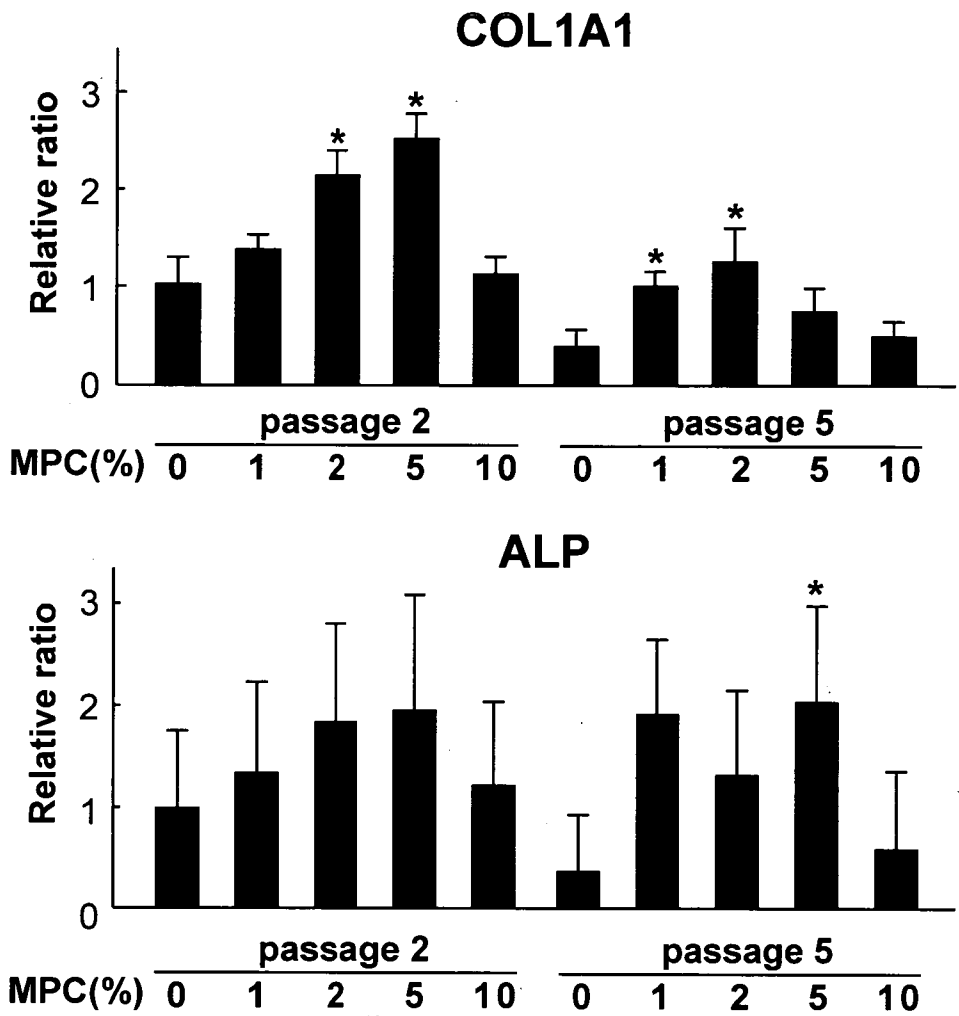
COL1A1
Relative ratio
3
2
1
0
*
*
*
*
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10
ALP
Relative ratio
3
2
1
0
*
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10

Figure 4

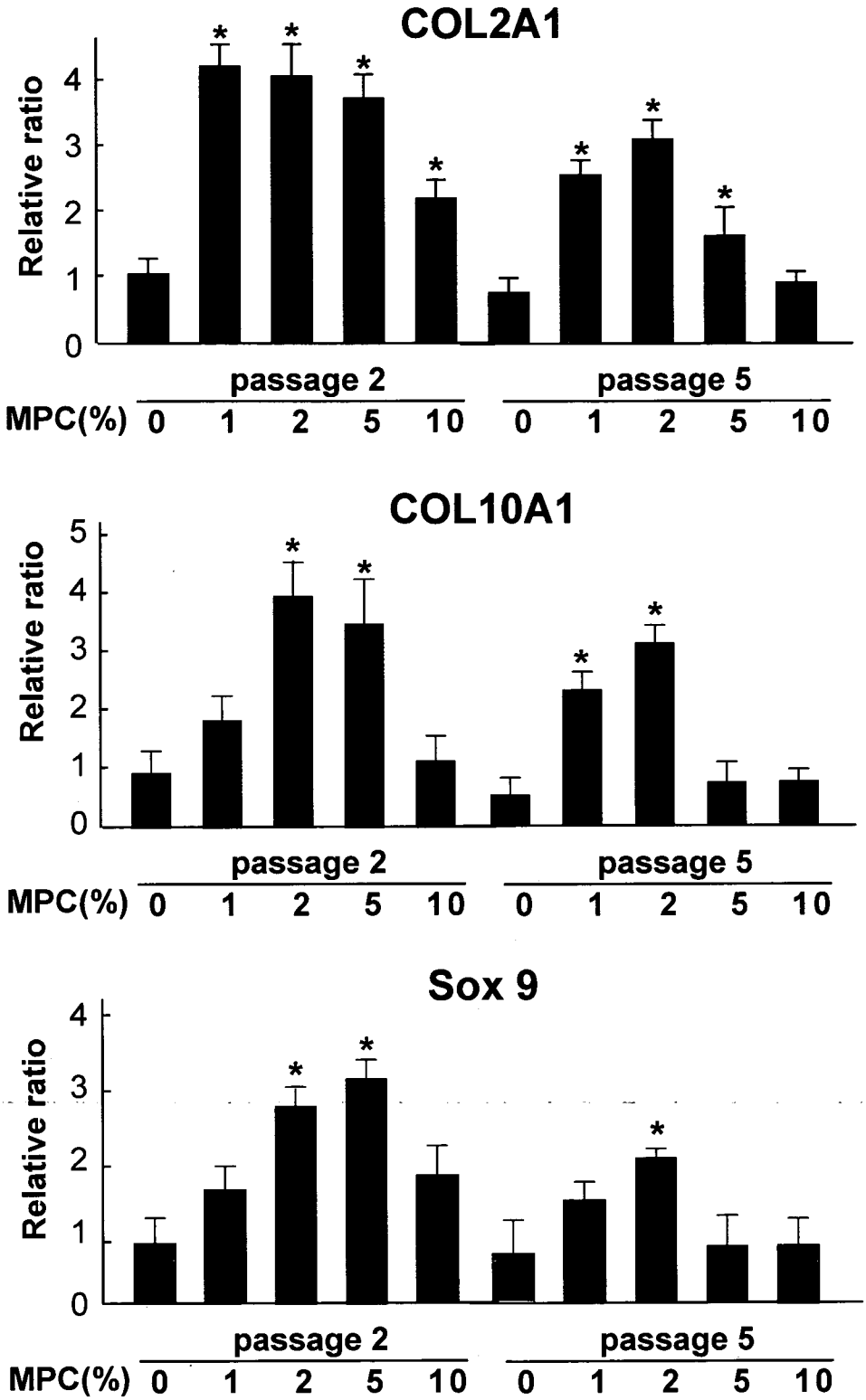
COL2A1
Relative ratio
0
1
2
3
4
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10
COL10A1
Relative ratio
0
1
2
3
4
5
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10
Sox 9
Relative ratio
0
1
2
3
4
passage 2
passage 5
MPC(%) 0 1 2 5 10 0 1 2 5 10

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)	-	-	-	-	-

Journal of Biomedical Materials Research Part A

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