1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

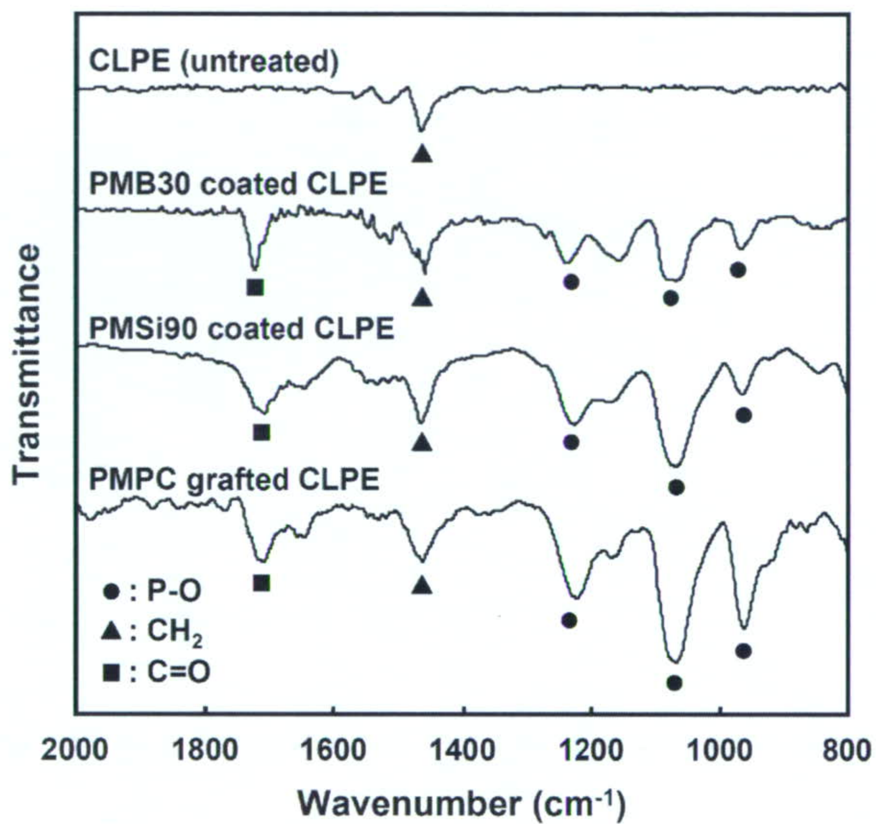

Figure 2. FT-IR/ATR spectra of untreated CLPE, MPC polymer coated CLPE, and PMPC grafted CLPE.
46x42mm (600 x 600 DPI)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

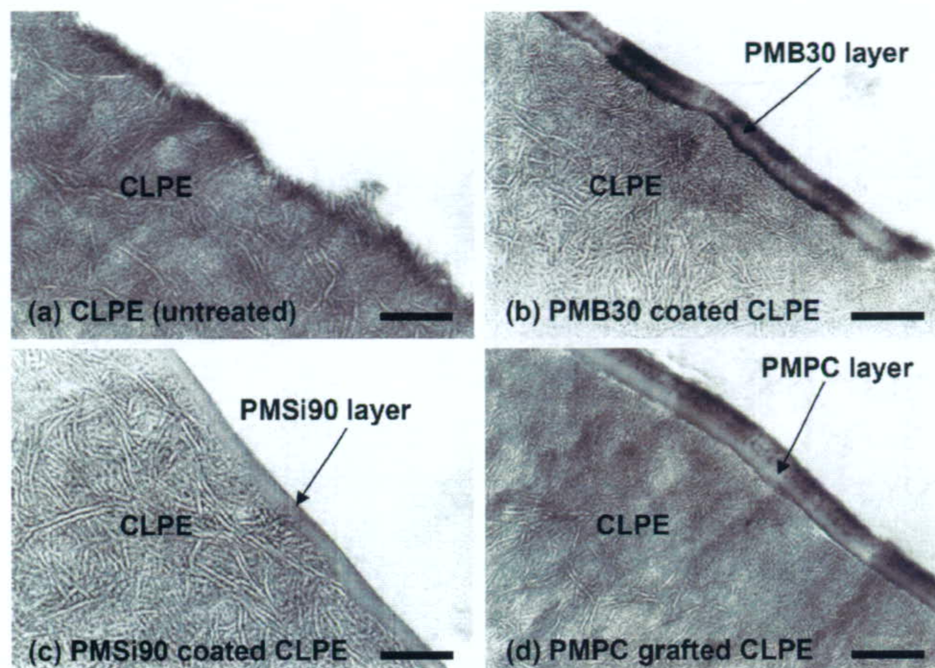

Figure 3. Cross-sectional TEM images of untreated CLPE, MPC polymer coated CLPE, and PMPC grafted CLPE. Bar: 200 nm.
49x35mm (600 x 600 DPI)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

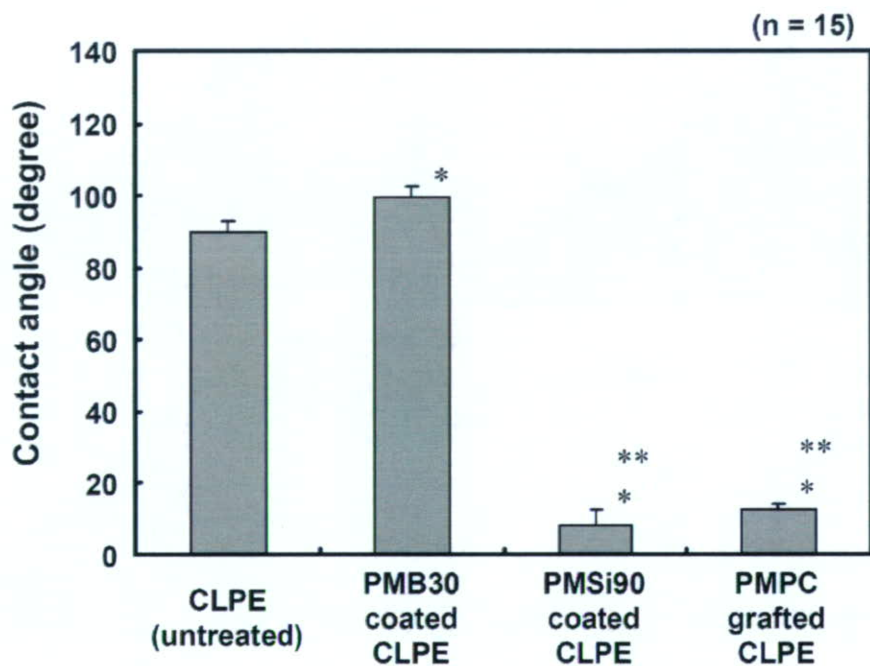

Figure 4. Static-water contact angle of untreated CLPE, MPC polymer coated CLPE, and PMPC grafted CLPE. Bar: Standard deviations. *: Significant difference (*p* < 0.001) as compared to the untreated CLPE, **: significant difference (*p* < 0.001) as compared to the PMB30 coated CLPE.
38x29mm (600 x 600 DPI)

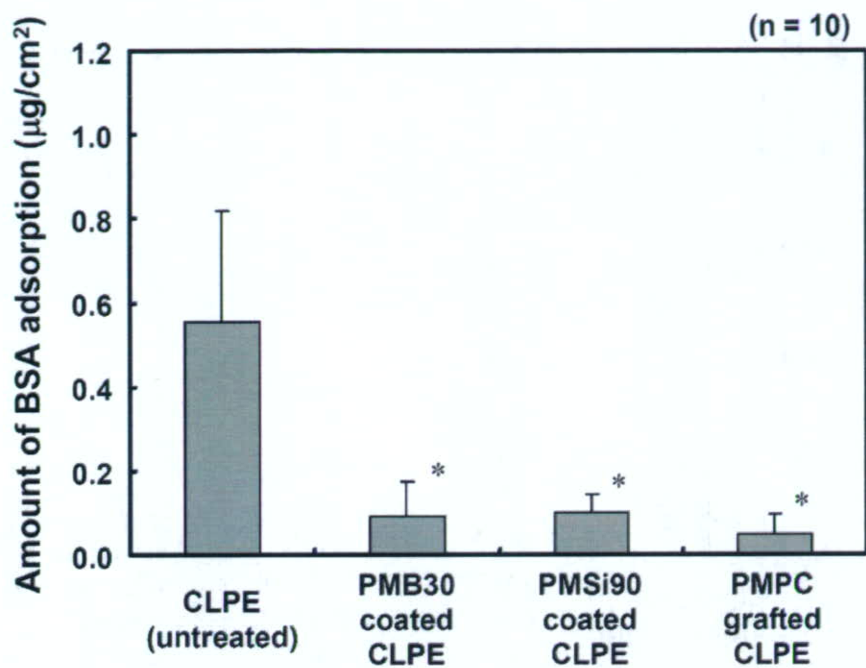

Figure 5. Amount of BSA adsorbed on the surfaces of the untreated CLPE, MPC polymer coated CLPE, and PMPC grafted CLPE. Bar: Standard deviations. *: Significant difference ($p < 0.001$) as compared to the untreated CLPE.
38x29mm (600 x 600 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

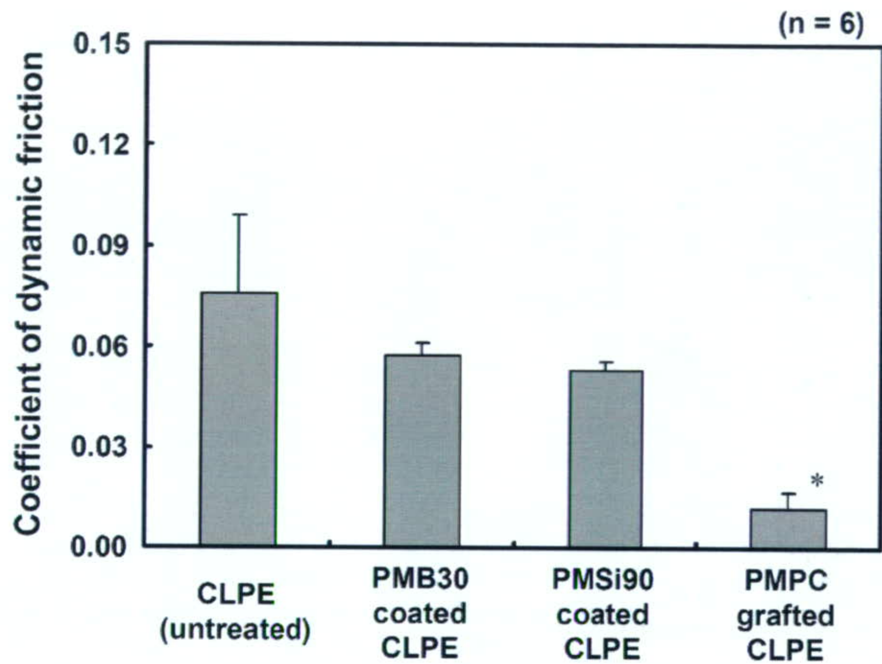

Figure 6. Coefficients of dynamic friction of untreated CLPE, MPC polymer coated CLPE, and PMPC grafted CLPE. Bar: Standard deviations. *: Significant difference ($p < 0.005$) as compared to the untreated CLPE.
38x29mm (600 x 600 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

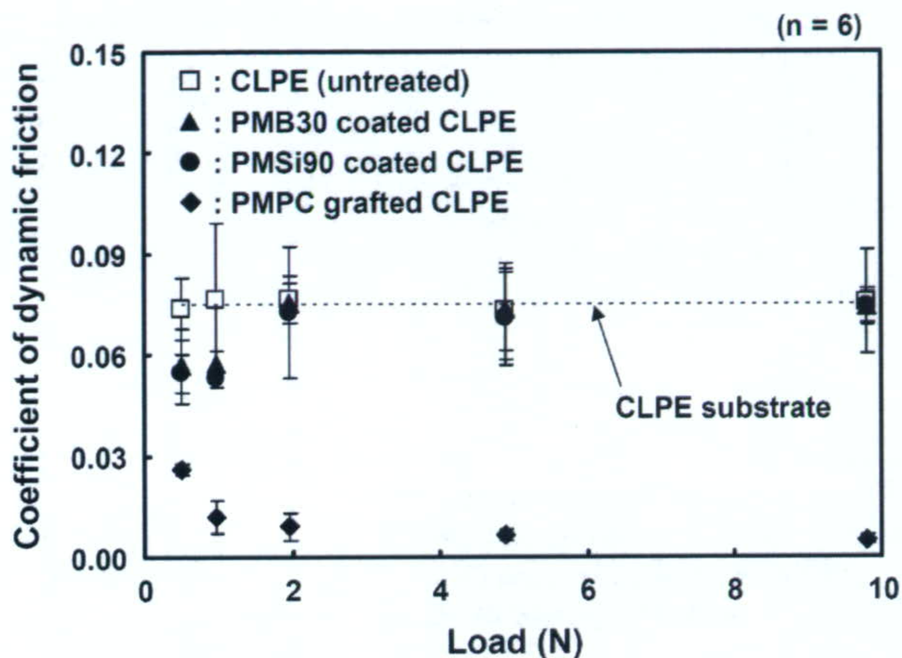

Figure 7. Coefficients of dynamic friction of the untreated CLPE, MPC polymer coated CLPE, and PMPC grafted CLPE as a function of loads in the ball-on-plate friction test. Bar: Standard deviations.
38x29mm (600 x 600 DPI)

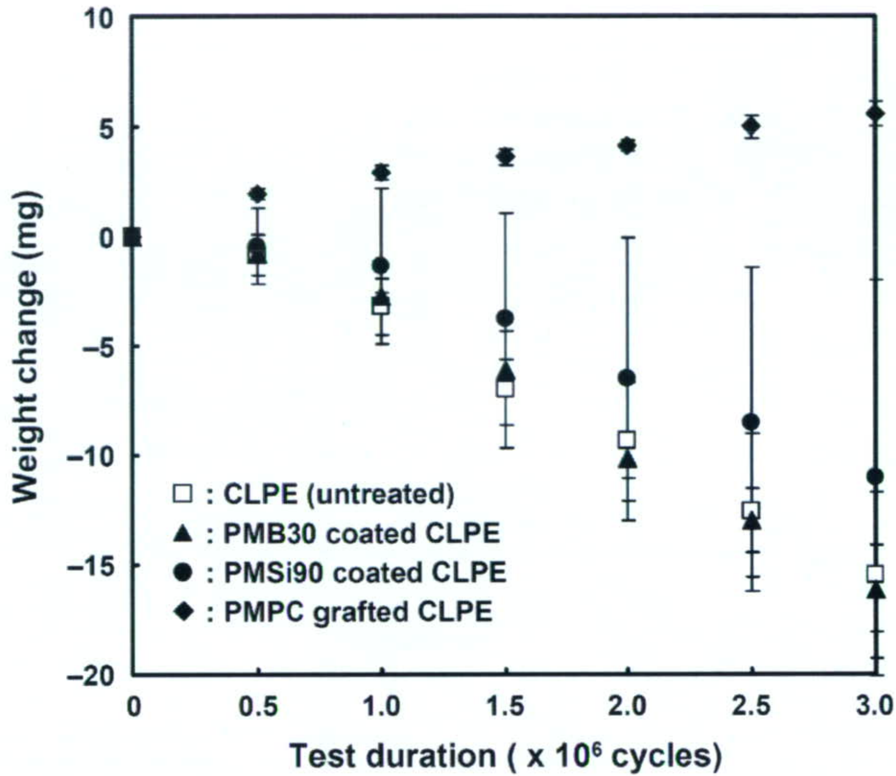

Figure 8. Weight change (volumetric wear) of the untreated CLPE, MPC polymer coated CLPE, and PMPC grafted CLPE in the hip joint simulator wear test. Bar: Standard deviations.
44x39mm (600 x 600 DPI)

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

G. Liu,[1,2] T. Ogasawara,[1,2] J. Watanabe,[3] K. Ishihara,[3] Y. Asawa,[1] Ul. Chung,[4] T. Moro,[5] Y. Takatori,[2] T. Takato,[2] K. Nakamura,[2] H. Kawaguchi,[2] K. Hoshi[1]

[1]Department of Cartilage & Bone Regeneration (Fujisoft), Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

[2] Department of Sensory & Motor System Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

[3]Department of Materials Engineering Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

[4]Bioengineering , School of Engineering, The University of Tokyo, Tokyo, Japan

[5]Center of Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Correspondence to: K. Hoshi; e-mail: pochi-tky@umin.net

Contract grant sponcer: Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Japan; contract grant numbers 18659593, 18592166.

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-selected MSCs. The number of MSCs that were attached to the MPC polymer-coated plates decreased with an increase in the density of MPC polymer (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 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, 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) polymer. It consists of biocompatible

phospholipid polymers that resemble the phospholipids of biomembranes and that can reduce protein adsorption or subsequent cell adhesion.[10-12] This material was evaluated for use in medical devices, for example, the surfaces of intravascular stents, intravascular guide wires, soft contact lenses, an artificial lung or artificial joint, some of which are already clinically available.[13-16]

We examined the selectivity of MSCs using MPC polymer-coated plates and evaluated the proliferation ability or differentiation potential of the MPC-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 polysthylene (PS) surface of the culture plates was performed by a photoinduced polymerization technique that produced a covalent bond between the MPC and PS polymers, as previously described.[17] After the PS surface of the culture plates (φ 2.2 cm) was washed twice with methanol using an ultrasonicator for 30 min, the membrane was immersed in an acetone solution containing benzophenone (1.0 g/dL) for 30 sec. The plates were dried under a dark condition for 1 h in vacuo. The plates were filled with ethanol, while ultrasonication was then done for 10 min to detach the benzophenone from the PS surface. This washing process was repeated seven times. The MPC solution was prepared using degassed pure water. The MPC solution at the concentration of 0-10% was placed in a glass tube and argon gas was passed through the solution for 3 min to eliminate any oxygen. The MPC solution was then placed on the plate coated with benzophenone, and the photopolymerization on the PS surface of the culture plate was carried out using a 500 W ultra-high pressure mercury lamp at 60°C. The wavelength was

selected using a Toshiba D-35 filter (350950 nm). Afterwards, the plates were washed with water then hot ethanol (50°C), and dried in vacuo for 15 h.

MSC preparation and selection by MPC polymer-coated plates

All procedures for the present experiments were approved by the ethics committee of the University of Tokyo Hospital (ethics permission #622). Figure 1 indicates the experimental design. Human MSCs were obtained from the femur of osteoarthritic patients who underwent total hip replacement at the University of Tokyo Hospital, after informed consent. Cells in bone marrow aspirates (100 μL/φ 2.2 cm dish) were seeded on MPC polymer-coated culture plates at the concentration of 0-10%, and cultured using the hMSC bullet kit (Cambrex, East Ruatherford, NJ) in a 37°C/5% CO_2 incubator. The cells were harvested by treatment using trypsin-EDTA solution. After the cell harvest of the primary culture from the MPC polymer-coated plates, the cells were re-seeded onto the conventional PS culture plates at a density of 5000 cells/cm^2. Passages were performed when the cells were approaching confluence. The medium was changed three times/week. The cell numbers were counted by a haematocytometer, while the viability of the cells was checked by trypan blue staining.

Flow cytometric analysis

Cells were harvested using trypsin-EDTA solution, centrifuged at 1500 x g for 5 min, and resuspended at 5 x 10^6 cells/ml in phosphate-buffered saline (PBS) containing 3% fetal bovine serum. Aliquots containing 10^5 cells were incubated with individual primary antibodies or control IgG for 30 min at room temperature. The cells were washed in PBS containing 3% fetal bovine serum and incubated with a fluorescent conjugated secondary antibody for 30 min at room temperature.

Samples were analyzed using a FACS LSL II (BD, Franklin Lakes, NJ). The following monoclonal antibodies were used: mouse monoclonal antibodies against human CD29 (integrin β1, BD), human CD34 (Chemicon, Victoria, Australia), human CD44 (hyaluronan receptor, Ancell, Bayport, MN), human CD45 (LCA, Cymbus, Chandlers Ford, UK), human CD105 (Endoglin, Ancell), CD166 (ALCAM, Ancell), normal mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA) and fluorescein isothiocyanate (FITC)-conjugated rabbit antibody against mouse IgG (Santa Cruz Biotechnology).

Osteogenic and chondrogenic induction for MSCs

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

Total RNA extraction and realtime RT-CR

The total RNA was isolated from MSC using the chaotropic Trizol method (Nippon-gene, Tokyo, Japan). The total mRNA (1 μg) was reverse transcribed using the Super Script reverse

transcriptase with a random hexamer (Takara Shuzo, Shiga, Japan). The full-length or partial-length cDNA of the target genes, including the PCR amplicon sequences, was amplified by PCR, cloned into pCR-TOPO Zero II or pCR-TOPO II vectors (Invitrogen, Carlsbad, CA), and used as standard templates after linearization. The QuantiTect SYBR Green PCR Master Mix (Qiagen, Hilden, Germany) was used, and the SYBR Green PCR amplification and real-time fluorescence detection were performed with an ABI 7700 Sequence Detection system (Foster City, CA). All reactions were run in quadruplicate. The sequences of the primers were 5'-CTCCTCGCTTTCCTTCCTCT-3' and 5'-GTGCTAAAGGTGC CAATGGT-3' for COL1A1; 5'-GAGTCAAGGGTGATCGTGGT-3' and 5'-CACCTTGGTCT CCAGAAGGA-3' for COL2A1; 5'-AGGAATGCCT GTGTCTGCT T -3' and 5'-ACAGGCCTACCCAAACATGA-3' for COL10A1; 5'- GACCCTTGACCCCCACAAT-3' and 5'- GCTCGTACTGCATGTCCCCT-3' for ALP; 5'-CATG AGCGAGGG CACTCC-3' and 5'-TCGCTTCAGGTCAGCCTTG-3' for Sox9; 5'-GAAG GTGAAGGTCGGAGTCA-3' and 5'-GAAGATGGTGATGGGATTTC-3' for GAPDH.

RESULTS

Selection using MPC polymer-coated plates

We first selected some subpopulations of the MSCs according to the degrees of the adhesiveness on the culture plates coated with different concentrations of the MPC polymer. Human bone marrow aspirates (approximately 0.1 mL) was seeded onto the culture plates with a 2.2 cm diameter coated with 0, 1, 2, 5 and 10% MPC solutions. For 3 days, the number of adherent cells on the plate surface had plateaued on all plates. At 3 days, the medium was changed together with the floating cells and were replaced by another medium. The adherent cells continued to be cultured for 4 more days on the same MPC polymer-coated plates, and then were harvested for cell counting.

The cells attached on the plate surface were observed to have a higher density on the dishes treated with a 0% MPC 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.