(45 s), 56°C/(45 s), and 72°C/(60 s) for 35 cycles. After amplification, 10 μl of each reaction was analyzed by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

Oligonucleotide primers corresponding to cDNA sequences for the mouse-specific osteocalcin were designed using mouse sequence (X04142). The site selected for mouse-specific osteocalcin has low (<60%) homology to human osteocalcin (X53698). Even if these primers could anneal to human cDNA, the PCR product size would be 419 or 339 bp, which differs from 443 bp of the mouse product. Furthermore, we confirmed that no PCR product was synthesized with these primers and human RNA samples containing osteocalcin mRNA. In the case of human-specific GAPDH (M33197), the antisense primer had no similar homology site to mouse GAPDH (M32599). Thus, the product amplified by these primers corresponds to human GAPDH.

Statistical analysis

Student's *t*-test was used.

RESULTS

Expansion of alveolar BMSCs in culture

We obtained alveolar bone marrow samples from 41 dental patients. BMSCs (fibroblast-like cells) in 29 samples adhered to the culture surface and proliferated in the presence of 10% FBS and bFGF in primary and secondary cultures. No BMSC expansion was observed with the other samples (Table 1). There was no sex difference regarding the expansion of alveolar BMSCs (Fig. 1A), but the BMSC expansion depended on patients' age (Fig. 1B): BMSCs from patients >50 years of age often showed only a few adherent cells in primary cultures, and these did not form colonies within 14 days (data not shown), suggesting an age-related decline in the number of alveolar BMSCs and/or their growth capability. Marrow samples obtained during the course of wisdom tooth extraction or surgery for bone fracture or jaw deformity showed a higher success ratio than did those obtained during surgery for dental implants or dental cyst extraction (Fig. 1C). It remains unknown whether the site of aspiration affects the success ratio (Table 1). The low success ratio for dental implants may be caused by the increased age of the patients (Table 1). Whereas the success ratio increased with an increase in the volume of obtained marrow samples (Fig. 1D), this relationship is questionable because some aspirates contain peripheral blood.

All BMSC lines obtained by ex vivo expansion were maintained at least until passages 3–4 and then stored in liquid nitrogen for differentiation assays; some lines underwent further successive passages for proliferation assays (Fig. 2). Passages were performed when cells were approaching confluence. The cell number was determined at the passage, and the cumulative cell number at the passage is shown in Fig. 2. Alveolar BMSC lines retained their proliferative capacity until passages 8–11. The growth rate during the logarithmic growth phase (1.0 ± 0.2 cell division/day; doubling time, 25 ± 4 h) and proliferative life span (36 ± 10 cell doublings) of alveolar BMSCs were similar to those (0.9 ± 0.1, 28 ± 5, and 33 ± 11, respectively) of iliac BMSCs, although both BMSC lines showed large interindividual variations (Fig. 2).

TABLE 1. ISOLATION OF BMSCs FROM ALVEOLAR BONE

Patients	*Age*	*Sex*	*Procedure*	*Initial quantity (ml)*	*Cell expansion*
a1	25	F	A	0.1	+
a2	29	M	A	0.1	+
a3	31	F	A	0.1	+
a4	24	F	A	0.1	+
a5	25	M	A	0.1	+
a6	19	F	A	0.1	+
a7	35	M	A	0.1	+
a8	19	F	A	0.1	+
a9	22	F	A	0.1	+
a10	14	F	A	0.1	+
a11	19	F	C	0.5	+
a12	20	F	B	3	+
a13	31	F	A	1	+
a14	19	M	B	1	+
a15	22	F	B	0.5	+
a16	25	F	A	0.1	+
a17	58	F	A	0.1	–
a18	59	M	C	0.1	–
a19	53	F	D	0.2	–
a20	53	M	D	0.5	+
a21	53	M	D	0.2	–
a22	17	M	C	0.2	–
a23	52	F	D	0.2	+
a24	12	F	E	0.4	–
a25	40	F	D	0.15	–
a26	41	M	D	0.5	+
a27	41	M	A	0.5	+
a28	57	F	D	0.5	–
a29	18	F	D	0.1	–
a30	53	M	E	0.2	–
a31	66	F	C	0.1	–
a32	71	F	D	0.4	–
a33	21	M	C	1	+
a34	51	M	E	0.2	+
a35	26	M	C	0.4	+
a36	6	F	C	0.1	+
a37	28	F	C	0.1	+
a38	36	M	C	0.2	+
a39	19	F	A	0.3	+
a40	15	F	B	0.15	+
a41	38	M	E	0.06	+

A, extraction of wisdom tooth; B, jaw deformity; C, fracture of mandible; D, dental implant; E, cyst extraction.

Cells in marrow aspirates were seeded and maintained as described in the Materials and Methods section. When marrow samples from 41 patients were examined, the expansion of BMSCs was observed in some cultures, depending on the patients' age.

Osteogenic differentiation of human alveolar BMSCs in vitro

In most cultures of alveolar BMSC lines (a1–a11) maintained in osteogenic conditions for 21–28 days, the mRNA levels of osteopontin, osteocalcin, bone sialoprotein, and ALP (Fig. 3A), as well as the calcification level estimated

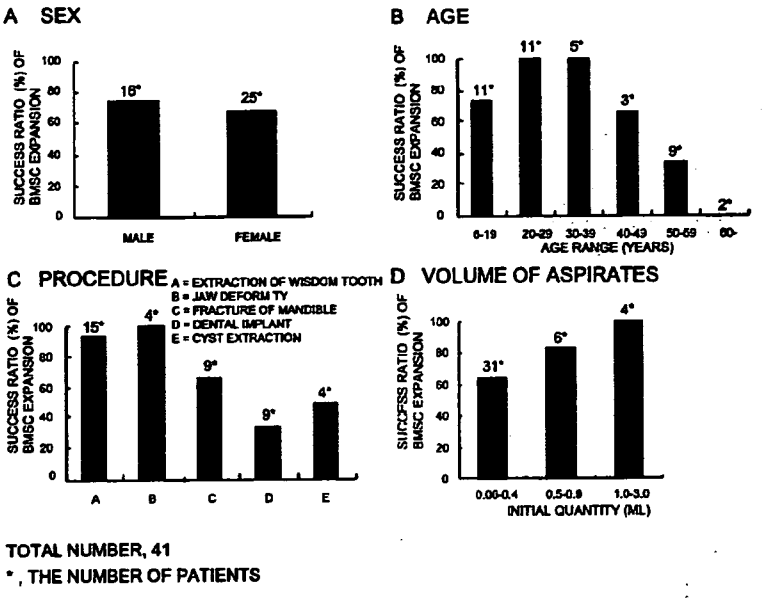

FIG. 1. Effects of sex, age, disease history, and marrow sample volumes on ex vivo expansion of alveolar BMSCs. Cells in marrow aspirates were seeded and maintained as described in the Materials and Methods section. *The number of patients examined.

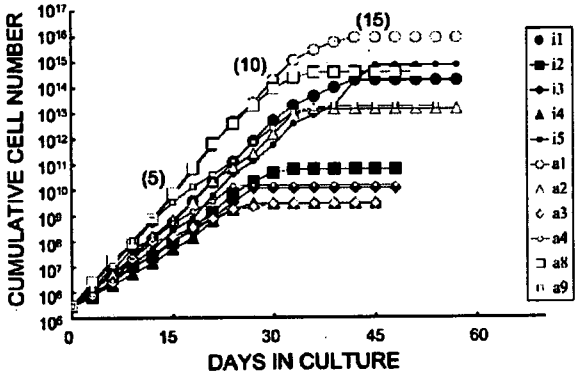

FIG. 2. The growth and proliferative life span of alveolar and iliac BMSCs. Alveolar (a) and iliac BMSC lines (i) obtained from the secondary cultures were seeded and passaged in 10-cm tissue culture dishes at 5000 cells/cm^2. The ages of donors (i1, i2, i3, i4, i5, a1, a2, a3, a4, a8, and a9) were 22, 24, 22, 32, 29, 19, 25, 29, 31, 24, and 19 years, respectively. Passages were performed when cells were approaching confluence. Each point shows the cumulative cell number at the passage. The number of passages is shown in parenthesis.

with alizarin red (Fig. 3B), were higher than those in undifferentiated cultures maintained in medium-A alone. The calcium level in a1–a11 cultures in osteogenic conditions was significantly higher than in undifferentiated cultures (Fig. 3C), but the degree of calcification varied among the lines. Lines a4, a5, and a7 showed the expression of osteopontin and bone sialoprotein at low levels and calcification at high levels on day 28. In contrast, lines a1, a6, and a9 showed expression of osteopontin and bone sialoprotein at moderate or high levels and calcification at low levels on day 28. The latter may represent a delayed calcification process.

Poor chondrogenic and adipogenic potentials of alveolar BMSCs

The differentiation potential of alveolar BMSCs was compared with that of iliac BMSCs using a12–a14 and i1–i3 lines. In the osteogenic conditions, ALP activity and calcium level in alveolar BMSC cultures were similar to those in iliac cultures (Fig. 4A). The chondrogenic potential of alveolar and iliac BMSCs was examined in pellet cultures, because chondrocyte differentiation takes place at high levels in pellet cultures.[17,18] Almost all iliac cells reorganized into a cartilage-like tissue that was stained purple with toluidine blue (Fig. 4B), whereas scarcely any metachromasia was observed with alveolar BMSCs, indicating poor chondrogenesis in the alveolar cell pellets. In the periphery of alveolar cell pellets, a few chondrocytes appeared (Fig. 4D), but the GAG level and ALP activity were lower in alveolar cultures than in iliac cultures (Fig. 4B). Under the adipogenic conditions, most iliac BMSC prosperously accumulated lipid droplets, whereas only a few adipocytes appeared in cultures with alveolar BMSC (Figs. 4C and 4E). Furthermore, alveolar cells showed a lower GAPDH, a marker for adipocytes, than did iliac cells on day 28. Some alveolar BMSC lines showed appreciable adipogenic potential on day 45, and their adipogenic potential was greater than that of fibroblasts (data not shown).

To compare alveolar BMSCs with iliac BMSCs without interindividual variations, we simultaneously isolated alveolar and iliac BMSCs from three beagle dogs. After osteogenic differentiation, ALP and calcium levels in the alveolar BMSC cultures were found to be similar to those in the iliac cultures (Fig. 5). However, under chondrogenic conditions, the alveolar BMSCs synthesized glycosaminoglycan or ALP, and these cells synthesized GAPDH under adipogenic conditions, but only marginally in both cases. In

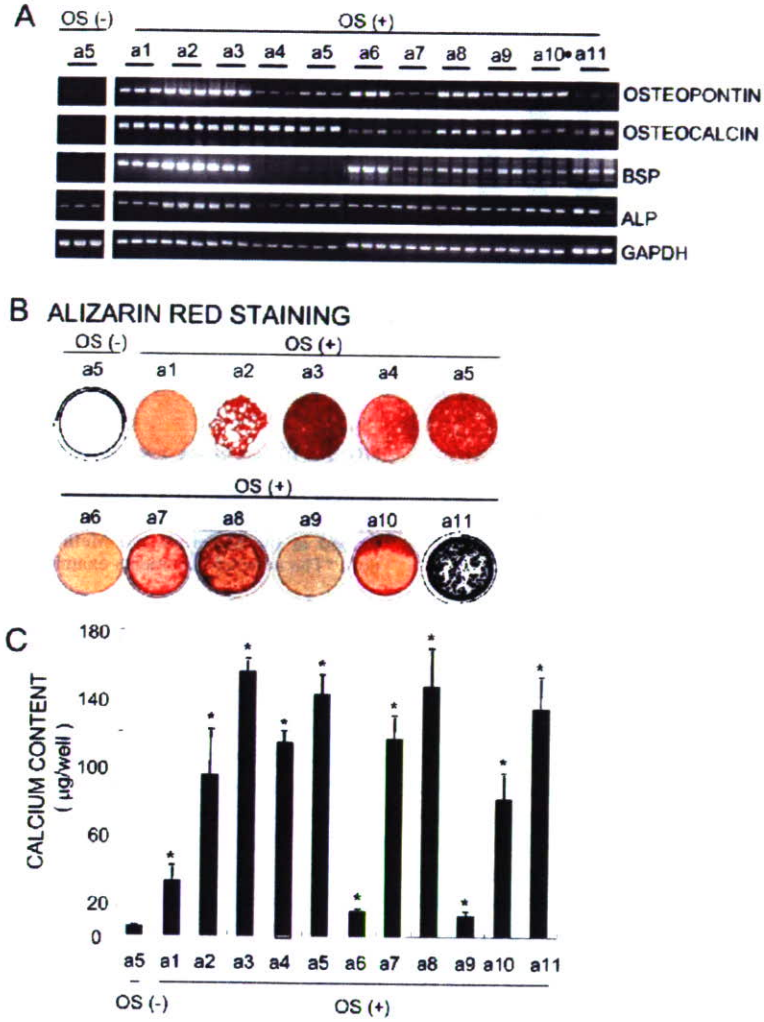

FIG. 3. The osteogenic potential of alveolar BMSC's. Alveolar BMSC's lines (a1–a11) obtained at passage 3 were induced to differentiate into osteoblasts by incubating with the osteogenic medium (OS+) for (B) 21 or (A and C) 28 days. In addition, line a5 was maintained in medium A (OS−) for (B) 21 or (A and C) 28 days. The expressions of osteopontin (OP), osteocalcin (OC), bone sialoprotein (BSP), and ALP mRNA in triplicate cultures was determined by RT-PCR on day 28. (B) Cultures maintained in the osteogenic medium (OS+) or in medium A (OS−) were stained with alizarin red on day 21. (C) The calcium level was determined on day 28. Values are means ± SD for four cultures. $^{*}p < 0.05$.

contrast, the iliac BMSC's underwent either chondrogenic or adipogenic differentiation under these conditions (Fig. 5).

Cell surface antigens

Previous studies have shown that several cell surface antigens, including ALCAM (activated leukocyte cell adhesion molecule), CD29 (integrin β-1), intercellular adhesion molecule (ICAM)-1, platelet-derived growth factor receptor (PDGFR), CD44, CD90, and CD105/SH2, are expressed in BMSC's and/or perichondrium mesenchymal stem cells.[9,19,20] In this study, we examined the expression of 25 cell surface antigens in alveolar and iliac BMSC's by FACS analysis: None of the cell surface antigens examined differed between alveolar and iliac BMSC's (Fig. 6). STRO-1 is a marker for BMSC's.[21] However, alveolar and iliac BMSC's showed STRO-1 expression at a low level (Fig. 6). Previous studies have also shown that STRO-1^{+} cells in undifferentiated human BMSC populations are only 7 ± 6%.[22] Therefore, STRO-1 may be progressively lost with time in these cultures.

Transplantation of alveolar BMSCs

Next we examined whether alveolar BMSC's could differentiate into bone tissue in vivo. Alveolar BMSC's were attached to β-TCP powder and transplanted into SCID mice: 8 weeks after transplantation, new bone formation was observed (Figs. 7B and 7C). In contrast, no bone formation was observed with implants of β-TCP alone (Figs. 7A). Histomorphometrical measurements showed significant differences in bone formation between cell-loaded and unloaded implants: in six transplants (18 fields) with alveolar BMSC's plus β-TCP, bone area was 22 ± 9% compared with 0 ± 0% in four implants (12 fields) with β-TCP alone (Table 2), proving that alveolar BMSC's do indeed enhance initial bone formation.

It was still unknown, however, whether transplanted BMSC's contributed to the bone formation, because host (mouse) cells could have induced bone formation in response to β-TCP. To address this issue, we used mAb against human vimentin that did not cross-react with mouse vimentin. The mAb to human-specific vimentin reacted with osteoblasts and osteocytes around and in new bone

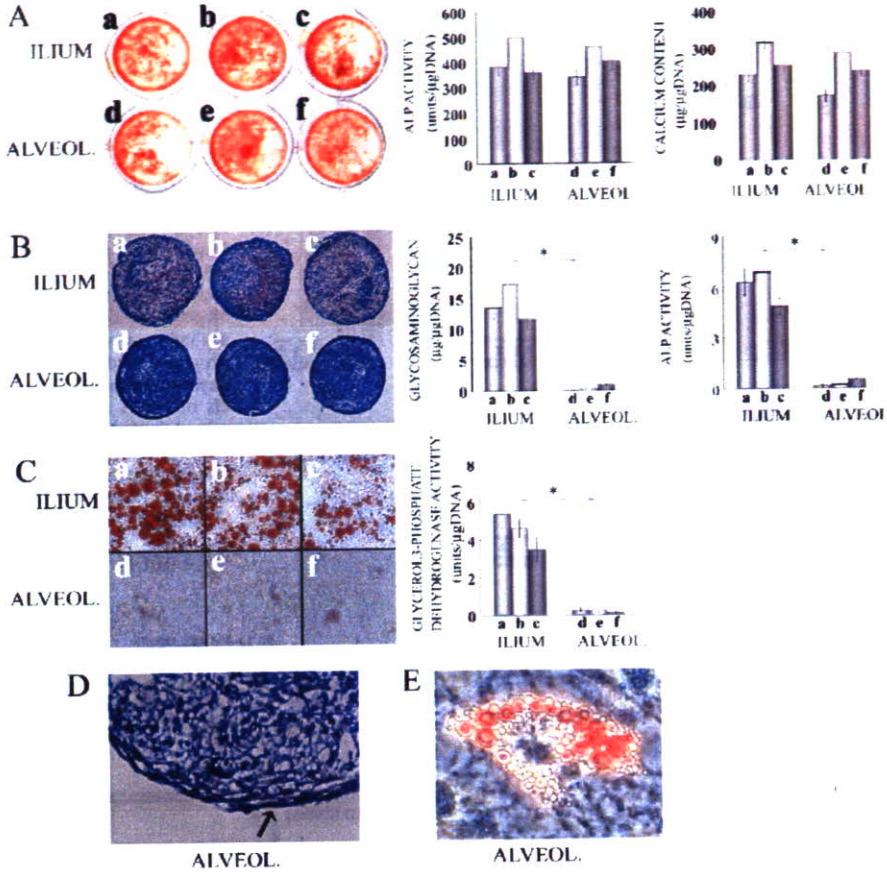

FIG. 4. The differentiation potentials of human alveolar and iliac BMSC's. (A) Human alveolar (a12, a14) and iliac BMSCs (i1, i3) obtained at passage 3 were transferred into the osteogenic medium. The cell layers were stained with alizarin red on day 21. ALP activity and the calcium content of the cell-matrix layers were determined on day 28. (B) Human alveolar and iliac BMSC's obtained at passages 3–4 were incubated in the chondrogenic status in pellet cultures for 28 days and stained with toluidine blue. The levels of GAG and ALP were determined on day 28. In the periphery of the alveolar BMSC pellet, stained with toluidine blue, a few chondrocytes were observed (D). (C) Human alveolar and iliac BMSC's obtained at passage 3 were maintained in the adipogenic status for 28 days. The cells were stained with oil red O. GAPDH activity was determined on day 28. Values are means ± SD for four cultures. $^*p < 0.05$. (E) Magnification of the alveolar BMSC culture maintained in the adipogenic status for 28 days: a few cells became adipocytes in alveolar cultures.

tissues: most osteoblasts and osteocytes, as well as some mesenchymal cells surrounding bone tissue, in the transplants with BMSCs were stained with the human-specific antibody (Figs. 7E and 7F) compared with no human vimentin+ cells in the implants with β-TCP alone (Figs. 7D). Thus, human alveolar BMSC's did in fact reorganize into new bone tissues. Furthermore, osteoblasts, osteocytes, and mesenchymal cells in or near bone tissues in the transplants of alveolar BMSC synthesized osteocalcin (Fig. 8).

To examine whether new bone formation would occur with other alveolar BMSC lines, we examined the expression of human osteocalcin and human GAPDH mRNA, using total RNA isolated from whole transplants. In almost all transplants (11/13) of three alveolar BMSC lines (3/3, 4/6, and 4/4), human osteocalcin and/or human GAPDH mRNA expressions were observed (Fig. 7G), and similar bone formation was observed with human iliac BMSC's (data not shown). In contrast, no human osteocalcin/GAPDH expression was detected in the implants of β-TCP alone, although mouse GAPDH was found in these implants. Unexpectedly, using mouse-specific osteocalcin primers, mouse osteocalcin mRNA was detected in some transplants of human alveolar BMSC's, suggesting that mouse mesenchymal cells partly contributed to bone formation during osteogenic differentiation of human BMSC's. However, no mouse osteocalcin mRNA was detected in the implants of β-TCP alone (Fig. 7G), indicating the absence of bone formation in whole implants of β-TCP alone. These findings indicate that in vivo bone formation in the transplants of alveolar BMSC's was much greater than that in the implant of β-TCP alone.

DISCUSSION

Alveolar BMSC's had the same fibroblastic shape as that reported for BMSC's isolated from the iliac crest, and their

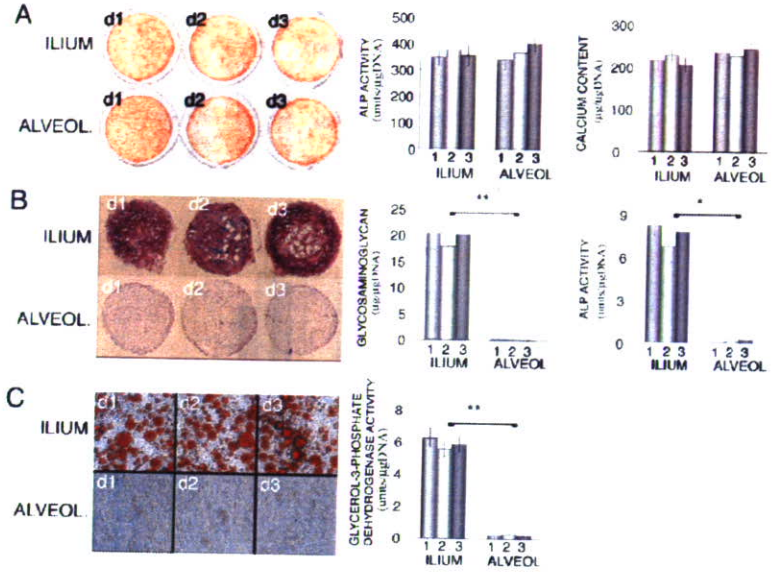

FIG. 5. The differentiation potentials of canine alveolar and iliac BMSCs. (A) Iliac and alveolar BMSCs were isolated from three dogs (d1–d3). Cells obtained from secondary cultures were incubated in the osteogenic status. The cell layers were stained with alizarin red on day 21. ALP activity and the calcium content of the cell-matrix layers were determined on day 28. (B) Iliac and alveolar BMSCs obtained from the secondary cultures were transferred into the chondrogenic medium in pellet cultures for 28 days and stained with toluidine blue. The levels of GAG and ALP activity were determined on day 28. (C) Alveolar and iliac BMSCs obtained from the secondary cultures were maintained in the adipogenic status for 28 days. The cells were stained with oil red O. GAPDH activity was determined. Values are means ± SD for four cultures. $^{*}p < 0.05$; $^{**}p < 0.01$.

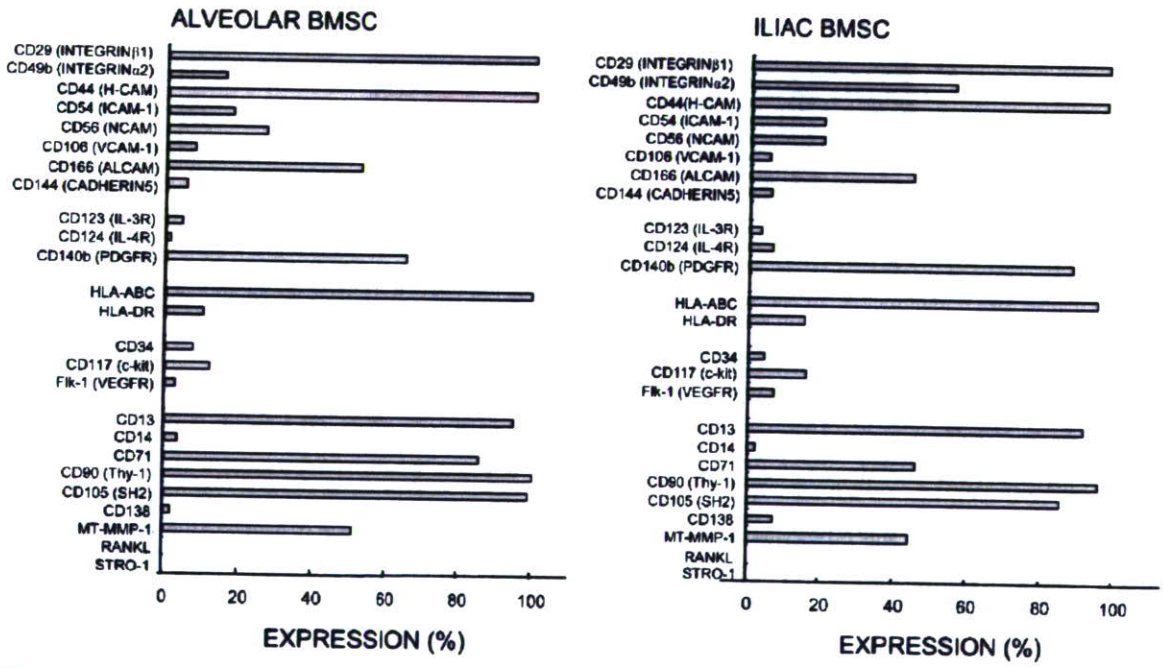

FIG. 6. Cell surface antigen expression by human alveolar and iliac BMSCs. Human alveolar and iliac BMSCs obtained at passage 3 (maintained in OS– medium) reacted with antibodies to cell surface antigens and were analyzed using a FACSCalibur cytometer.

proliferative and osteogenic potentials were similar to those of iliac BMSCs. However, alveolar BMSCs hardly differentiated into chondrocytes, and their adipogenic potential was less than that of iliac BMSCs, at least in the standard differentiation medium: we do not know at present whether other growth factors not included in the medium might enhance the chondrogenic or adipogenic differentiation of these cells. However, we rarely observed cartilage callus formation at the site of a jaw fracture, which may be caused by the poor chondrogenic potential of alveolar BMSCs. The physiological significance of the low adipogenic potential of alveolar BMSCs is not known, but, in any case, we showed here for the first time that topologically different bone marrows contain BMSCs with different features.

Precisely why alveolar and iliac BMSCs have different features remains unknown. BMSCs, like hematopoietic stem cells,[23,24] might have migrated from some bones to others,[20] because it has been suggested that BMSCs mi

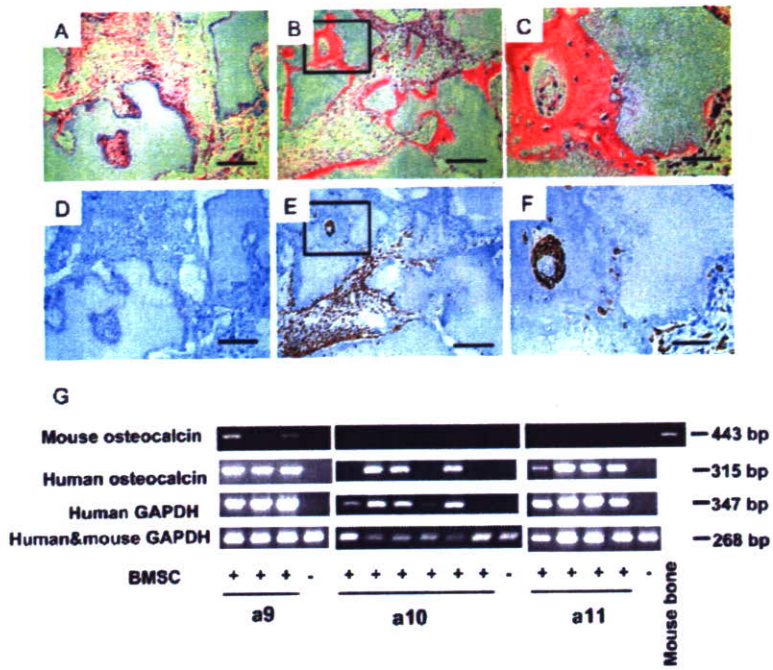

FIG. 7. In vivo differentiation of human alveolar BMSCs after transplantation. Three lines of alveolar BMSCs (a9, a10, a11) obtained at passages 3–5 were used. Implants of (A and D) β-TCP powder alone or (B, C, E, and F) β-TCP + BMSC (a9) were stained with (A–C) H&E or (D–F) human-specific anti-vimentin mAb. (G) RT-PCR analysis of transplants of three alveolar BMSC lines was performed for detection of human cells using human-specific GAPDH and osteocalcin primers and mouse cells using human and mouse GAPDH primers and mouse-specific osteocalcin primers. Bar = (A, B, D, and E) 200 and (C and F) 50 μm.

TABLE 2. BONE AREA IN THE MIDDLE SECTIONS OF TRANSPLANTS WITH ALVEOLAR BMSCS

Implants	*BMSCs*	*Area (%)*	*SD*	
1	-	0	0	
2	-	0	0	
3	-	0	0	
4	-	0	0	
1	+	21.4	7.3	*
2	+	13.7	3.5	*
3	+	16.2	6.6	*
4	+	32.9	6.8	*
5	+	28.7	2.6	*
6	+	19.5	9.9	*

* The areas of bone-like tissue were significantly different ($p < 0.0001$) from those not containing BMSCs.

The values are means ± SD for three fields in the middle sections of the transplants of BMSCs (a9) or b-TCP alone.

grate from bone marrow to injured tissues.[25,26] And BMSC-like cells with osteogenic, adipogenic, and chondrogenic potentials have, in fact, been found in the blood.[27] It is likely that the features of BMSCs are modulated by the components of the adjacent bone marrow, such as growth factors/cytokines and extracellular matrix: different bones may have different marrow components.

In this study, we examined the effects of age, sex, disease history, aspiration site, and volume of aspirate and found a significant correlation between age and success ratio for BMSC expansion. This is a revealing observation, because the decrease in skeletal bone formation and rate of fracture repair observed with aging is reportedly caused by a decrease in numbers of BMSCs and/or their osteogenic capacity or changes in their secretion of cytokines, such as interleukin–11 and insulin-like growth factor binding protein-3.[28,29] However, there have been conflicting reports on the effect of age on human BMSCs. Stenderup et al.[30] reported that there was no age-related difference in the number and proliferative capacity of human osteogenic cells derived from marrow aspirates, and Oreffo et al.[31] found no age-related difference in the colony-forming efficiency of BMSCs or in ALP$^+$ colony-forming efficiency, although they did find a reduction in the size of BMSC colonies with age. On the other hand, Nishida et al.,[32] Mueller and Glowacki,[8] and D'Ippolito et al.[7] showed an age-related decline in the osteogenic potential of BMSCs from, respectively, human iliac crest, femur, and vertebral bone. In this study, we found an age-related, marked decline in the proliferative capacity of alveolar BMSCs. Alveolar BMSCs may be more affected by age than iliac BMSCs. In any case, considering the age-dependence of the proliferative potential of alveolar BMSCs, alveolar BMSCs isolated from young patients during wisdom tooth extraction could be preserved in liquid nitrogen at −196°C for future clinical use.

Transplantation of β-TCP plus alveolar BMSCs induced bone formation in the skin of SCID mice. Furthermore, the alveolar BMSC-derived osteoblasts and osteocytes synthesized osteocalcin adjacent to or in newly formed bone, and all bone tissues formed on the surface of β-TCP powder (Fig. 7 and data not shown), suggesting that the contact between BMSCs and the calcium phosphate scaffold enhanced osteogenic differentiation even in the nonskeletal tissue. Mouse mesenchymal stem cells also contributed to bone formation in the transplants of human BMSCs, perhaps because growth factors released by a large number of transplanted BMSCs enhanced osteogenic differentiation

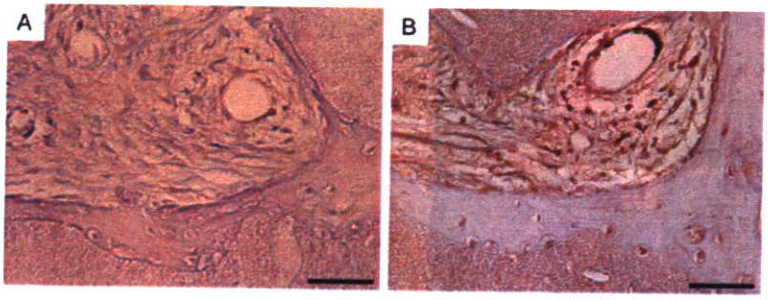

FIG. 8. Immunohistochemical photographs of alveolar BMSC transplants with anti-osteocalcin antibody. Specimens were immunostained (B) with or (A) without primary antibody (anti-osteocalcin). Bar = 50 μm.

of a small number of endogenous mesenchymal cells in vivo. These findings suggest that alveolar BMSCs have a strong osteogenic potential in the presence of appropriate scaffolds and that the number of BMSCs/mesenchymal cells near scaffolds is important for bone formation in vivo.

We feel that the most important significance of this study is the introduction of alveolar BMSCs as a cell source for regenerative medicine, especially because alveolar BMSCs can be isolated with minimal pain to the patient. In addition, these cells have a shown osteogenic potential both in vitro and in vivo. Furthermore, their poor adipogenic potential may decrease unfavorable fat formation during tissue regeneration at the site of transplantation. Transplantation of bone marrow or BMSCs has been shown to promote bone formation in patients with osteogenesis imperfecta or long bone defect,[33,34] and we are now investigating whether transplantation of alveolar BMSCs can promote regeneration of alveolar bone in patients with periodontal disease.

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Transplantation of autologous rabbit BM-derived mesenchymal stromal cells embedded in hyaluronic acid gel sponge into osteochondral defects of the knee

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Background

Mesenchymal stromal cells (MSC) have the potential to differentiate into distinct mesenchymal tissues including cartilage, suggesting that these cells are an attractive cell source for cartilage tissue engineering approaches. Various methods, such as using hyaluronan-based materials, have been employed to improve transplantation for repair. Our objective was to study the effects of autologous transplantation of rabbit MSC with hyaluronic acid gel sponges into full-thickness osteochondral defects of the knee.

Methods

Rabbit BM-derived MSC were cultured and expanded with fibroblast growth factor (FGF). Specimens were harvested at 4 and 12 weeks after implantation, examined histologically for morphologic features, and stained immunohistochemically for type II collagen and CD44.

Results

The regenerated area after autologous transplantation of hyaluronic acid gel sponge loaded with MSC into the osteochondral defect at 12 weeks after surgery showed well-repaired cartilage tissue, resembling the articular cartilage of the surrounding structure, of which the histologic score was significantly better than that of the untreated osteochondral defect. In the regenerated cartilage, type II collagen was found in the pericellular matrix of regenerative chondrocytes, while CD44 expression in the regenerative tissue could not be revealed.

Discussion

These data suggest that the autologous transplantation of MSC embedded in hyaluronan-based material may support chondrogenic differentiation and be useful for osteochondral defect repair.

Keywords

autologous cell transplantation, fibroblast growth factor (FGF), hyaluronic acid gel sponge, mesenchymal stem cells.

Introduction

Full-thickness lesions of articular cartilage are capable of being repaired in young animals [1,2]. In injured joint cartilage, progenitor cells from the BM can proliferate and differentiate within the injured osteochondral defects [3]. Adult cartilaginous tissues contain populations of stem cells that have the capacity for renewal after trauma, disease and aging [4]. However, the capacity of articular cartilage for repair is limited, and frequently is insufficient for full-thickness repair. Then the untreated injuries of articular cartilage do not heal, with inferior tissue-possessing mechanical properties resulting in osteoarthritis of the joint [5–8].

Mesenchymal stromal cells (MSC) are undifferentiated pluripotential cells capable of differentiating into many cell types and may be a suitable autogenous cell source for

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articular cartilage repair [4,9]. MSC obtained from adult human contribute to the regeneration of mesenchymal tissues such as bone, cartilage, fat, muscle, tendon and marrow stroma [4]. MSC thus have been used for re-establishing chondrogenesis by being implanted in osteochondral defects of rabbit [5,6,10]. However, regeneration of cartilage tissue is so far not ideal because the homing of the cell in the required area has not been sufficient. Recently, several studies have approached the problem of articular cartilage repair by providing the repair site with BM-derived progenitor cells combined with biocompatible carrier materials [5,6,11,12].

Among biomaterial carriers as scaffolds onto which cells are seeded are fibrin, polymers of polyglycolic and polylactic acids, alginate, collagen gels and Gelfoam (a purified gelatin sponge), which have been constructed as three-dimensional matrices and tested in different animal models [13–15]. A collagen gel used as the delivery vehicle for the BM-derived progenitor cells led to incomplete integration of the *de novo* differentiated tissue with subsequent deterioration of the repair site [11]. A hyaluronan-based scaffold allows not only the expression of specific extracellular matrix molecules by human chondrocytes grown onto them, but also a down-regulation of degenerative cartilage molecules in chondrocytes [16]. Hyaluronan-based polymers, such as ACP sponge made of cross-linked hyaluronan or HYAFF-11 made of benzylated hyaluronan, have the ability to enhance the natural healing response. Hyaluronan-based polymers placed into defects provide an appropriate scaffolding and favorable microenvironment for the reparative process [11,13,17,18]. Hyaluronan-based scaffolds support the chondrogenic differentiation of BM-derived MSC. A marrow-loaded matrix has been developed for the repair of articular cartilage defects [11,12].

Our previous report showed that MSC can maintain their multilineage differentiation potential even after many cycles of cell division in the presence of fibroblast growth factor-2 (FGF-2) [19]. FGF-2 increased the growth rate and the life span of rabbit MSC in monolayer culture [19]. In the present study, a hyaluronic acid (HA) gel sponge, which was freeze-dried hyaluronic acid gel, was used to fill the cartilaginous region of the defect. The useful effects of this high molecular weight hyaluronan, with an average molecular weight of 1900 kDa, on cartilage degeneration have been investigated in a rabbit model of osteoarthritis [20,21]. This study examined the value of BM-derived MSC expanded *in vitro* in the presence of FGF-2 and HA gel sponge as a scaffold for the repair of osteochondral defects of the articular cartilage of the rabbit. Three months later, MSC expanded in the presence of FGF-2 embedded in the high-molecule HA gel sponge exhibited a significant regenerative effect in histologic assessments.

Methods

Isolation of MSC

MSC were isolated from the ilium of 12–14-week-old Japanese White rabbits (NIBS, Tokyo, Japan), weighing an average of 2.3 kg. All operations were performed under general anesthesia. The rabbits were anesthetized by an intramuscular injection of xylazine (20 mg/kg of body weight) and ketamine hydrochloride (30 mg/kg of body weight). BM with blood of the ilium was aspirated by myelocentesis. After the BM samples (0.5 mL/100-mm dish) were centrifuged to remove heparin, cells were seeded on 100-mm tissue culture dishes and maintained at 37°C under 5% CO_2 in air in 10 mL DMEM supplemented with 10% FBS and antibiotics (medium A). Three days after seeding, floating cells were removed and the medium was replaced by fresh medium A. Thereafter, attached cells were fed with fresh medium A every 3 days. The cultures were divided into two groups: supplementation with or without 1.0 ng FGF-2/mL. Passages were performed twice at 5×10^3 cells/cm^2 in 100-mm dishes when cells were approaching semi-confluence. After two passages of the cells, rabbit MSC were suspended into a freezing medium, Cell banker (Nippon Zenyaku Kogyo Co. Ltd, Fukushima, Japan), frozen and stored at −80°C for 6 months. After 6 months of cryopreservation, cells were recovered and cultured in the same medium as that used before cryopreservation. Once the culture reached semi-confluence, the cells were subjected to autologous transplantation. MSC were harvested with 0.05% trypsin-EDTA and absorbed into HA gel sponge or atelocollagen gel at 1×10^6 cells/100 μL DMEM. These were incubated overnight at 37°C under 5% CO_2 in air in 1 mL DMEM in 16-mm culture dishes before transplantation.

Implant

Two different delivery vehicles were used to fabricate an implant for repair of the full-thickness osteochondral defects. One implant was HA gel sponge. The degradable

hyaluronic acid material used was freeze-dried hyaluronate solution. Hyaluronic acid (an average molecular weight of 1900 kDa) solution was freeze-dried to prepare the HA gel sponge (Denki Kagaku Kogyo KK, Tokyo, Japan). The HA gel sponge was a column (5 mm in diameter) and became soluble 2 weeks after incubation in medium A. The other delivery vehicle was 3% atelocollagen gel (Koken, Tokyo, Japan), used for comparison with the HA gel sponge. To prepare the three-dimensional collagenous material for the delivery vehicle, atelocollagen containing 1×10^6 MSC was incubated in a glass ring 5-mm in diameter at 37°C under 5% CO_2 in air overnight. HA gel sponge materials without MSC underwent the same *in vitro* processing as those loaded with MSC.

Surgical procedure and autologous implantation of MSC on the osteochondral defect

Bilateral surgery was performed under sterile conditions on the 36–38-week-old rabbits, according to the Institutional Animal Care and Use Committee-approved protocol. The rabbits were anesthetized by the same procedure as used for BM harvesting. A medial parapatellar approach was made and the patella was luxated laterally to expose the femoral articular surface of the patellofemoral joint of rabbits. A full-thickness defect of diameter 5 mm and depth 2–3 mm on the articular cartilage of the patellar groove of the distal femur was made with a hand drill. All debris was removed from the defect with a curette. Before transplantation, the joints were rinsed with sterile saline thoroughly. The HA gel sponge, with or without loading of autologous MSC, was randomly implanted into the left or right knee. For comparison with the HA gel sponge, a three-dimensional collagenous material with loading of autologous MSC was implanted. The animals were divided into five groups, as shown in Table 1. In seven rabbits, empty delivery vehicles were used and the animals also underwent sham operations as a negative control (group 1, control). In nine rabbits, the HA gel sponge without loading of MSC was inserted into the defect (group 2, HS). In seven rabbits, the HA gel sponge loaded with autologous MSC grown without FGF-2 was inserted into the defect (group 3, HS + MSC − FGF). In 17 rabbits, the HA gel sponge loaded with autologous MSC grown with FGF-2 was inserted into the defect (group 4, HS + MSC + FGF). For comparison with the HA gel sponge, three-dimensional collagenous material with loading of autologous MSC grown with FGF-2 was implanted in 14 animals (group 5, collagen + MSC + FGF). The patella was repositioned, and the capsule, deep fascia, subcutaneous tissues and skin were then repaired with 4–0 nylon sutures. Postoperatively, all rabbits were allowed to move freely in the cage. Severe infection, requirement of additional medication, wound breakdown or premature death were defined as individual stopping rules [22].

Table 1. Study design

Group	Treatment
1 (control)	Empty delivery vehicles also underwent sham operations as a negative control
2 (HS)	HA gel sponge without loading of MSC was inserted into the defect
3 (HS + MSC − FGF)	HA gel sponge loaded with autologous MSC grown without FGF-2 was inserted into the defect
4 (HS + MSC + FGF)	HA gel sponge loaded with autologous MSC grown with FGF-2 was inserted into the defect
5 (collagen + MSC + FGF)	Three-dimensional collagenous material with loading of autologous MSC grown with FGF-2 was inserted into the defect

Approval for the animal study was obtained from the ethics committee of Gunma University (Maebashi, Japan) before starting the experiment.

Histologic evaluation

The rabbits were killed at 4 and 12 weeks after the operation. Tissue samples of the distal femora were thawed and fixed in 10% buffered paraformaldehyde. After decalcification with buffered 10% EDTA, the samples were dehydrated and embedded in paraffin. Sections were cut sagitally at a thickness of 5 μm and stained with toluidine blue, safranin-O and hematoxylin and eosin. Histomorphologic findings for each section were evaluated according to the histologic grading scale for defective cartilage, described by Wakitani *et al.* [5]. Assessment was performed by a blinded assessor based on the following parameters: cell morphology, matrix-staining, surface regularity, thickness of cartilage and integration of donor with host. The total score ranged from 0 to 14 points, with decreasing points indicating better repair of tissues.

The histologic scores were compared with one-factor analysis of variance (ANOVA) on ranks, followed by Newman-Keuls all-pairwise multiple comparison procedure to identify differences among groups [11]. All statistical tests were performed with the Statmate software package (ATMS Co., Ltd., Tokyo, Japan). Statistical significance corresponded to $P < 0.05$.

Immunohistochemical analyzes

For phenotypic characterization, immunohistologic stains were performed on 5-μm paraffin sections. For immunohistochemistry, serial sections were used for detection of type II collagen and CD44. Sections were deparaffinized with xylene, rehydrated with decreasing solutions of ethanol and rinsed three times with PBS, and pretreated with 1% BSA for 30 min to block any non-specific reaction. Serial sections were used for the detection of type II collagen with a specific Ab. Type II collagen was immunolocalized with a MAb to mouse anti-human collagen type II (1:100; Daiichi Fine Chemical, Toyama, Japan). After incubation overnight with the primary Ab at 4°C, sections were rinsed five times in PBS and then incubated with biotinylated anti-mouse Ig for secondary Ab. They were rinsed three times with PBS, and treated for 60 min with streptavidin solution (Dako, Hamburg, Germany). Visualization was performed with diaminobenzidine and counterstaining with Mayer's hematoxylin. For negative controls, the primary Ab was omitted according to the immunohistochemical protocol.

For immunohistochemical staining of CD44, the sections were incubated with proteinase K (Dako) to expose certain epitopes masked during fixation, followed by a washing procedure [23]. Endogenous peroxidase activity was quenched by treating tissue sections with 3% H_2O_2 for 30 min. After washing, the sections were incubated with 3% BSA in PBS to block non-specific binding. After rinsing three times with PBS, the sections were incubated overnight at 4°C with a 1:200 dilution of a monoclonal mouse anti-rabbit CD44v6 Ab (BMS125; Bender MedSystems, Vienna, Austria). The sections were washed three times with PBS and then treated with anti-mouse IgG for 30 min at room temperature. To visualize Ab binding, after sections were washed three times with PBS, the staining was developed by incubation with aminoethylcarbazole chromogen substrate for 30 min, and the reaction was stopped by rinsing in distilled water [23]. They were rinsed three times with PBS and treated for 60 min with avidin biotin complex solution (VECTASTAIN ABC KIT; Vector Laboratories Inc., Burlingame, CA, USA). Visualization was performed in the same way as that performed for collagen type II staining. For positive controls, sections were made from femoral cartilage slices obtained from the osteoarthritic knee at surgery for total knee replacement.

Results

After three passages of culturing, the MSC grown with FGF-2 became more spindle-shaped than MSC grown without FGF-2. FGF-2 also increased the growth rate of MSC in monolayer cultures. After three passages of culturing, the MSC grown with FGF-2 were approximately 10^2 times as great as without FGF-2. Expanded MSC had *in vitro* differentiation potential: chondrogenic, osteogenic and adipogenic potentials, as evaluated by the protocol of Pittenger *et al.* [4] These results were same as those described previously by Tsutsumi *et al.* [19] (data not shown).

Macroscopic findings

At 4 weeks after surgery, the edges of the defects could be determined in all the knees. Among the groups, few differences were revealed. The representative gross appearance of the repair tissue at 12 weeks after surgery is shown in Figure 1. Debris of either the HA gel sponge or atelocollagen gel material was not observed in the knee joint, and there were no signs of synovitis or infection in the tissues surrounding the implants. For all procedures, the osteochondral defect was filled by repair tissue. The regenerated area of the control group (group 1) was red or dark brown. Irregular tissue with depression was noted and the margin of the defect was clearly differentiated from surrounding normal cartilage (Figure 1A). Grafted tissue of group 2 was smoother and more obvious with pale tissue than that of group 1. At 12 weeks after surgery in the groups containing MSC treated with and without FGF (groups 3, 4 and 5), the regenerated area showed smooth, consistent, glistening white tissue that resembled articular cartilage, although the color incorporation into surrounding normal tissue in group 4 was most evident (Figure 1).

Histologic evaluation

In the control group (group 1), at both 4 and 12 weeks the lesions were covered with connective tissue (Figure 2A and

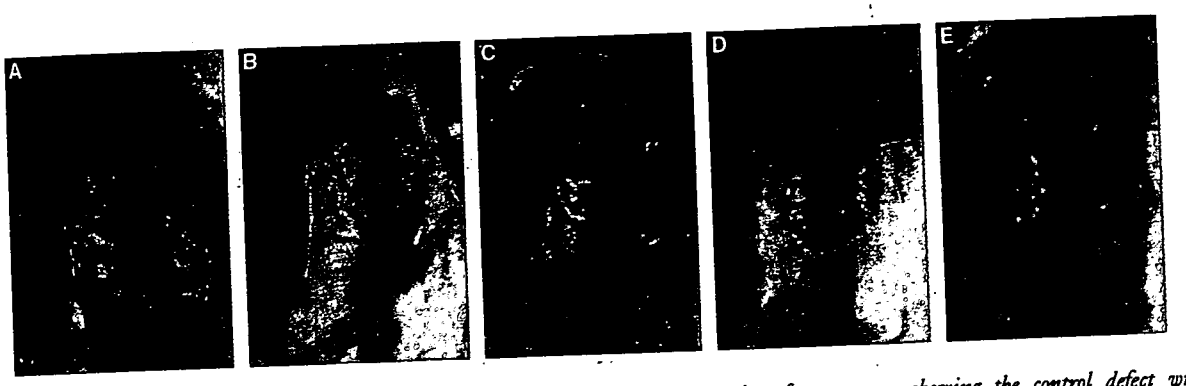

Figure 1. Macroscopic appearance of the rabbit medial femoral condyle at 12 weeks after surgery, showing the control defect without transplantation (A), HA gel sponge alone (B), HA gel sponge loaded with autologous MSC grown in the absence (C) or presence (D) of FGF-2, and atelocollagen gel sponge loaded with autologous MSC grown in the presence of FGF-2 (E).

3A). None of the untreated defects healed completely with hyaline cartilage. Adjacent cartilage tissue showed degenerative changes, including clustering and reduced cellularity at 4 and 12 weeks after surgery. At 12 weeks after surgery, the surface of the regenerative tissue varied from fibrous to fibrocartilagenous tissue (Figure 4A and Figure 5A). There was little metachromatic staining. The subchondral area showed reactive vascular formation, suggesting a regenerative reaction.

In the transplanted groups, HS or collagenous material was replaced with autologous reactive tissues in all rabbits. In contrast to the control group, cartilage-like tissue, similar to adjacent normal cartilage, appeared and replaced the defect. Compared with the surrounding articular cartilage, there were more cells and the arrangement seemed to be more irregular. The adjacent normal cartilage showed little degenerative change, as seen in the control group. The border of the regenerated cartilage

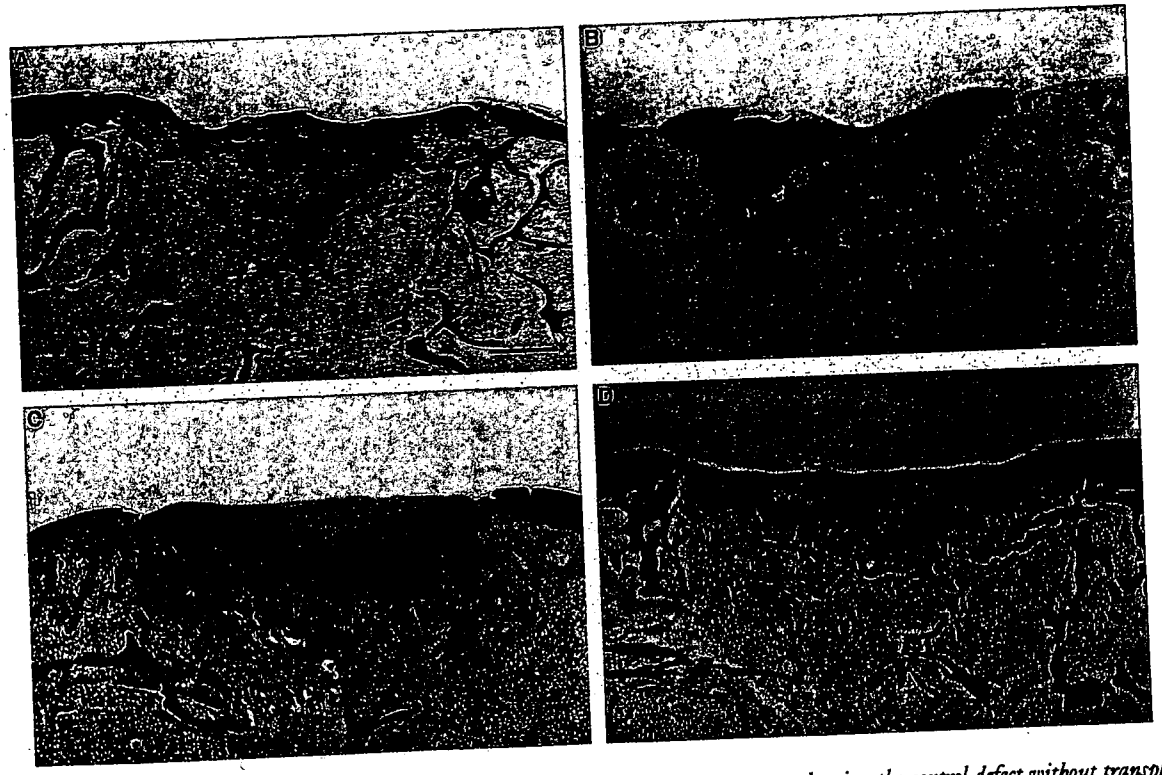

Figure 2. Histologic findings of the articular cartilage of adult rabbits at 4 weeks after surgery showing the control defect without transplantation (A), HA gel sponge alone (B) and HA gel sponge loaded with autologous MSC grown in the absence (C) or presence (D) of FGF-2. Bar indicates 1 mm.

was clearly distinguishable. At 4 weeks, all groups showed the regenerative tissue with a rim of hypertrophic chondrogenic cells (Figure 2B–D) and few characteristic differences were detected among the groups. At 12 weeks, among the four transplanted groups no significant differences were observed qualitatively; they showed more clusters of cartilage occupying the defect,with reduced thickness compared with normal tissue (Figure 3B–D). The defects of groups 3 and 4 at 12 weeks had a rim of chondrogenic cells at the interface with the host tissue. The central portion of the repair tissue had a low density of the cells. The top layer varied, and consisted of either hyaline-like cartilage or fibrocartilage tissues. At a higher magnification, chondroid tissue seemed to be more often observed in the repair tissue when the HS material was loaded with MSC than in repair tissue without MSC loading (Figure 4B–D). The well-repaired cartilage showed a relatively smooth surface without depression in group 4 at 12 weeks. At a higher magnification, the synthesis of extracellular matrix was revealed by toluidine blue and safranin-O (Figure 4D and 5B). Metachromatic staining was distinct in the deep zone of regenerated cartilage, compared with the superficial zone. The subchondral plate was thicker or similar to that of surrounding normal cartilage. Histologic findings in group 5, in which cells were embedded in collagen, seemed to be similar to those observed in group 4, while matrix staining was less evident than group 4.

Results according to the histologic grading scale described by Wakitani *et al.* [5] are shown in Table 2. As shown in Figure 6, there was no significant difference in the total points among groups at 4 weeks. In contrast, at 12 weeks the mean total histologic score was reduced in treated groups. In particular, the score of group 4, which was treated with transplanted HA gel sponge loaded with autologous MSC grown with FGF-2, 4.0 ± 1.4, was significantly improved compared with that of group 1, 8.5 ± 1.3 ($P < 0.05$). This may have been because of the improvement of the category cell morphology, in which there was a significant difference between groups 1 and 4 ($P < 0.01$; Table 2).

Immunohistochemical analyzes

Because the effect of hyaluronic acid transplantation on the regeneration of cartilage tissue was demonstrated 12 weeks after transplantation on histology, immunohisto-

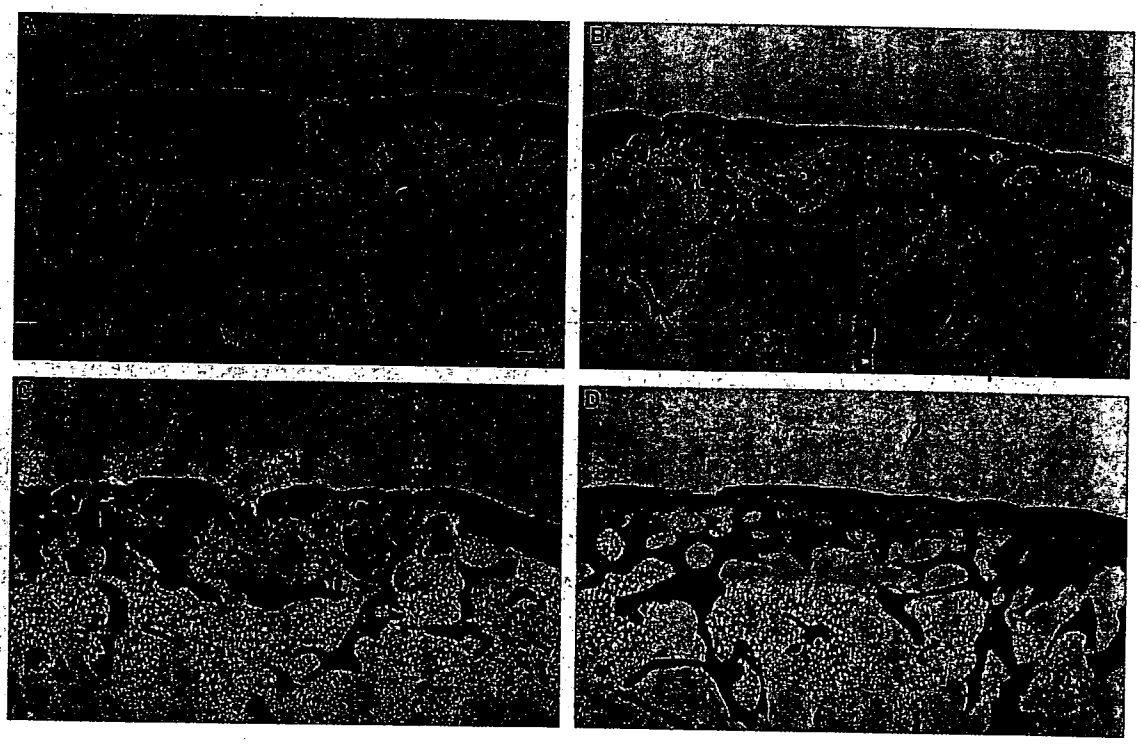

Figure 3. Histologic findings of the articular cartilage of adult rabbits at 12 weeks after surgery showing the control defect without transplantation (A), HA gel sponge alone (B) and HA gel sponge loaded with autologous MSC grown in the absence (C) or presence (D) of FGF-2. Bar indicates 1 mm.

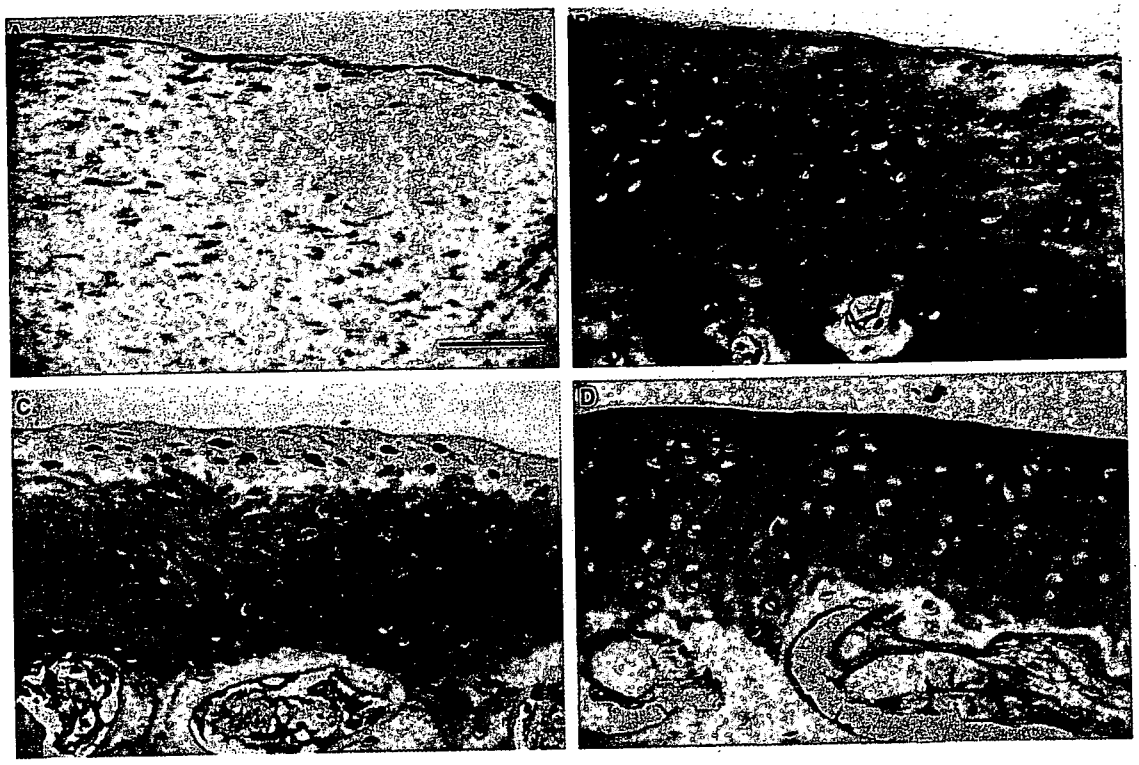

Figure 4. High-magnitude histologic findings of the articular cartilage of adult rabbits at 12 weeks after surgery showing the control defect without transplantation (A), HA gel sponge alone (B) and HA gel sponge loaded with autologous MSC grown in the absence (C) or presence (D) of FGF-2. Bar indicates 100 μm.

chemical analyzes were performed for the tissue specimen obtained at that time. In the regenerative area of the control group, little type II collagen was detected (Figure 7A). In contrast, type II collagen was accumulated in the pericellular and extracellular matrix cartilage in groups 2, 3 and 4, while staining for type II collagen was more intense on chondrocytes and the matrix around lacunae in normal cartilage (Figure 7B). The staining was denser in the matrix than in surrounding articular cartilage.

In order to investigate the role of CD44, a receptor for hyaluronan on the cell surface, in the chondrogenic cells embedded in hyaluronate gel, the expression of the receptor was evaluated by immunohistochemistry. Unexpectedly, in both the control and experimental groups the CD44 protein was not detected (Figure 8B). To examine

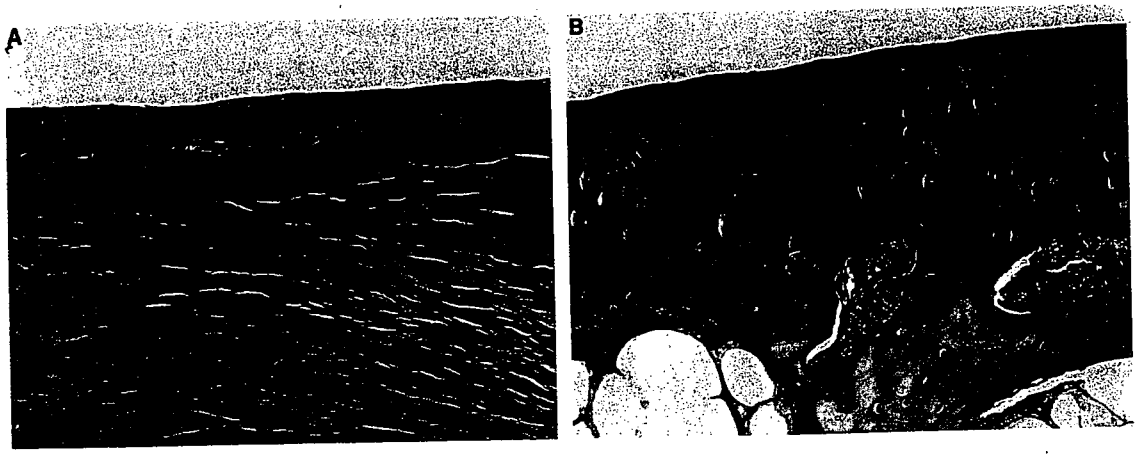

Figure 5. Safranin-O staining of hyaline cartilage of rabbits in the control group (A) and the group with autologous transplantation of HA gel sponge with rabbit MSC treated with FGF-2 (B) at 12 weeks after surgery. Bar indicates 100 μm.

Table 2. Results of the histologic grading scale

Group	Average of score (SD)											
	4 weeks						12 weeks					
	1	2		3		4	1	2		3		4	
Category	($n=3$)	($n=6$)	P^*	($n=3$)	P^*	($n=9$)	P^*	($n=4$)	($n=3$)	P^*	($n=4$)	P^*	($n=8$)	P^*
Cell morphology	2.7 (0.6)	2.3 (0.8)	NS	2.0 (0.0)	NS	2.4 (1.3)	NS	2.8 (0.5)	1.3 (0.6)	NS	1.5 (0.6)	NS	0.9 (0.6)	< 0.01
Matrix staining	2.7 (0.6)	2.0 (0.6)	NS	1.3 (0.6)	NS	2.2 (1.0)	NS	2.0 (0.0)	1.0 (0.0)	NS	1.3 (0.5)	NS	0.5 (0.8)	NS
Surface regularity	1.7 (1.2)	2.3 (0.8)	NS	1.3 (0.6)	NS	1.8 (0.7)	NS	1.0 (0.8)	1.3 (0.6)	NS	1.0 (0.8)	NS	0.8 (0.5)	NS
Thickness of cartilage	1.3 (0.6)	1.7 (0.4)	NS	1.0 (0.0)	NS	1.2 (0.7)	NS	1.8 (0.5)	1.0 (0.0)	NS	0.8 (0.5)	NS	0.9 (0.4)	NS
Integration of donor with host	1.3 (0.6)	1.3 (0.5)	NS	2.0 (0.0)	NS	1.0 (1.0)	NS	1.0 (0.0)	1.7 (0.6)	NS	1.3 (1.0)	NS	1.0 (0.5)	NS
Total	9.7 (2.5)	9.2 (2.5)	NS	7.7 (0.6)	NS	8.7 (3.1)	NS	8.5 (1.3)	6.3 (1.2)	NS	5.8 (1.5)	NS	4.0 (1.4)	< 0.05

NS, no significant difference.
**No probability of significant difference from the control group according to Newman-Keuls all-pairwise multiple comparison procedure.*

Figure 6. Total histologic grading score of repaired tissues at the rabbit knee joint. Asterisk indicates a significant difference according to Newman-Keuls all-pairwise multiple comparison procedure ($P < 0.05$*). Bars indicate SD.*

whether the lack of staining was the result of the absence of the protein or a technical failure, human cartilage tissue obtained from osteoarthritic knee was used as a positive control, because chondrocytes in the osteoarthritic cartilage clearly expressed CD44. Immunohistochemical staining of CD44 showed marked staining of the cell membrane and, in many cases, weaker staining of the cytoplasm (Figure 8A), suggesting that hyaluronan may not induce CD44 expression in the stem cells differentiating chondrocytes embedded in hyaluronic acid.

Discussion

In the present study, full-thickness repair of cartilage tissue of the knee was not sufficient with no treatment for the defect. Transplantation of hyaluronic acid alone did not induce a significant effect on the regeneration of the full-thickness lesion either. Only in rabbits treated with FGF-2-expanded autologous stem cells embedded within hyaluronic acid gel was full-thickness repair of cartilage tissue obtained sufficiently.

The effect of hyaluronic acid alone on regeneration of cartilage tissue as a scaffold was not significantly evident in the histologic scoring system used in the present study. In a previous report, hyaluronic acid-based biomaterials have been used as scaffolds for defects of articular cartilage, and the histologic score was significantly better than that obtained for untreated defects [11]. The reason why hyaluronic acid gel alone did not have a significant effect in the present study is uncertain at present. Possibly differences in the materials of the hyaluronic acid may be related to the insufficient regenerative effect. However, the

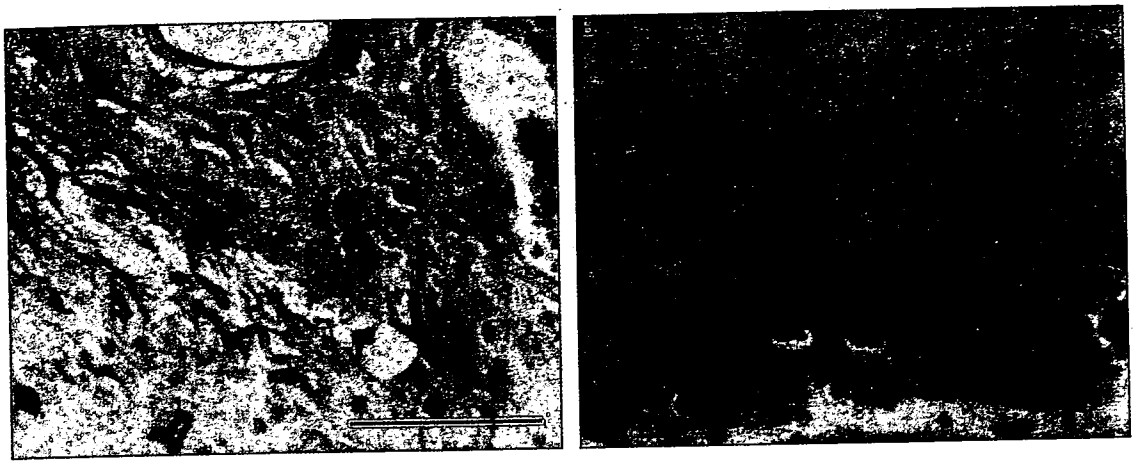

Figure 7. Immunologic localization of type II collagen in repaired tissues in the control group (A) and the group with autologous transplantation of HA gel sponge with rabbit MSC treated with FGF-2 (B) at 12 weeks after the operation. Bar indicates 100 μm.

useful effects of the present high molecular weight hyaluronic acid on cartilage degeneration have been shown in a rabbit osteoarthritis model [20,21]. Alternatively, defect size, which was larger in the present study than the other investigations, may have lead to an insufficiency in the hyaluronic acid-regenerative effect. Shapiro *et al.* [1] described small (three-millimeter-diameter) full-thickness lesions in very young animals as fully capable of repair. In contrast, large defects, with either partial thickness penetrating the cartilage alone or full thickness accessing the subchondral bone, are generally incapable of natural repair. In the present study, the defect size created was larger (5-mm diameter) than previous reports. It is therefore suggested that the capability of hyaluronic acid-assisted regeneration of cartilage tissue may not be enough for sufficient recovery.

In the present study, the regenerated area after autologous transplantation of HA gel sponge loaded with MSC into the osteochondral defect showed effective repair that resembled articular cartilage at the macroscopic and microscopic level. Solchaga *et al.* and Gao *et al.* [11,12] demonstrated that hyaluronic acid-based scaffolds support osteogenic and chondrogenic differentiation of BM-derived MSC; this marrow-loaded matrix was then developed for the repair of articular cartilage defects. Hyaluronic acid-based biomaterials used for cartilage repair allow not only the expression of specific extracellular matrix molecules by human chondrocytes grown onto them, but also a down-regulation of some catabolic factors as matrix metalloproteinases, MMP-1 and MMP-13 and nitric oxide [16,24]. Chondrocyte apoptosis is reduced during the growth of the cells onto the biomaterial

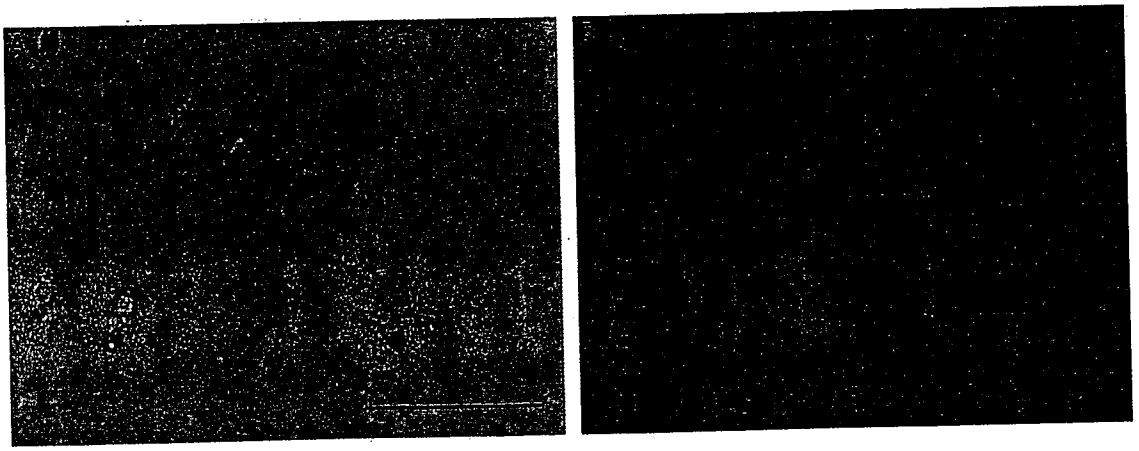

Figure 8. Immunostaining for CD44 in repaired tissues in the control group (A) and the group with autologous transplantation of HA gel sponge with rabbit MSC treated with FGF-2 (B) at 12 weeks after the operation. Bar indicates 100 μm.

[16]. Wakitani *et al.* [5] demonstrated that MSC transplantation with the collagen gel effectively repaired the defect of articular cartilage, in which histologic recovery was dominant in the cell morphology. Similarly in the present study, autologous MSC grown with FGF-2 transplantation with the use of HA gel sponge significantly improved the reparative capability of articular cartilage, as measured by histologic analyzes, especially for cell morphology. It is suggested that the HA sponge may enable MSC to induce an ability to differentiate into cartilaginous tissue and supply appropriate conditions for cartilage regeneration.

CD44 has been identified as the principal cell surface receptor for hyaluronic acid [25]. The hyaluronan receptor CD44 has been implicated in cell adhesion molecules, and serves as the critical link for the retention of hyaluronan–proteoglycan aggregates with the chondrocyte cell surface [26–28]. In several investigations, CD44 was expressed in chondrocytes from normal and osteoarthritic tissue in human cartilage [28]. Tibesku *et al.* [23] reported that chondrocytes in rabbit normal articular cartilage did not express CD44, or expressed it only at a very low percentage, and suggested that CD44 did not play an essential role in normal cartilage. In contrast, Ostergaard *et al.* [27] analyzed human femoral heads and found significantly up-regulated expression of CD44 in the deep zone of osteoarthritis cartilage. In the experiments, over the time–course of osteoarthritis of the rabbit, significantly more chondrocytes expressed CD44, suggesting that the augmentation of CD44 expression is related to the pathogenesis of osteoarthritis [23]. The present results, showing clear expression of the receptor in the cartilage chondrocytes in the osteoarthritic knee, corresponded well to these previous reports. On the other hand, the induction of receptor expression in response to hyaluronic acid in the regenerative cartilage is controversial. Addition of hyaluronic acid in biomaterial favors the adhesion of chondrocytes to their extracellular matrix, and results in a significant increase in $CD44^+$ cell number compared with in the absence of hyaluronic acid [29]. In contrast, however, the findings that the mRNA level of CD44 is unaffected by treatment with hyaluronan oligosaccharides was confirmed by flow cytometry analysis [26]. Grigolo *et al.* [16] reported that chondrocytes grown onto a hyaluronic acid-based scaffold redifferentiate and express their original phenotype, but reduce the production and expression of many factors including CD44, which are involved in cartilage degradation. In the present study, expression of CD44 in the regenerative tissue could not be determined. These results suggest that CD44, possessing a harmful role in regenerative cartilage tissue, may not be associated with communication between regenerative chondrocytes and the surrounding hyaluronic acid. Instead, other receptors such as RHAMM, may be involved in the regenerative process performed by MSC.

In a previous study, we showed the retention of the chondrogenic and osteogenic potentials of FGF-exposed MSC during proliferation *in vitro*. Also, FGF stimulated the growth rate and life span of rabbit and human MSC in monolayer cultures [19]. The velocity of chondrocyte migration was accelerated in the presence of FGF and it was not affected by the addition of hyaluronic acid alone. However, simultaneous administration of hyaluronic acid and FGF showed augmented effects. On the other hand, the velocity of synovial cell migration was enhanced by hyaluronic acid but not by FGF. Hyaluronic acid had a chemokinetic effect on synovial cells and FGF had the same effect on chondrocytes [30]. The results of our studies showed that autologous MSC grown with FGF transplantation with the use of HA gel sponge significantly improved the reparative capability of articular cartilage, as measured by histologic analyzes. It is suggested that FGF may stimulate the MSC motility necessary for osteochondral regeneration occurring at defects filled with hyaluronic acid gel.

In addition to its bionic lubricating function, hyaluronan has various biochemical actions, including anti-inflammation by suppressing proliferation of synovial cells and lymphocytes, synthesis of prostaglandin E_2, and protection of the cartilage matrix from degradation. Thus intra-articular administration of hyaluronic acid is often used clinically for patients with osteoarthritis or rheumatoid arthritis, and the effects are well documented [20]. The present finding that autologous transplantation of rabbit BM-derived MSC with HA gel sponge can effectively regenerate osteochondral defects has merit in cell therapy and regenerative therapy for the restoration of damaged or diseased articular cartilage.

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Ⅱ.臨床に向けた応用

軟骨修復に用いられる間葉系幹細胞の基本的性質

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Key words：MSC, cartilage repair, bone marrow, marker genes, osteocalcin

Basic step

自家/他家のMSC移植による再生医療

骨髄間葉系幹細胞(mesenchymal stem cell；MSC)は，各種の骨髄[1]，骨膜，脂肪組織，滑膜などから分離でき，軟骨，骨，脂肪へ分化する以外に，筋肉，セメント，歯周靱帯[2]，腱にも分化できる。また神経，肝臓実質細胞にも分化できる広い可塑性をもつ。したがって，軟骨欠損以外に，骨欠損，歯周病，心筋梗塞，難治性皮膚潰瘍などの治療に自家MSCが臨床応用されている。さらに動物モデルでは，脳梗塞や腎臓疾患などにも有効であるとされている。

一方，MSCは臓器移植での拒絶反応を抑制する[3]。自家MSC以外に，他家のMSCを再生医療で使用する試みが米国で進行しているのは，MSCが拒絶反応を抑制するためである。他家MSCを用いると，拒絶や感染のリスクはあるが，患者ごとの安全性/品質検査が必要でなくなるのでコスト面で有利である。

MSCの性質はわかっているか？

MSCの移植が骨/軟骨再生に有効であることはよく知られているが，それではMSCがどのような細胞であるかというと明確な定義はない。主として臨床面からMSCの研究が進んできたため，基礎的なMSCの研究が遅れている。

そして，MSCが幹細胞か前駆細胞かについてもいまだに議論がある。幹細胞とは，一般に，

Characterization of transplantable mesenchymal stem cells that are useful for cartilage regeneration

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