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Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells?

Gun-Il Im M.D.*, Yong-Woon Shin M.D. and Kee-Byung Lee M.D.
Hallym University Hospital, Orthopaedics, 896 Pyong-Chon Dong, Anyang, 431-070 Republic of Korea

Summary

Objective: Adipose tissue-derived mesenchymal stem cells (ATMSCs) have been shown to differentiate into bone, cartilage, fat or muscle. However, it is not certain that ATMSCs are equal to bone marrow-derived mesenchymal stem cells (BMMSC) for their bone and cartilage forming potential. The purpose of this study was to answer the question.

Methods: BMMSCs were obtained from the medullary canal of femur and ATMSCs were isolated from the fat harvested during liposuction procedures. After cell expansion in culture media and two passages, the immunofluorescent studies for STRO-1 and CD34 were performed to characterize the BMMSCs and ATMSCs. Osteogenesis was induced on a monolayer culture with osteogenic medium containing dexamethasone, β-glycerophosphate and ascorbate. After 2–3 weeks, alkaline phosphatase (AP) and Von Kossa staining were done. To test for chondrogenesis, mesenchymal stem cells (MSCs) were cultured in a pellet culture and in a fibrin scaffold with a chondrogenic medium (CM) containing transforming growth factor-β_2 and insulin-like growth factor-I. After 4 weeks, Safranin-O staining and immunohistochemical staining for type II collagen were done to evaluate the chondrogenic differentiation and the matrix production. A histological scale was used to semiquantitatively assess the degree of chondrogenesis.

Results: Both BMMSCs and ATMSCs were STRO-1 positive and CD34 negative. On the test of osteogenesis, the osteoblastic differentiation of ATMSCs as demonstrated by AP staining was much less than that of the BMMSCs ($P = 0.002$). The amount of matrix mineralization shown by Von Kossa staining also showed statistical differences between the two MSCs ($P = 0.011$). On the test for chondrogenesis by the pellet culture ATMSCs showed much weaker presentation as chondrogenic cells in both cell morphology and the matrix production. The histological score was 6.5 (SD1.3) for the BMMSCs, and 4.3 (SD1.6) for the ATMSCs cultured in CM, which was statistically significant ($P = 0.023$). The results from fibrin gel paralleled those from the pellet culture in general.

Conclusion: The results of our study suggest that the ATMSCs may have an inferior potential for both osteogenesis and chondrogenesis compared with the BMMSCs, and these cast doubts on the value of adipose tissue as a source of MSCs.

Key words: Mesenchymal stem cell, Osteogenesis, Chondrogenesis, Adipose tissue, Bone marrow.

Introduction

The loss of musculoskeletal tissue creates a major challenge to orthopaedic or plastic surgeons. New techniques involving the implantation of cells and tissue engineered construct are being developed to improve bone and cartilage repair[1–9]. The ideal cell needed for tissue engineering must be immunocompatible and have a self-regenerative potential. Multi-lineage stem cells have both these characteristics, so they possess excellent therapeutic potential for tissue regeneration[1,2,4]. Although the use of embryonic stem cells is appealing because of their pluripotentiality, their practical use is limited due to ethical concerns and government regulations[10]. Mesenchymal stem cells (MSCs) from adults are highly clonogenic cells capable of self-regeneration and multi-lineage differentiation into bone, cartilage, fat, and muscle [1,11–13]. Recent evidences have suggested that stem cells residing in one tissue can contribute to the repair of another because of a previously unrecognized plasticity[13]. Although bone marrow provides the most universal source of MSC, other tissues such as periosteum, muscle, synovial membrane and adipose tissue also appear to possess MSCs[1,14–21]. Adipose tissue is particularly attractive because of its easy accessibility and abundance[20–22].

Adipose tissue-derived mesenchymal stem cells (ATMSCs) obtained from lipoaspirates have been shown to have the multi-lineage potential to differentiate into adipogenic, chondrogenic, myogenic and osteogenic cells[21,23,24]. Even a differentiation into the neurogenic phenotype has been demonstrated as well[20]. ATMSCs mineralized their extracellular matrix (ECM), and increased expression of osteocalcin and alkaline phosphatase (AP)[25]. ATMSCs produced cartilage matrix molecules in alginate culture and also *in vivo* when implanted subcutaneously in nude mouse[26]. However, it is not well established whether ATMSCs have the same potential for osteogenesis and chondrogenesis as bone marrow-derived mesenchymal stem cells (BMMSCs). Thus, the purpose of this study

*Address correspondence and reprint requests to: Dr Gun-Il Im, Hallym University Hospital, Orthopaedics, 896 Pyong-Chon Dong, Anyang, 431-070 Republic of Korea. Tel: 82-31-380-1886; Fax: 82-31-476-1886; E-mail: gunil@hallym.ac.kr
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was to investigate whether ATMSCs are equal to BMMSC in regards to their bone and cartilage forming potential.

Materials and methods

The materials used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

PROCUREMENT OF SAMPLES

Bone marrow samples used for the isolation of MSCs (BMMSCs) were obtained from eight patients (mean age: 65 years, range: 52–73 years) undergoing total hip replacement. The adipose tissue samples used for MSCs (ATMSCs) were isolated from lipoaspirates generated during the elective liposuction procedures for six patients (mean age: 55 years, range: 44–65 years). This study was approved by the institutional review board of our university, and an informed consent was obtained from all the individuals included in the study.

CELL ISOLATION AND CULTIVATION

BMMSCs were isolated from fresh bone marrow samples as described elsewhere[9,11,27,28]. Briefly, bone marrow samples (10 ml) were mixed with 0.3 ml of heparin to prevent coagulation, and then this was diluted with 20 ml of phosphate buffered saline (PBS). The cells were fractionated on a Lymphoprep® density gradient (Axis-Shield, Oslo, Norway), by centrifuging at 600 g for 10 min. The interface mononuclear cells were isolated, and these cells were washed with PBS, and then erythrocyte (RBC) lysis buffer (0.154 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM ethylenediamine tetra-acetic acid (EDTA)) was added to destroy the RBC contaminants. The cells were washed twice by centrifugation (600 g) in PBS. One million cells were seeded in F-12/Dulbecco's Modified Eagle's Medium (DMEM) (Gibco BRL, Green Island, NY, USA) containing 10% fetal calf serum (FCS: Gibco BRL) and 1% antibiotics (penicillin 100 U/ml, streptomycin 0.1 mg/ml, amphotericin B 0.25 mg/ml: Gibco BRL) at 37°C in a humidified atmosphere with 5% CO_2.

The ATMSCs were isolated from lipoaspirate. Briefly, the lipoaspirates were washed three times with PBS and the matrix was digested with 1.5 mg/ml collagenase, and then filtered through a 250-μm nylon mesh. The erythrocytes were removed using erythrocyte lysis buffer. The remaining cells were seeded in culture flasks and maintained under conditions identical to those used for the BMMSCs.

The cells were cultured in DMEM/Ham F-12 supplemented with 10% FCS, 1% antibiotics, and the cultures were maintained at 37°C in a humidified atmosphere and 5% CO_2. After 48 h, the cultures were washed with PBS to remove the nonadherent material. During the expansion period, the medium was replaced twice a week. When the cells had reached 80% of confluence, they were detached from the culture dish using 0.25% trypsin containing 1 mM EDTA (Gibco BRL), and they were washed with PBS, counted and plated again. After culturing through passage 2, the cells were suspended in cryo-preservation medium containing 90% FCS and 10% dimethylsulfoxide. As there were differences in the growth kinetics of two types of MSCs, care was taken to ensure that cells have the same population doubling in each passage.

INVESTIGATION OF CELL MARKER BY IMMUNOFLUORESCENT STAINING

As the isolation of MSCs was solely based on the adherence on plastic surface, immunofluorescent stainings for cell surface antigens were done in order to further characterize the cells[29]. STRO-1, a cell surface antigen used to select for multipotential bone marrow stromal cell, and CD34, a cell surface marker of hematopoietic lineage cells, were investigated. Cells on the passage 3 were plated on eight-well chamber slides on the density of 10^5 cells per chamber, and grown until they reached confluence. They were washed with 0.01 M PBS three times and then fixed with absolute methanol at −20°C for 20 min. They were next washed with 0.01 M PBS three times for 5 min each in a shaker and then incubated with 0.2% Triton X-100 in PBS for 20 min at room temperature; this was followed by incubation with 10% goat serum in PBS for 1 h at room temperature. Primary antibodies of STRO-1 (R&D systems, Minneapolis, MN, USA) and CD34 (Lab Vision-Neomarkers, Fremont, CA, USA) were applied for 1 h at 37°C, and then washed with 0.01 M PBS three times for 5 min each in shaker. FITC-labeled secondary antibody (goat anti-mouse: Zymed, CA, USA) was applied for 1 h at 37°C, then washed with PBS. The slides were then mounted and examined with confocal microscopy. Mononuclear cells isolated from human peripheral blood were used as negative control for STRO-1 and positive control for CD 34.

INDUCTION OF OSTEOGENIC DIFFERENTIATION

The ATMSCs and BMMSCs at passage 3 were analyzed for their capacity to differentiate toward the osteogenic pathway. To induce differentiation, the ATMSCs and BMMSCs were cultured with specific induction media. The osteogenic medium (OM) was composed of DMEM/F-12 solution containing 1% FCS, 0.1 μM dexamethasone, 50 μM ascorbate-2-phosphate, 10 mM β-glycerophosphate, 1% antibiotic/antimycotic. DMEM/F-12 solution containing 1% FCS, 1% antibiotic/antimycotic was used as the control medium for each condition.

The cells were incubated in OM for 2–3 weeks in a 12-well plate at the density of 10^5 cells per well, and they were then examined for osteoblastic differentiation by AP staining, and for the ECM calcification by Von Kossa staining. To detect AP activity, the cells were rinsed with PBS and fixed with citrate–acetone–formaldehyde solution for 30 s at room temperature. The cells were rinsed with distilled water, and then incubated in an alkaline-dye mixture (0.08 mg/ml Naphthol AS-BI phosphate, 0.1 mg/ml Fast blue BB base; Sigma-Aldrich) at room temperature for 15 min. The cells were washed several times with distilled water and counterstained with 0.5% neutral red solution. For Von Kossa staining, the cells were fixed with 4% paraformaldehyde for 1 h at room temperature. The cells were rinsed with distilled water and then overlaid with a 1% (w/v) silver nitrate solution in the absence of light for 30 m. The cells were washed several times with distilled water and then developed under UV light for 1 h. Finally, the cells were counterstained with 0.1% eosin in ethanol. The specimens were then reviewed with a microscope. The percentage of positive cells was obtained with AP staining and the number of mineralization nodules was counted in Von Kossa staining, both by examining 5 high power fields.

INDUCTION OF CHONDROGENIC DIFFERENTIATION

Three-dimensional pellet and fibrin gel culture

For the induction of chondrogenesis in three-dimension, both pellet culture and fibrin gel culture were used. For the pellet culture, passaged MSCs were centrifuged at 500 g for 5 min. Cells were resuspended at a density of 1×10^6 cells/ml in DMEM/F-12 medium supplemented with 1% insulin-transferrin-selenium (ITS) (Gibco BRL), 10^{-7} M dexamethasone, 50 mM ascorbate-2-phosphate, 50 μM L-proline, 1 mM sodium pyruvate. One milliliter of cell suspension was aliquoted into 15 ml polypropylene centrifuge tubes, then spun in a benchtop centrifuge at 500 g for 5 min. Then, 5 ng/ml of transforming growth factor (TGF)-β_2 (R&D Systems), and 100 ng/ml of insulin-like growth factor (IGF)-I (R&D Systems) were added to the medium. This combination constituted chondrogenic medium (CM) and the medium without growth factors was used as the control medium. The tubes were placed in the incubator at 37°C in a humidified atmosphere of 95% air and 5% CO2 for up to 4 weeks. The caps of the tubes were loosened to allow air exchange. Medium was changed every third day. For fibrin gel culture, ATMSC and BMMSC suspension at the passage 3 were recovered and centrifuged. The cell pellets were resuspended in equine fibrinogen (200 mg/ml) to form a mixture containing 1×10^7 cells/ml and 2 TIU/ml of aprotinin. Each fibrin disk was polymerized by combining 0.25 ml calcium activated bovine thrombin with 0.25 ml of the fibrinogen–cell suspension[19]. The resulting three-dimensional fibrin disk contained 100 U of thrombin, 100 mg of fibrinogen, 2.5×10^6 cells and 0.5 TIU of aprotinin in a total culture volume of 0.5 ml. The disk thickness was 4 mm. Disks were covered with 0.5 ml of the same CM as used in the pellet culture. The disks were cultured in the incubator at 37°C in a humidified atmosphere of 95% air and 5% CO2 for up to 4 weeks. Fresh medium was added every third day.

Histological analysis

After 4 weeks of culture, the pellets and the fibrin disks were fixed in 4% formaldehyde solution for 3 h, then dehydrated with 100% ethanol, washed with xylene, and then embedded with paraffin. Sections with a thickness of 4 μm were cut from the paraffin block and coated on the glass slide. Safranin-O staining was performed to assess the degree of proteoglycan synthesis. The tissue sections were deparaffinized with xylen and ethanol. Then 1% aqueous Safranin-O was added for 30 min, and washed with distilled water; 0.2% fast green was added for 3 min, then the sections were washed with distilled water and with serial concentration of 70%, 80%, and 95% ethanol. The immunohistochemical staining for type II collagen was performed to test if the cells function as chondrocytes. The tissue sections were deparaffinized, treated with trypsin for 15 min, then 3% hydrogen peroxide was added to suppress the endogenous peroxidase activity. Polyclonal antibody against type II collagen (Chemicon, Temecula, CA, USA) was allowed to react with the tissue section at the room temperature for 1 h. Then biotinylated anti-mouse antibody (Zymed) was added for 30 min, and then streptavidin peroxidase (Dako, Glostrup, Denmark) was applied. 3-Amino-9-ethylcarbazole was used as chromogenic agent and counterstaining was done with Mayer's hematoxylin. A semiquantitative scoring system modified from that used by Grogan *et al.*[30] was used to evaluate and compare the difference in the degree of chondrogenesis from the pellet culture (Table I). It evaluates the neocartilage in three categories: (1) the uniformity and darkness of Safranin-O stain and immunohistochemical stain for type II collagen; (2) the distance between cells and the amount of matrix produced; (3) the cell morphology represented. Each category has scores ranging from 0 to 3, higher scores indicating satisfactory chondrogenesis. When there is a discrepancy in the Safranin-O and type II collagen staining, their average score was used for the first category. Normal human articular cartilage with subchondral bone from femoral head was used as the control for the staining.

Table I
The semiquantitative histological grading system to evaluate tissue engineered cartilage

Scoring categories	Score
1. Uniformity and darkness of Safranin-O–Fast Green stain and immunohistochemical staining for type II collagen	
No Stain	0
Weak staining of poorly formed matrix	1
Moderately even staining	2
Even dark stain	3
2. Distance between cells/amount of matrix produced	
High cell densities with no matrix in between	0
High cell densities with little matrix in between	1
Moderate cell density with little matrix	2
Low cell density with moderate distance between cells and an extensive matrix	3
3. Cell morphologies represented	
Condensed/necrotic/pycnotic bodies	0
Spindle/fibrous	1
Mixed Spindle/fibrous with rounded chondrogenic morphology	2
Majority rounded/chondrogenic	3
Total score	9

STATISTICAL ASSESSMENT

Statistical analysis was carried out by the Mann–Whitney *U* test. Statistical significance was indicated by *P* values <0.05.

Results

CULTIVATION AND PASSAGING OF HUMAN MESENCHYMAL STEM CELL CULTURES

Nonadherent cells were removed from the dish during medium changes and the subsequent passaging. Typically, 80–90% of confluence was reached by day 14 in BMMSC cultures and by day 10 for the ATMSC culture. Cells at the third passage were used for the experiments.

CHARACTERIZATION OF MSCs BY IMMUNOFLUORESCENCE

The results from passage 3 cells showed that almost all of the BMMSCs and ATMSCs were STRO-1 positive although the intensity of STRO-1 was stronger for the BMMSCs; the signals for CD34 were very faint in both BMMSCs and ATMSCs while the peripheral blood monocytes that were used as the control were very weakly positive for STRO-1 and strongly positive for CD34. This confirmed that the passage 3 cells derived from both bone marrow and adipose tissue had chracteristics of MSCs and

there was not a population of hematopoietic lineage cells (Fig. 1).

DIFFERENCES IN OSTEOGENESIS

Induction of AP activity

After 2 weeks of culture in OM, MSC cultured with OM underwent a change in cellular morphology from spindle-shape to a more polygonal appearance accompanied by a significant increase in AP activity. BMMSCs underwent a much higher increase in AP activity compared with ATMSCs (Fig. 2). After 3 weeks of culture, the AP activity further increased, and the difference between BMMSCs and ATMSCs was still detectable. There were significant differences in the number of positive cells after both 2 weeks and 3 weeks ($P = 0.002$, Table II).

Von Kossa staining

After 2 weeks of culture, mineralization was barely detectable in either the BMMSCs or ATMSCs. After 3 weeks, mineralizing nodules were detectable in both BMMSCs and ATMSCs. The mineralization was more marked in BMMSCs compared with ATMSCs although the difference was less prominent than with the AP staining (Fig. 3). However, there was a statistically significant difference in the number of mineralization nodules ($P = 0.011$, Table II).

DIFFERENCES IN CHONDROGENESIS

On the three-dimensional fibrin culture, the appearances of cells were of similarly round shape although the ATMSCs are more conglomerated (Fig. 4).

The cells after 4 weeks of pellet culture did not have the appearance of chondrocytes although the area of low density with abundant ECM was found in BMMSCs treated with CM [Fig. 5(A, B)]. These appearances were not found in ATMSCs treated with CM [Fig. 5(C, D)]. Safranin-O staining showed distinct proteoglycan production in BMMSCs treated with CM compared to BMMSCs grown in the control medium [Fig. 5(E, F)]. However, the treatment with CM did not lead to such a difference in ATMSCs [Fig. 5(G, H)]. This pattern was quite similarly reproduced in the immunohistochemical staining for type II collagen [Fig. 5(J–M)]. The histological score was 6.5 (SD1.3) for the BMMSCs cultured in CM, and 4.3 (SD1.6) for the ATMSCs treated with CM, which was statistically significant ($P = 0.023$).

In fibrin gel culture, the shape of BMMSCs was round and looked like chondrocytes in lacunae [Fig. 6(A)]. When TGF-β_2 and IGF-I were not given, fewer cells were found and the size of the cells was smaller [Fig. 6(B)]. ATMSCs were found in large number in the scaffold. However, only a small percentage of cells had round shape, located in lacuna-like structure [Fig. 6(C, D)]. BMMSCs cultured in the CM showed a considerable degree of metachromasia, especially at the pericellular area [Fig. 6(E)]. Without TGF-β_2 and IGF-I, the metachromatic staining appeared much fainter [Fig. 6(F)]. ATMSCs had metachromatic staining on the

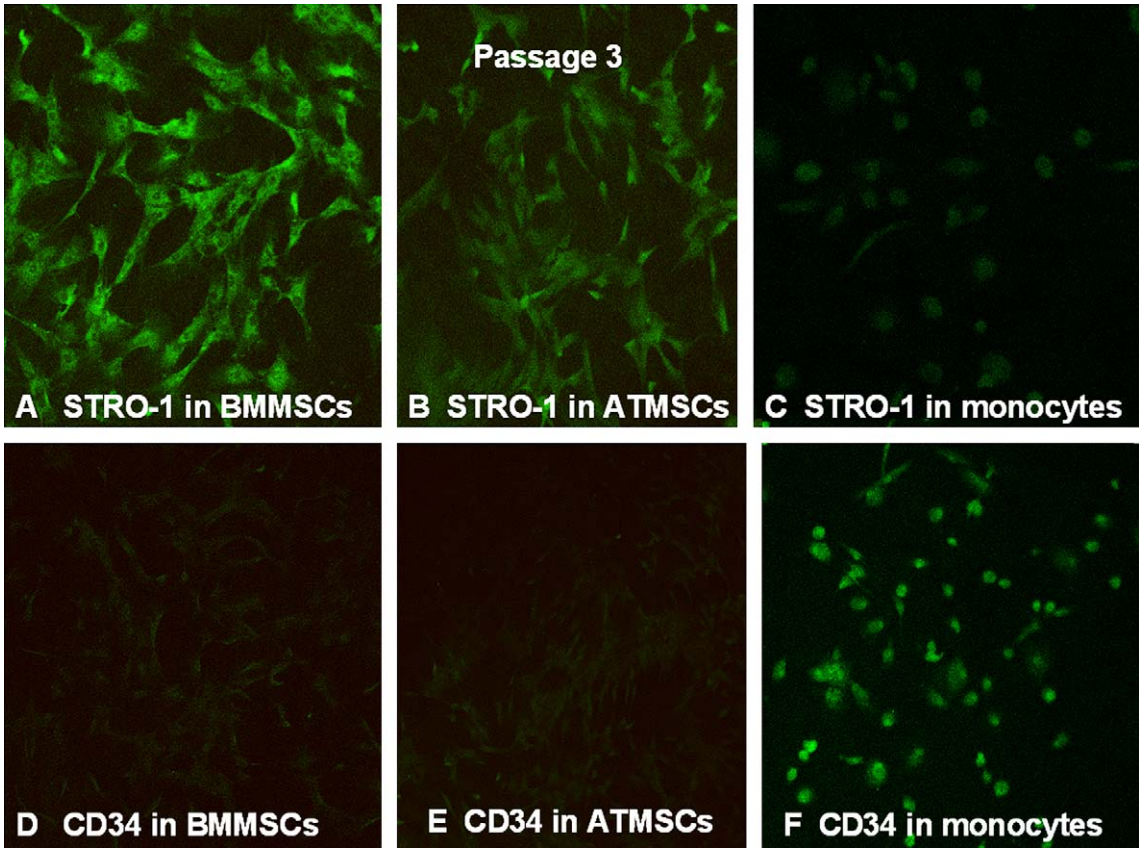

Fig. 1. Immunofluorescent staining for STRO-1 in BMMSCs (A); ATMSCs (B); peripheral blood monocyte (C), and CD34 in BMMSCs (D); ATMSCs (E); peripheral blood monocyte (F).

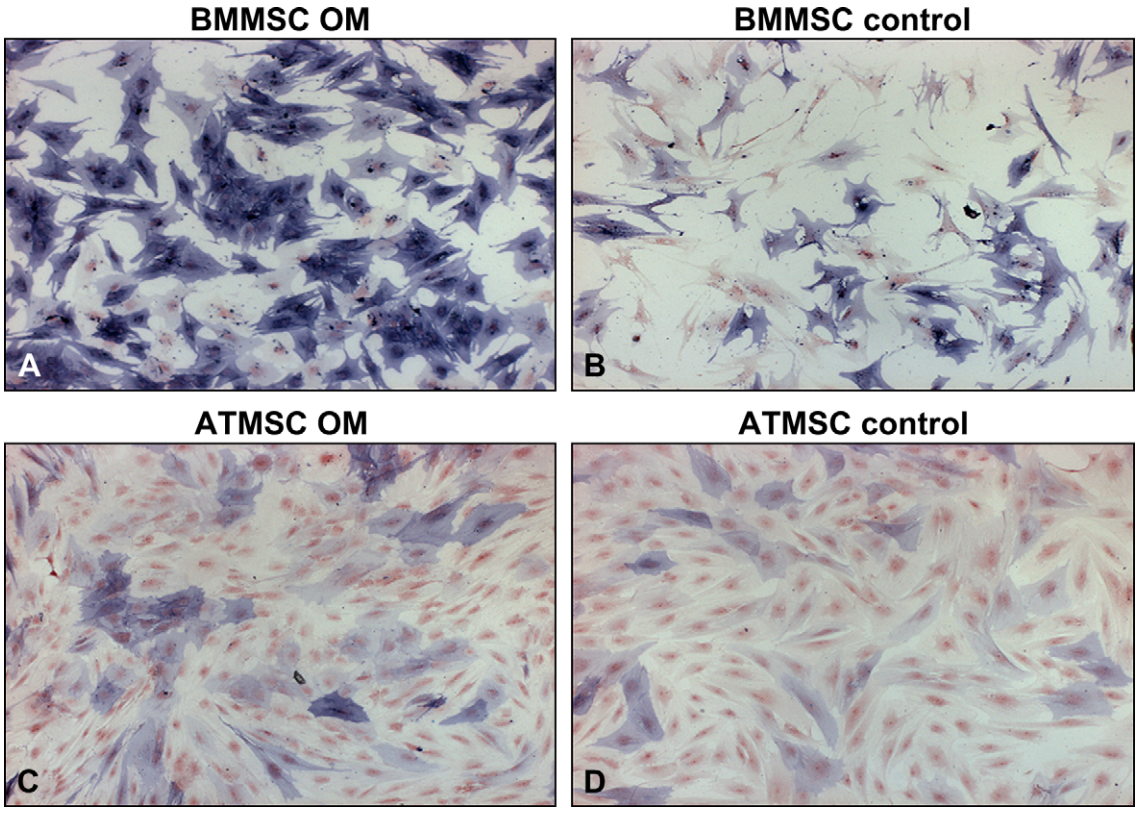

Fig. 2. AP staining of BMMSCs after 2 weeks of culture in OM (A); control medium (B), and ATMSCs after 2 weeks of culture in OM (C); control medium (D).

matrix, but this lacked the strong pericellular staining observed with the BMMSCs [Fig. 6(G)]. Without TGF-β_2 and IGF-I, metachromatic staining of matrix was hardly observed in the ATMSCs [Fig. 6(H)]. In the immunohistochemical staining for type II collagen of the BMMSCs cultured in CM, the expression was generalized throughout the matrix with a stronger pericellular expression [Fig. 6(I)]. Even without TGF-β_2 and IGF-I, some degree of positive staining was observed [Fig. 6(J)]. For the ATMSCs, very weak staining was observed irrespective of growth factor administration [Fig. 6(K, L)].

Discussion

Although bone marrow provides a universal source of MSCs, adipose tissue also possesses abundant and easily accessible MSCs. Recent advances in cosmetic surgery add to its advantage with huge amount of available fatty tissue. It may have a further advantage when the morbidity associated with large volume bone marrow harvests is taken into the consideration[31,32].

Zuk *et al.*[20] first identified that fibroblast-like cell obtained from lipoaspirate could differentiate into adipogenic, chondrogenic, myogenic and osteogenic cells under appropriate medium conditions. However, it was not certain from their study that the ATMSCs have the same capacity for tissue regeneration as BMMSCs. Subsequent studies have suggested that there might be differences in the cellular characteristics and differentiation potential between BMMSCs and ATMSCs[18,33]. CD marker antigens were found to differ between BMMSCs and ATMSCs: CD49d was expressed only in ATMSCs and CD 106 vice versa[20,34]. Wickham *et al.*[33] also showed that ATMSCs isolated from the human patellar fat pad differed from BMMSCs in the cell surface antigen profile. Winter *et al.*[18] demonstrated that though the multi-lineage potentials of BMMSCs and ATMSCs are similar in cell morphology and histology, there were minor differences in the marker gene expression for the diverse differentiation pathways. An additional important finding from their study was that only the BMMSCs responded to a shift to high-density three-dimensional cell culture. However, a recent study by de Ugarte *et al.*[35] that compared cell yield, growth kinetics, cell senescence and multi-lineage differentiation capacity showed no significant difference between the BMMSCs and ATMSCs isolated from the same person.

With these unanswered questions and inadequate informations, the authors tried to compare the osteogenic

Table II
Details of osteogenesis

	Percentage of AP positive cells cultured in OM		Number of mineralization nodules per high power field after 3 weeks of culture in OM
	2 weeks	3 weeks	
BMMSCs ($n = 8$)	85 (2)%	92 (3)%	4.2 (0.9)
ATMSCs ($n = 6$)	28 (4)%	45 (4)%	2.8 (0.6)
P-value	0.002*	0.002*	0.011*

Standard deviations are given in parenthesis. *$P < 0.05$.

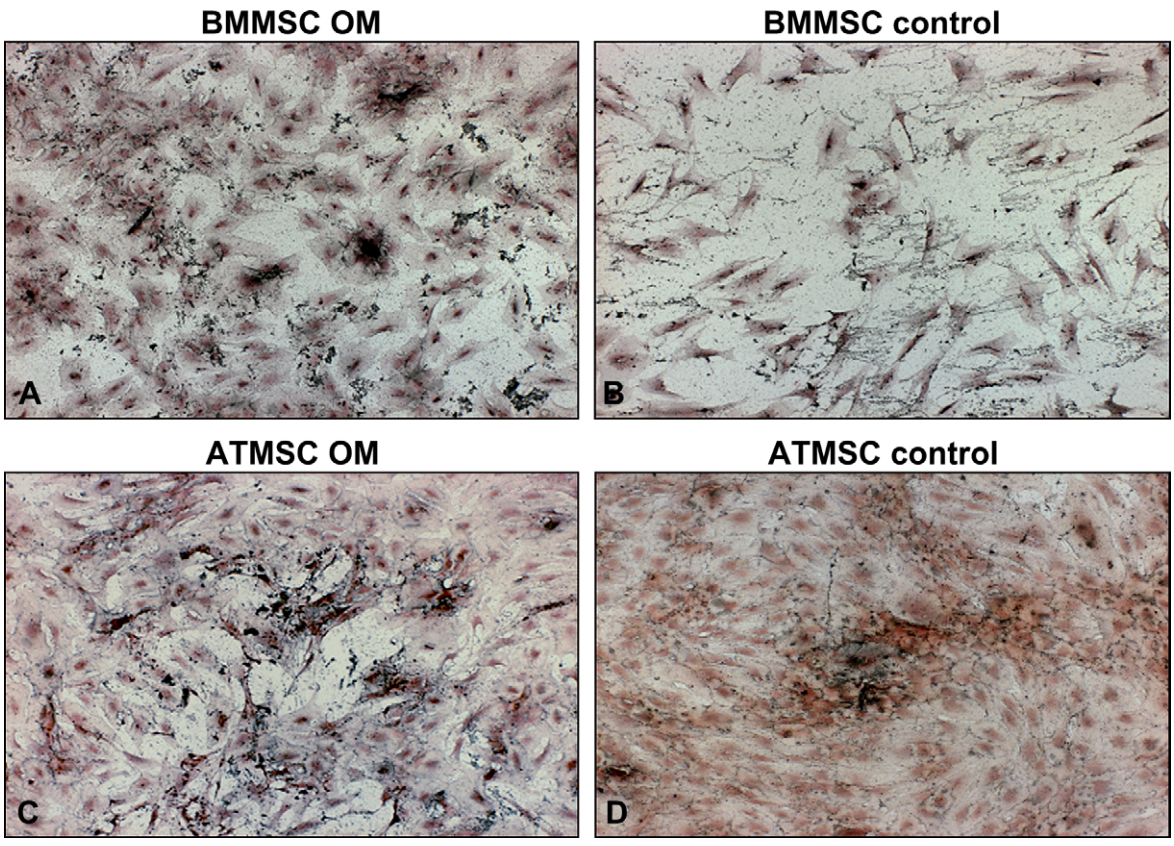

Fig. 3. Von Kossa staining of BMMSCs after 3 weeks of culture in OM medium (A); control medium (B), and ATMSCs after 3 weeks of culture in OM (C); control medium (D).

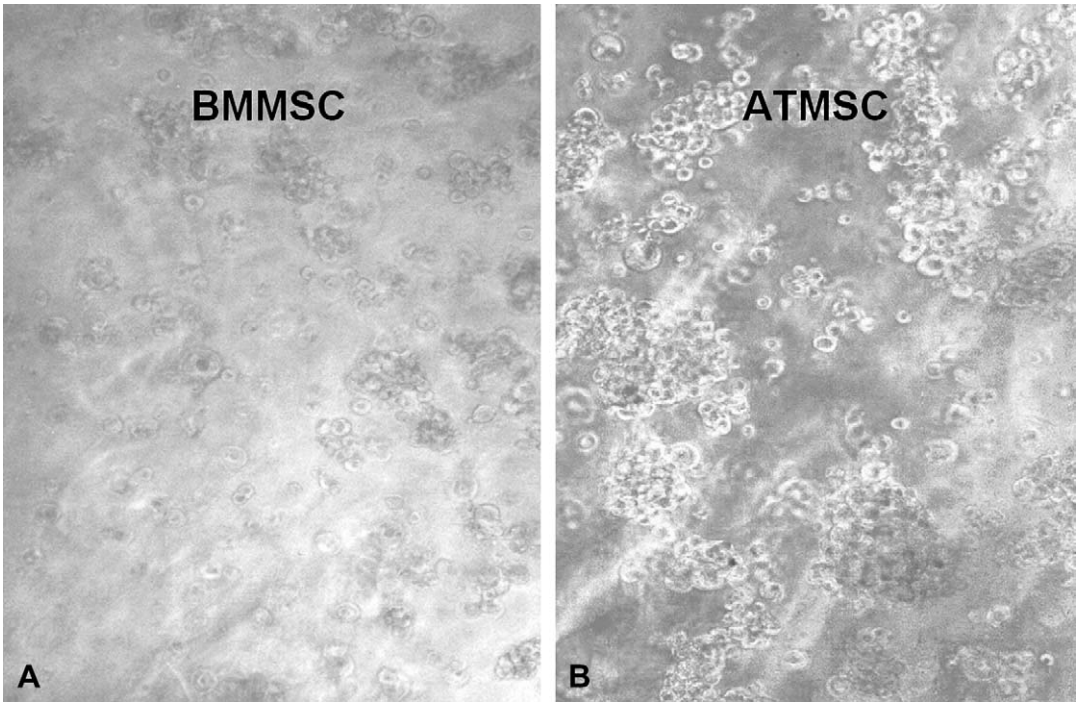

Fig. 4. The appearance of BMMSCs (A) and ATMSCs (B) in the fibrin culture (5 days after seeding).

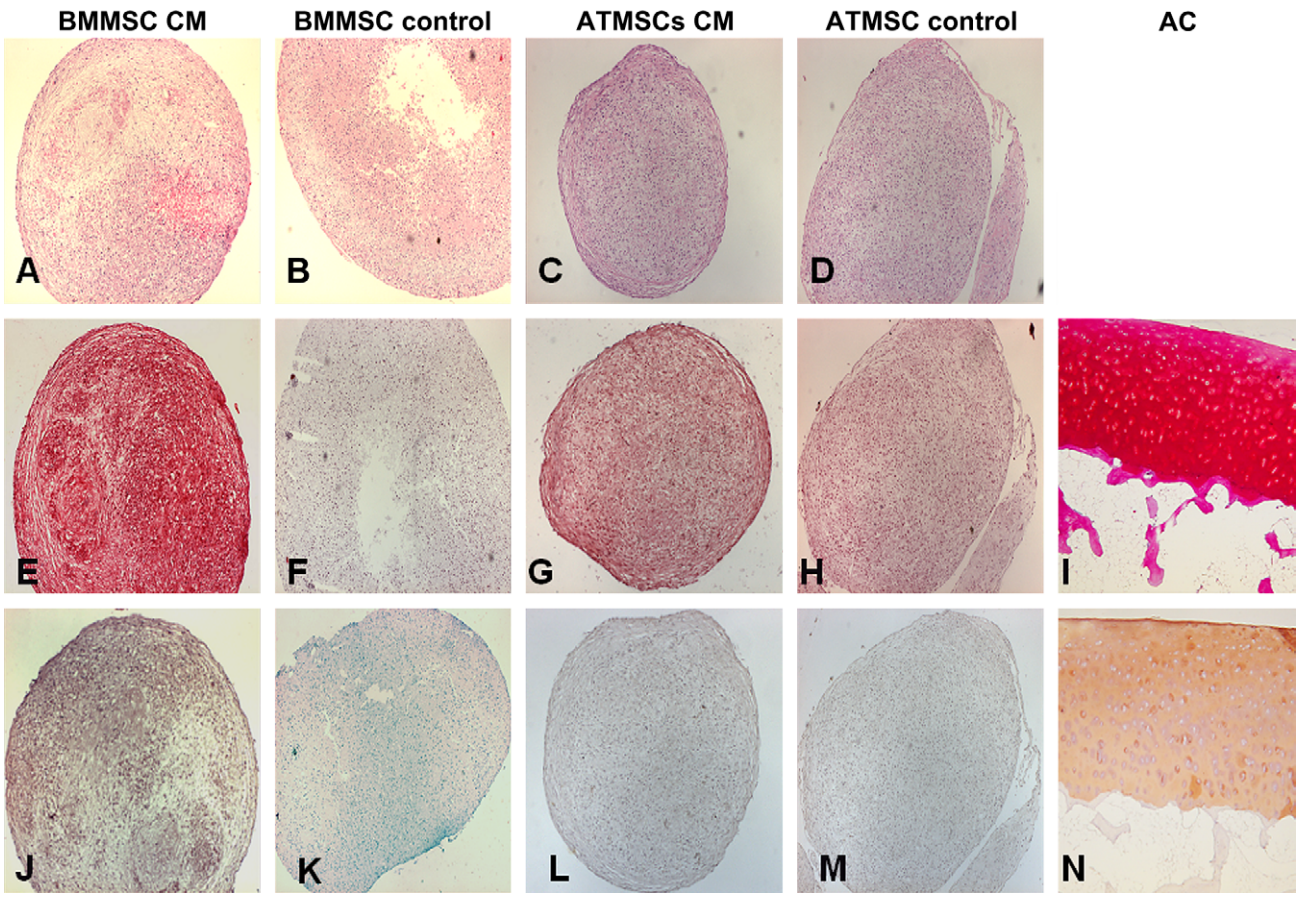

Fig. 5. Chondrogenesis in the pellet culture. Hematoxylin eosin staining of BMMSCs cultured in CM (A); in control medium (B), ATMSCs cultured in CM (C); in control medium (D) after 4 weeks of culture. Safranin-O staining of BMMSCs in CM (E); in control medium (F), ATMSCs cultured in CM (G); in control medium (H). Immunohistochemical staining for type II collagen of BMMSCs in CM (J); in control medium (K), ATMSCs cultured in CM (L); in control medium (M). Normal human articular cartilage and subchondral bone were used as the control for Safranin-O staining (I) and type II collagen staining (N).

and chondrogenic potentials of ATMSCs with those of BMMSCs. The expression of STRO-1 and CD34 by the immunofluorescent study illustrated a qualitatively comparable pattern between BMMSCs and ATMSCs.

On test for osteogenesis *in vitro*, the osteoblastic differentiation of ATMSCs as demonstrated by AP staining was less than that of the BMMSCs. The amount of matrix mineralization shown by Von Kossa staining also showed statistical differences between the two MSCs. It is suggested that ATMSCs will differentiate into osteoblasts under the appropriate conditions but less effectively when compared with the BMMSCs. Our results differ from those of de Urgarte *et al.*[35] who reported no statistically significant changes in AP activity between BMMSCs and ATMSCs. They used passage 4 cells and DMEM medium, whereas we used passage 3 cells and DMEM−F-12 medium. It could be speculated that ATMSCs may require a more defined, different medium for an effective osteogenesis.

On test for chondrogenesis by the pellet culture ATMSCs showed much weaker presentation as chondrogenic cells in both cell morphology and the matrix production.

The scores from the semiquantitative histological grading system accordingly demonstrated a significant difference between the two cells. The fibrin gel culture gave another opportunity to examine the appearance of ATMSCs as compared to BMMSCs. The results in general paralleled those from the pellet culture. One interesting point is that when TGF-β_2 and IGF-I were not added, ATMSCs hardly showed metachromasia whereas BMMSCs had some proteoglycan production without these factors. This may mean that ATMSCs were absolutely dependent on these factors for chondrogenesis while the chondrogenesis per se was even less perfect with the given amount of growth factors as compared with the BMMSCs. The overall results of our study do not corroborate the results of previous studies that suggested equal or comparable capacity of ATMSCs for osteogenesis and chondrogenesis[20,34,35]. Our results are backed up by a recent study by Yoshimura *et al.*[36] that showed that bone marrow- or synovium-derived stem cells had greater chondrogenic and osteogenic potential than adipose tissue-derived stem cells. Another study from the same group demonstrated that cells derived from subcutaneous fat have inferior potential in proliferation and chondrogenesis to those from infrapatellar fat pad[37]. This lends support to the supposition that ATMSCs may have an inferior potential to differentiate into the cartilage. However, the authors' results do not exclude the possibility that a different combination of growth factors may induce a more effective chondrogenesis from ATMSCs.

There are limitations of this study that preclude definite conclusions. First, we had different donors for BMMSCs and ATMSCs. The authors had originally planned to get

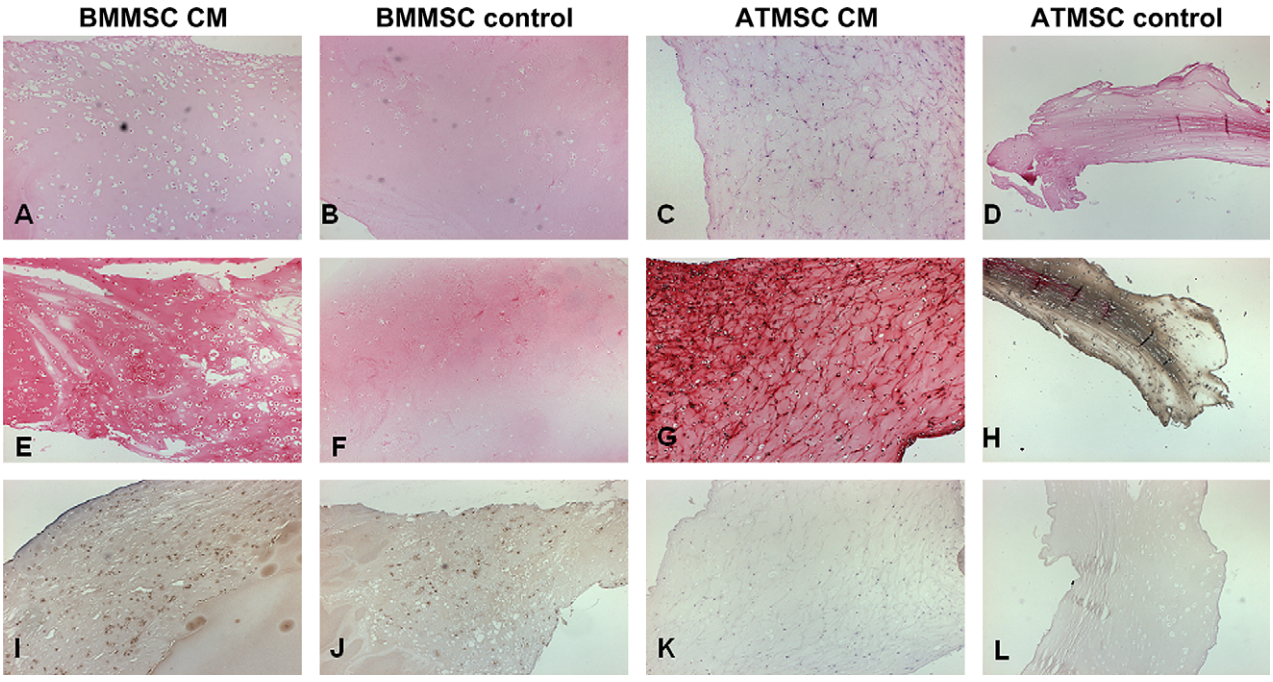

Fig. 6. Chondrogenesis in the fibrin gel culture. Hematoxylin eosin staining of BMMSCs cultured in CM (A); in control medium (B), ATMSCs cultured in CM (C); in control medium (D) after 4 weeks of culture. Safranin-O staining of BMMSCs in CM (E); in control medium (F), ATMSCs cultured in CM (G); in control medium (H). Immunohistochemical staining for type II collagen of BMMSCs in CM (I); in control medium (J), ATMSCs cultured in CM (K); in control medium (L).

both bone marrow and adipose tissue from the patients undergoing total hip arthroplasty. However, we found it difficult to harvest adequate fatty tissue without causing a wound problem. Advanced age is known to affect the proliferation and differentiation potential of MSCs[38–41]. If we had got the BMMSCs from the same age group as ATMSC donors, it would have amplified the overall differences in the results between them. Second, the gene expression profiles were not thoroughly investigated using a quantitative PCR technique. This will be pursued in the following study.

In conclusion, the results of this study show that ATMSCs differ from BMMSCs in the osteogenic and chondrogenic potentials. When equal amount of bioactive factors is given, the ATMSCs have inferior capacity to differentiate into bone or cartilage, suggesting the limited utility of ATMSCs as a source of cells needed for tissue engineering of bone and cartilage. As a further step forward, a search for the culture conditions that would induce a successful osteogenesis or chondrogenesis from ATMSCs is warranted.

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