The Stem Cell Potential and Multipotency of Human Adipose Tissue-Derived Stem Cells Vary by Cell Donor and Are Different from Those of Other Types of Stem Cells

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Key Words

Fat tissue · Adipose tissue-derived stem cells ·
Mesenchymal stem cells · Donor · Cell yield ·
Cell proliferation

Abstract

Human adipose tissue-derived mesenchymal stem cells (AT-MSCs) from various sites are applied in tissue engineering and cell therapy. The condition of AT-MSCs depends on the donor's age, body mass index (BMI), and gender. AT-MSCs from 66 human donors were analyzed, and the cells were sorted according to donor age (10–19 years: n = 1; 20–29 years: n = 5; 30–39 years: n = 12; 40–49 years: n = 22; 50–59 years: n = 12; 60–69 years: n = 9, and 70 years or older: n = 5), BMI (under 25, 25–30, and over 30), and gender (19 males and 48 females). Additionally, AT-MSCs were compared to bone marrow MSCs and chorionic tissue-derived MSCs. We measured the MSC yield, growth rate, colony-forming units, multipotency, and surface antigens. AT-MSC proliferation was greater in cells isolated from individuals aged less than

30 years compared to the proliferation of AT-MSCs from those over 50 years old. BMI was correlated with osteogenic differentiation potency; increased BMI enhanced osteogenesis. Adipogenic differentiation was more strongly induced in cells isolated from donors aged less than 30 years compared to those isolated from other age groups. Also, a BMI above 30 was associated with enhanced adipogenic differ-

Abbreviations used in this paper

AT-MSCs	adipose tissue-derived mesenchymal stem cells
BM	bone marrow
BMI	body mass index
BM-MSCs	bone marrow mesenchymal stem cells
CFU	colony-forming units
CFU-F	colony-forming unit formation
CT-MSCs	chorionic tissue-derived mesenchymal stem cells
DMEM	Dulbecco's modified Eagle's medium
ELISA	enzyme-linked immunosorbent assay
FBS	fetal bovine serum
IRB	institutional review board
MSCs	mesenchymal stem cells
PBS	phosphate-buffered saline
SVF	stromal vascular fraction

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entiation compared to cells isolated from individuals with a BMI below 25. Bone marrow MSCs were strongly induced to differentiate along both osteogenic and adipogenic lineages, whereas AT-MSCs predominantly differentiated into the chondrogenic lineage. Therefore, the type of regeneration required and variations among potential donors must be carefully considered when selecting MSCs for use in applied tissue engineering or cell therapy.

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Introduction

Mesenchymal stem cells (MSCs) have recently been isolated from a variety of tissues and are used in many tissue engineering and cell therapy applications. MSCs are not associated with any ethical issues, and immunological rejection is not a problem when the donor and recipient genotypes are identical [Arrigoni et al., 2009]. MSCs can be obtained from bone marrow (BM), the placenta, and the umbilical cord. However, MSCs can only be obtained at low levels from these sources. In contrast, adipose tissue-derived MSCs (AT-MSCs) are readily available in abundance from the stromal vascular fraction (SVF) of tissue in various bodily locations [De Ugarte et al., 2003; Zhe Jin et al., 2008; Lee et al., 2012]. AT-MSCs possess differentiation abilities equivalent to those of BM-MSCs and exhibit multipotent characteristics such as the ability to undergo osteogenic [de Girolamo et al., 2007], chondrogenic [Lin et al., 2005], adipogenic [Gronthos et al., 2001], and neurogenic [Kingham et al., 2007; Lim et al., 2010] differentiation [Cavallo et al., 2011e]. A recent clinical study found that renal transplantation of MSCs, in an effort to ensure donor hyporesponsiveness, enhanced tissue regeneration. Administration of AT-MSCs admixed with hematopoietic stem cells was more effective than the addition of hematopoietic stem cells alone [Vanikar et al., 2010]. Adipose tissue SVF transplantation has been used to induce bone repair [Varma et al., 2007].

Although AT-MSCs are used in cell therapy and tissue engineering, these cells vary depending on the tissue source. The cell yield, the extent of multipotency, and the cell growth rate may differ by cell source [Fraser et al., 2007; Jurgens et al., 2008]. Human adipose tissue can be obtained from several sites including the abdomen, hip, and thigh. Cells from the latter two regions are obtained in higher yields and have greater differentiation capacities than abdominal fat cells [Jurgens et al., 2008]. Colony-forming unit formation (CFU-F) assay is a popular method used to evaluate stem cell capacity, and CFU-F

results have varied when the MSC stem cell capacity has been tested. It is thought that donor features influence the characteristics of tissue-derived MSCs. Furthermore, AT-MSCs from the same site exhibit differences in cell yield depending on the harvest depth [Fraser et al., 2007] and vary in osteogenic differentiation potential when cultured in 2- or 3-dimensional environments [Leong et al., 2006]. In addition, AT-MSCs isolated from pregnant women exhibit higher proliferation rates [Ng et al., 2009].

Donor variation in the context of AT-MSC stem cell potential, yield, growth rate, and multipotency was studied. The 66 donors varied in terms of gender, age, and body mass index (BMI). In this study, the effects of other factors were minimized, including the experimental environment (i.e. temperature, humidity, and atmospheric and culture process) and differential operator handling when evaluating the cell yield, CFU-F, growth rate, and multipotency (i.e. adipogenic, osteogenic, and chondrogenic differentiation capacities). Additionally, AT-MSC potential and multipotency were compared to those of BM-MSCs and chorionic tissue-derived MSCs (CT-MSCs). Typically, MSCs can be induced into adipogenic, chondrogenic, osteogenic, and neurogenic differentiation. AT-MSCs reportedly exhibit a lower regenerative capacity but a higher differentiation capacity than BM-MSCs [Kingham et al., 2007]. In addition, another report found that both AT-MSCs and BM-MSCs exhibited enhanced osteogenic differentiation [Cavallo et al., 2011e]. Due to these findings, variations among donors were explored and we found that AT-MSC capacities may be influenced by different factors.

Materials and Methods

Collection of AT-, BM-, and CT-MSCs

MSCs from donors were obtained in accordance with Seoul St. Mary's Hospital of Korea Institutional Review Board (IRB) guidelines (IRB permit No. KC11TNSI0186). AT-MSCs were collected from waste adipose tissues obtained during surgery from 66 donors, and donor data were documented. We analyzed AT-MSCs from 66 human donors by donor age (10–19 years: n = 1; 20–29 years: n = 5; 30–39 years: n = 11; 40–49 years: n = 22; 50–59 years: n = 12; 60–69 years: n = 9, and over 70 years: n = 5), BMI (under 25, 25–30, and over 30), and gender (19 males and 48 females). The BMI of 11 patients was excluded due to the absence of height and weight data. Three BM-MSC cell lines (the CMC-MASTER cell line) were purchased from the Catholic Institute of Cell Therapy (CIC; Seoul, Korea). Primary CT-MSCs were obtained from the Seoul St. Mary's Hospital of Korea Department of Obstetrics and Gynecology (n = 5 patients) in accordance with the requirements of the Seoul St. Mary's Hospital of Korea IRB (IRB permit No. KC09WZZZ0173).

Isolation and Culture of AT-MSCs

Adipose tissue was washed 3 times with phosphate-buffered saline (PBS; Wisent Inc., Canada), chopped into small pieces, and digested in PBS with 0.1% (w/v) type I collagenase (Sigma-Aldrich, St. Louis, Mo., USA) for 30 min at 37°C in a Cellticator (Medican, Seoul, Korea). The digested adipose tissue was washed 3 times in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Grand Island, N.Y., USA) with 10% (v/v) fetal bovine serum (FBS; Wisent Inc.) and 1% (v/v) of an antibiotic-antimycotic solution (Gibco-BRL). The sample was centrifuged for 3 min at 350 g. The supernatants were discarded and the cell pellets were suspended in DMEM and passed through a strainer with a 100-µm pore diameter (Becton Dickinson, San Jose, Calif., USA). The filtered cells were centrifuged for 3 min at 350 g and the supernatants were discarded. The cell pellets were suspended in DMEM. The cells were counted using a hemocytometer, seeded in culture dishes at a cell density of 7,000 cells/cm², and incubated at 37°C under 5% (v/v) CO₂. CT-MSCs were cultured in minimum essential medium alpha with 10% (v/v) FBS, 1% (v/v) of an antibiotic-antimycotic solution, and 1% (w/v) GlutaMAX™ (Gibco-BRL). When the cell monolayers reached 80–90% confluence, the cells were subcultured after treatment with trypsin-EDTA (Gibco BRL). Both the cell viability and the growth rate were evaluated at passages 3–5. All of the culture medium was replaced every third day.

Morphology and Growth Identification

To assess the growth rates (doubling times) of MSCs, cells were seeded in 48-well plates at a cell density of 9,000 cells/well and incubated for 3 days at 37°C under 5% (v/v) CO₂ in the appropriate culture media. We measured optical densities using a Cell Count Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) solution mixed with a 10-fold excess of DMEM. The culture medium in the plates was exchanged for this solution, and the MSCs were then incubated for 2 h at 37°C under 5% (v/v) CO₂. Next, the supernatants (100 µl) were transferred into the wells of a fresh 96-well plate and the absorbance values were read at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Spectramax PLUS384; Molecular Devices, Sunnyvale, Calif., USA).

CFU-F Assay

To measure CFUs, MSCs were seeded into 55-cm² culture dishes (1,000 cells) and incubated for 3 weeks at 37°C under 5% (v/v) CO₂ in the appropriate culture media. Next, the culture medium was discarded and the cells were washed 3 times with PBS. The cells were fixed in methanol (Duksan, Ansan, Korea) for 5 min, and the methanol was then removed. To stain fixed MSCs, a solution of 0.5% (w/v) crystal violet (Sigma-Aldrich) was added for 5 min and then washed out with PBS and distilled water. The stained cells were dried at room temperature and colonies were counted. A colony was defined as a cluster of more than 40 cells. The culture medium was replaced every third day.

Multipotency: Osteogenesis, Chondrogenesis, and Adipogenesis

MSCs (passages 3–5) were subjected to osteogenic, adipogenic, and chondrogenic differentiation. Cells (4,000 cells/well) were seeded in 12-well plates and incubated in the appropriate medium at 37°C under 5% (v/v) CO₂. When the cells reached 70% confluence, the culture medium was changed to differentiation medium. The cells were then incubated for 2 weeks at 37°C under 5% (v/v) CO₂. The adipogenic and chondrogenic differentiation media

were from a Stempro Adipogenic Differentiation Kit (Gibco-BRL) and a Stempro Chondrogenic Differentiation Kit (Gibco-BRL), respectively. The osteogenic differentiation medium used was: DMEM supplemented with 10% (v/v) FBS, 1% (v/v) of an antibiotic-antimycotic solution, 0.1 mM dexamethasone (Sigma-Aldrich), 10 mM glycerol-2-phosphate (Sigma-Aldrich), and 37.5 µl/ml ascorbic acid-phosphate (Sigma-Aldrich). The differentiation media was changed every fifth day.

Staining: Adipogenic, Osteogenic, and Chondrogenic Differentiation

Adipogenic differentiation was assessed by Oil Red O staining. Cells were washed with PBS, fixed in absolute propanediol (Sigma-Aldrich) for 6 min at room temperature, and stained with 0.5% (w/v) Oil Red O solution (Sigma-Aldrich) for 1 h at room temperature. After 3 gentle washes with 85% (v/v) propanediol, the cells were treated with absolute propanediol for 1 min and then washed with PBS and distilled water. Stained cells were suspended in PBS and photographed under an Olympus CKX41 microscope (NY Microscope Co., Hicksville, N.Y., USA). To quantify staining, the stain was eluted into 4% (v/v) Nonidet®P-40 (Amresco, Solon, Ohio, USA), and the solutions were diluted with isopropanol prior to measurement of absorbance at 520 nm using an ELISA reader.

To measure osteogenic differentiation, the extent of calcium deposition was assessed by staining with Alizarin Red S. Cultured cells were washed twice with PBS and fixed in 10% (v/v) formalin (Sigma-Aldrich) for 10 min at room temperature. After washing, cells were treated with 2% (w/v) Alizarin Red S (Sigma-Aldrich) for 10 min, suspended in PBS, and photographed under an Olympus CKX41 microscope. To quantify staining, the stain was eluted into a solution of 10% (w/v) cetylpyridinium chloride (Sigma-Aldrich). Absorbance was read at 550 nm using an ELISA reader.

Chondrogenic differentiation was analyzed using Alcian Blue staining. Cultured cells were washed with distilled water and fixed in 0.1 M HCl for 5 min at room temperature. A 1% (w/v) solution of Alcian Blue (Sigma-Aldrich) was added and the cells were incubated for 24 h in the dark. The cells were then treated with 0.1 M HCl (Daejung, Busan, Korea) for 5 min and washed with PBS. Cells were next treated with a 0.1% (w/v) solution of Nuclear Fast Red (Sigma-Aldrich) for 3 min and washed with PBS. The stained cells were suspended in PBS and photographed under an Olympus CKX41 microscope. To quantify staining, the stain was eluted into a solution of 6 M guanidine hydrochloride (Sigma-Aldrich), and the absorbance was read at 620 nm using an ELISA reader.

Cell Membrane Antigen Identification: Flow Cytometry

MSCs (passage 4) were subjected to flow cytometric analysis. The cells were trypsinized and resuspended in Hanks' balanced salt solution (Wisent Inc.) with 2% (v/v) FBS. The cells were next incubated with monoclonal fluorescein isothiocyanate-conjugated antibodies against CD90, CD13, human lymphocyte antigen class II (HLA-DR; Becton Dickinson), and CD105 (AbD Serotec, Oxford, UK) and PE-conjugated antibodies against CD34, CD73, CD45, and CD146 (Becton Dickinson) for 30 min on ice in the dark. Cells stained with anti-isotype IgG1 served as a negative control. After washing with Hanks' balanced salt solution, the cells were fixed in 0.5% (w/v) paraformaldehyde (Sigma-Aldrich) and analyzed via flow cytometry (Becton Dickinson). Cell surface markers were detected and quantitated using Summit software, version 5.2 (Beckman Coulter, Brea, Calif., USA).

Table 1. AT-MSCs from 66 patients

No.	Gender	Age, years	Weight, kg	Height, cm	No.	Gender	Age, years	Weight, kg	Height, cm
1	F	52	59.02	150.9	35	F	46	58	154
2	F	57	55	153	36	F	63	64	163
3	F	47	51	155	37	F	62	47	150
4	F	44	63.8	156.1	38	F	47	74.5	155
5	M	22	95	164	39	F	47	77	155.1
6	F	55	68	160	40	F	38	101	183
7	F	30	109.6	164	41	F	52	58	160
8	F	59	49	159	42	M	72	46	161
9	F	38	66.2	156.2	43	M	55	71	N/A
10	F	38	101	183	44	F	31	55	N/A
11	F	48	56.3	157.4	45	F	49	61.5	163.3
12	F	25	37.5	157	46	F	41	73	164
13	F	61	42	158	47	F	41	73	164
14	M	26	N/A	N/A	48	F	41	73	164
15	F	36	54.4	155	49	F	25	62	N/A
16	F	54	51.25	158.7	50	F	67	67	N/A
17	F	67	53	149	51	M	72	49	160
18	M	39	N/A	N/A	52	M	42	49	160
19	F	67	53	149	53	M	72	49	160
20	F	41	82.8	172.5	54	M	72	49	160
21	F	36	57.25	150.9	55	F	45	71	155
22	F	39	60.2	165.4	56	M	45	71	N/A
23	M	61	65	164.5	57	F	37	54	N/A
24	M	49	89.4	167.9	58	M	55	71	N/A
25	F	64	61	161	59	M	55	71	N/A
26	F	43	72	162	60	F	39	53	158
27	F	40	52.55	157.4	61	F	43	70	163
28	M	18	76	184	62	F	57	66	160
29	F	22	52	158	63	M	47	70	168
30	F	43	57	N/A	64	F	52	62	163
31	M	72	49	160	65	M	37	81	177
32	F	68	55	160	66	F	56	54.3	161.9
33	M	40	63	173					
34	F	44	53	155					

M = Male; F = female; N/A = not available.

Results

Interindividual Variation in AT-MSCs

Adipose tissues were collected from 66 donors categorized in terms of BMI, gender, and age (table 1). The BMI of 11 patients was excluded due to the absence of the patients' height and weight. Thus, the BMI was analyzed in 55 donors.

MSC Yield from Adipose Tissue

AT-MSCs were isolated from adipose tissue obtained from various sites on the 66 donors. The MSC yield was much greater for donors aged less than 30 years compared to older donors; the yield gradually fell

as the donor age rose. Patients aged 10–29 years yielded twice the number of cells compared to those aged 50–59 years ($p < 0.0001$; fig. 1a). The yield was not affected by gender or BMI (fig. 1b, c). Patients with a BMI below 25 yielded more cells than those with a BMI of 25–29 ($p = 0.05$).

Proliferation of MSCs from Adipose Tissue

We examined AT-MSC proliferation by donor age, gender, and BMI. Cells from donors aged less than 30 years proliferated readily and those from donors over 50 years of age proliferated slowly ($p < 0.0001$; fig. 2b). The proliferation rates of primary cells and those of cells at all passages up to passage 4 were similar (fig. 2a). AT-MSCs

from males proliferated more rapidly than those from females ($p < 0.05$; fig. 2c). The BMI was not associated with the proliferation rate (fig. 2b).

CFU-F Assay

AT-MSC self-renewal ability was evaluated using a CFU-F assay and did not vary by age, gender, or BMI (fig. 3a–c).

Cell Membrane Antigens of AT-MSCs

AT-MSCs were positive for stem cell markers (CD13, CD73, CD90, and CD105). Conversely, AT-MSCs were negative for hematopoietic markers (CD34 and CD35) and an immune cell marker (HLA-DR). Surface marker expression levels did not vary by donor age, gender, or BMI (fig. 4a–c). Positive markers were expressed in at least 80% of cells, and negative markers were expressed in less than 6% of cells.

Multipotency of AT-MSCs: Osteogenic and Adipogenic Differentiation

AT-MSCs were evaluated in terms of their capacity to exhibit osteogenic and adipogenic differentiation. The extent of osteogenic differentiation fell somewhat as the donor age rose, but this trend did not reach statistical significance (fig. 5a). Osteogenic differentiation was not affected by donor gender (fig. 5b). However, an increased BMI was significantly associated with a rise in the extent of osteogenic differentiation ($p < 0.005$; fig. 5c). Adipogenic differentiation was more strongly induced in cells from donors under rather than over 30 years of age (fig. 6a). Additionally, a BMI over 30 was associated with a higher level of adipogenic differentiation than that noted in those with a BMI below 25 (fig. 6c). Gender did not affect the extent of adipogenic differentiation (fig. 6b).

Multipotency of AT-, BM-, and CT-MSCs

The multipotencies of various types of MSCs, including AT-, BM-, and CT-MSCs, were evaluated in terms of osteogenic, chondrogenic, and adipogenic differentiation (fig. 7a). BM-MSCs could be strongly induced to differentiate into the osteogenic and adipogenic lineage, and AT-MSCs could be induced to differentiate into the chondrogenic lineage. CT-MSCs appeared to differentiate less readily than AT- and BM-MSCs (fig. 7b–d).

Fig. 1. Human AT-MSCs were isolated from adipose tissue obtained from 66 donors. **a** The AT-MSC yield of donors less than 30 years of age ($p < 0.0001$) was considerably greater. **b** The AT-MSC yield did not differ by gender. **c** The AT-MSC yield was increased for donors with a BMI >25 but <29 .

Fig. 2. AT-MSC proliferation doubling time. **a** The doubling time decreased with each passage (0–4). **b** The doubling time increased with donor age. AT-MSCs isolated from donors less than 30 years of age had an increased proliferation rate compared to those from

donors over 50 years of age ($p < 0.0001$). **c** The AT-MSC doubling time did not differ by donor gender. **d** The AT-MSC doubling time not differ by donor BMI.

Discussion

Adipose tissue is abundant and easy to acquire; MSCs therein can be used in tissue engineering and stem cell therapy [Fraser et al., 2008; Gimble et al., 2011]. Donor factors, including height, weight, gender, and age, influence the properties of AT-MSCs. Many studies do not consider the AT-MSC isolation site or donor factors, but several studies have shown that the properties of AT-MSCs depend on such parameters [Aust et al., 2004; de Girolamo et al., 2009; Faustini et al., 2010]. In addition, the SVF may be affected by both the adipose tissue preparation method and the chopping regimen [Mojallal et al., 2008].

We examined AT-MSCs from 66 donors and observed that the cell properties varied. Possible influences of BMI, age, and gender on cell yield, proliferative capacity, stem cell properties, and multipotency were explored. Furthermore, the multipotency of AT-MSCs was compared with that of BM- and CT-MSCs under the same experimental conditions.

Faustini et al. [2010] reported that neither donor age nor BMI affected the cell yield when preparing AT-MSCs from 125 donors. De Girolamo et al. [2009] found that AT-MSCs from young donors were obtained in higher yields compared to those from older donors; our results were similar. The cell yield and cell ability may differ due to varied methods used to separate cells from tissue and

Fig. 3. AT-MSC colony forming units (CFUs) compared according to donor characteristics. AT-MSC CFUs classified by donor age (a), donor gender (b), and donor BMI (c). CFUs did not differ by age, gender, or BMI.

Fig. 4. Flow cytometry of AT-MSCs compared according to donor characteristics. Flow cytometry of AT-MSCs classified by donor age (a), donor gender (b), and donor BMI (c). Flow cytometry results did not differ by age, gender, or BMI.

Fig. 5. Osteogenic differentiation of AT-MSCs compared according to donor characteristics. **a** The osteogenic differentiation of AT-MSCs did not differ by donor age. **b** The osteogenic differentiation of AT-MSCs did not differ by donor gender. **c** The osteogenic differentiation of AT-MSCs was increased in donors with a BMI >30 compared to donors with a BMI <25 ($p < 0.005$).

the primary culture protocols used. Recent reports have been contradictory regarding whether or not BMI and donor age affect the cell yield [Aust et al., 2004; Griesche et al., 2010]. Differences in tissue separation and chopping methods may explain these differences [Oedayraj-singh-Varma et al., 2006; Griesche et al., 2010].

AT-MSCs from 66 donors were evaluated under identical conditions. The cell yield fell significantly with increasing donor age, and the cell growth was more vigorous when cells were isolated from younger rather than older donors. Neither the cell yield nor the growth rate was affected by BMI. CFU-F was decreased when the age of the donors increased, while proliferation was increased. We compared the connectivity of the CFU and proliferation rate and ensured the relevance of these and the BMI. CFU-F was low when the proliferation ability was high in donors less than 30 years of age. Additionally, the proliferation and CFU-F ability were decreased when a high BMI resulted in a high cell yield.

Cell surface antigens were analyzed by flow cytometry to demonstrate the stem cell capacity of AT-MSCs. In a previous study, AT-MSCs isolated from the hip formed twice the number of colonies compared to cells isolated from the abdomen [Fraser et al., 2007]. We observed no significant differences in cells from different isolation sites. However, we confirmed that age, gender, and BMI influenced cellular properties [Jurgens et al., 2008; Bieback et al., 2009]. CD13 and CD90 were expressed on the surfaces of AT-MSCs, and CD45 and HLA-DR were absent [Aust et al., 2004]. In a previous study, CD73 and CD105 were present and CD34 was absent [Baptista et al., 2009]. Therefore, all AT-MSCs expressed CD13, CD73, CD90, and CD105 but did not express hematopoietic markers [Jurgens et al., 2008; Bieback et al., 2009; Griesche et al., 2010; Goedecke et al., 2011]. Adipose tissue SVF contains hematopoietic cells [Varma et al., 2007; Faustini et al., 2010], but these cells are eliminated upon subculture (typically, by passage 3).

The multipotency of AT-MSCs was explored by inducing osteogenic and adipogenic differentiation. A previous study found that the extent of adipogenic differentiation was not affected by donor age, although osteogenic differentiation was more strongly induced in cells from younger rather than older donors [de Girolamo et al., 2009]. We found that the extent of osteogenic differentiation was significantly greater in cells from donors with a BMI greater than 25 as compared to those from donors with BMI below 25. In addition, osteogenic differentiation decreased somewhat with donor age, although this association did not reach statistical significance. Adipo-

Fig. 6. Adipogenic differentiation of AT-MSCs compared according to donor characteristics. **a** The adipogenic differentiation of AT-MSCs was increased in donors under 30 years of age compared to donors aged 30–39 years ($p < 0.005$), 40–49 years ($p < 0.005$), 50–59 years ($p < 0.05$), and over 60 years ($p < 0.001$). **b** The adipogenic differentiation of AT-MSCs did not differ by donor gender. **c** The adipogenic differentiation of AT-MSCs was increased in donors with a BMI >30 compared to donors with a BMI <25 ($p < 0.001$) or 25–30 ($p < 0.05$).

genic differentiation was induced to a greater extent in cells from donors under 30 years of age than in cells from donors over 30 years of age, and adipogenic differentiation increased with elevated BMI. Neither osteogenic nor adipogenic differentiation was affected by donor gender.

AT-MSC ability was examined by comparing the multipotency of BM-MSCs and CT-MSCs. BM-MSCs engaged in osteogenic and adipogenic differentiation more readily than did CT- or AT-MSCs. Furthermore, AT-MSCs readily differentiated along the chondrogenic lineage. CT-MSCs did not readily differentiate. Therefore, MSCs to be used in tissue engineering must be carefully selected with regard to the type of differentiation required and variation in properties caused by BMI, age, and gender.

The cell yield of donors aged less than 30–39 years was considerably greater, and cells from donors under 29 years of age proliferated readily compared to cells from those over 40 years of age. An increased BMI was significantly associated with an increased osteogenic differentiation of AT-MSCs, and a BMI over 30 was associated with a higher level of adipogenic differentiation than a BMI below 25. Therefore, MSCs must be selected after careful consideration of the type of regeneration required, and variations among potential donors should be examined when MSCs are to be used in applied tissue engineering or cell therapy.

Fig. 7. Osteogenic, adipogenic, and chondrogenic differentiation of MSCs from various origins. **a** Photomicrographs of differentiated MSCs. **b** Quantification of the osteogenic differentiation of MSCs from various origins after 14 days of culture. BM-MSCs were strongly induced to differentiate into the osteogenic lineage compared to CT- and AT-MSCs ($p < 0.001$). **c** Quantification of the adipogenic differentiation of MSCs from various origins after

14 days of culture. BM-MSCs were strongly induced to differentiate into the adipogenic lineage compared with CT- and AT-MSCs ($p < 0.0001$). **d** Quantification of the chondrogenic differentiation of MSCs from various origins after 14 days of culture. AT-MSCs were strongly induced to differentiate into the chondrogenic lineage compared with CT- and BM-MSCs ($p < 0.0001$).

Acknowledgments

This research was supported by a grant for 'the Center for Evaluating Next-Generation Stem Cell-based Therapeutics (CENST)'

supported by the National Institute of Food and Drug Safety Evaluation, an affiliate of the Ministry of Food and Drug Safety (14172 CENST 974) and a Seoul R&BD Program of the Seoul government of Korea (No. SS110011C0211601).

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