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Comparison of the osteogenic differentiation capacity of adipose tissue derived mesenchymal stem cells from humans and rats

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Abstract: Mesenchymal stem cells (MSCs) can be found in many types of adult tissues such as the bone marrow, adipose, placenta, liver, and periosteum. Recently, adipose tissue-derived MSCs (ADMSCs) have become one the most preferred MSC types because of their fast proliferation rate, abundance, and high plasticity for variable cell types. It is known that ADMSCs are able to differentiate into various cells, including osteoblasts, so they are quite promising for orthopedic clinical trials. The present study aimed to compare the osteogenic differentiation conditions of MSCs from human adipose tissue (hADMSC) and those of MSCs from rat adipose tissue (rADMSC). Therefore, differentiation experiments with five different media and two (human and rat) ADMSC types were performed and the mineralization responses of hADMSCs and rADMSCs were different.

Key words: Osteogenic differentiation, mesenchymal stem cell, adipose tissue, human, rat

1. Introduction

Many people of all ages all over the world suffer from serious bone diseases such as bone fractures, bone tumors, and osteoporosis. The limited treatment options for these disorders indicate a need for new therapies. Cellular therapy is one of the promising methods in orthopedic clinical trials (Pytlik et al., 2009; Seong et al., 2010). To date, the most popular cells used in cellular therapies are mesenchymal stem cells (MSCs). They can be isolated from different adult tissues such as the bone marrow, placenta, liver, adipose, periosteum, and testes (Vater et al., 2011). Adipose tissue is one of the most preferred sources because it can be obtained by less invasive methods and in larger quantities than the other adult tissues (Liu et al., 2007). Adipose tissue-derived MSCs (ADMSCs) have a great plasticity for variable cell types. For the characterization of ADMSC plasticity, one of the most widely accepted methods is differentiating them into osteoblasts, chondrocytes, and adipocytes in vitro (Vater et al, 2011).

Recently, several experiments have been performed to demonstrate the existence of adipose tissue-derived progenitors that give rise to bone (Liu et al., 2007; Arrigoni et al., 2009; Song et al., 2011; Çapkın, 2012). It was reported in these studies that various bioactive factors, including dexamethasone, ascorbic acid, and β-glycerophosphate, induced osteogenic differentiation in both human and rat ADMSCs. In addition, it became known that the osteogenic potential of ADMSCs was affected by the concentration of these bioactive factors (Seong et al., 2010).

The aim of the present study was to compare the osteogenic differentiation conditions of MSCs from human adipose tissue (hADMSC) and those of MSCs from rat adipose tissue (rADMSC) in order to explore the potential usage of allogeneic and xenogeneic MSCs for bone tissue engineering applications and to provide data on which further research in this area can be conducted.

2. Materials and methods

All cell culture materials and differentiation reagents were purchased from Greiner Bio-One (Germany) and Sigma Chemical Co. (USA) respectively, unless otherwise noted. Alpha minimum essential medium (MEM) (HyClone, SH30007.04), Dulbecco's modified Eagle's medium (DMEM) (high glucose) (FG0435), fetal bovine serum (FBS) (S0115), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (L1613), gentamicin (A2710), phosphate buffered saline (PBS) (L1835), Hank's balanced salt solution

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(HBSS) (L2045), trypsin/ethylenediaminetetraacetic acid (EDTA) solution (L2163), and collagenase type-I (C1-28) were obtained from BioChrom AG (Germany).

2.1. Isolation and cultivation of hADMSCs

The isolation procedure of hADMSCs was performed with the approval of Ege University's Human Research Ethics Committee. Adipose tissue biopsies were kindly provided by Prof Dr Erol Mir from Celal Bayar University.

hADMSCs were obtained from white adipose tissues surrounding the kidneys of two children who were younger than the pubertal age. Biopsies were immediately transported to the Ege University Animal Cell Culture and Tissue Engineering Laboratory in HBSS at 4 °C. While harvesting the ADMSCs, the biopsies were washed with HBSS previously. After the tissue was discarded from capillaries, it was minced into little pieces. These tissue pieces were treated with 0.075% (w/v) collagenase type-I for 1 h at 37 °C and shaken every 10 min. This was followed by enzymatic digestion; the suspension was centrifuged at 1800 rpm for 10 min and the supernatant containing the adipose cells was poured. This washing process was repeated three times. The cell pellet obtained after the washing steps was resuspended in culture medium (DMEM, 10% FBS, 10 µg/mL gentamicin) and then centrifuged at 1800 rpm for 10 min. Following centrifugation, the cell suspension was filtered through 70 µm of cell strainer (BD Biosciences, USA). Cells that passed through the filter were suspended in sufficient volume of culture medium, plated on tissue culture flasks, and then incubated at 37 °C in an incubator with a humidified atmosphere of 5% CO_2. Cell culture medium was changed every 3 days.

2.2. Isolation and culturing of rADMSCs

The isolation procedure of rADMSCs was performed with the approval of the Ethics Committee for Animal Experiments of Ege University.

rADMSCs were obtained from the white adipose tissues surrounding the kidneys of two 4-month old Sprague Dawley rats, provided by the Center for Experimental Animal Production at Ege University (İzmir, Turkey). The harvesting protocol for the rADMSCs was the same as the protocol for the human adipose tissue described in the previous section.

2.3. Osteogenic differentiation and histological investigations

hADMSCs and rADMSCs were plated in six-well tissue culture plates with a concentration of 6000 cells/cm^2 in control medium. After 48 h, when the cells reached 70%–80% confluency, their medium was changed with differentiation medium and they were incubated for 4 weeks. The differentiation medium exchange was performed every 2–3 days. In the present study five different osteogenic medium compositions were used (Table) (Colter et al., 2001; Liu et al., 2007; Hildebrandt et al., 2009; Pytlik et al., 2009; Çapkın, 2012; Seong et al., 2010; Song et al., 2011; Vater et al, 2011).

At the end of the incubation period, osteogenic mineral deposition into the bone extracellular matrix was determined by Alizarin Red S (Lee et al., 2008) and Von Kossa (StemPro Osteogenesis Differentiation Kit Protocol, GIBCO/Invitrogen, USA) staining. In both of these staining protocols, after washing with PBS, the cells were fixed with 4% paraformaldehyde (USB, USA, 19943) for 30 min. Following fixation, the washing step with PBS was performed again. For staining with Alizarin Red S, the fixed cells were incubated with 2% Alizarin Red S (Sigma, A5533) solution (pH 4.1–4.3) for 2–3 min and then washed with distilled water three times. Mineral deposits within the cell layers were stained bright red.

For the Von Kossa staining method application, the fixed cells were incubated with 5% silver nitrate solution (AppliChem, Germany, A3944,0025) for 30 min and then washed with PBS three times. Following this washing step, the cells were incubated under UV light for 1 h and then washed with distilled water three times. For elimination of nonspecific background staining, cells were treated with 5% sodium thiosulfate (Kimetsan, Turkey, STS/01CP/051003) for 5 min and washed with distilled water three times. Staining results were captured by an inverted phase-contrast microscope (Olympus, Japan). The secretion of the calcified extracellular matrix was observed as dark brown or black deposits with Von Kossa staining.

Table. Osteogenic differentiation medium compositions.

Medium No.	Dexamethasone (Sigma, D2915)	B-Glycerophosphate (Sigma, G9891)	L-Ascorbic acid (Sigma, A8960)	Cell culture medium
1	100 nM	10 mM	200 µM	DMEM 10% FBS, 2 µg/mL gentamisin
2	100 nM	10 mM	50 µM	DMEM 10% FBS, 2 µg/mL gentamisin
3	100 nM	10 mM	300 µM	DMEM 10% FBS, 2 µg/mL gentamisin
4	10 nM	10 µM	50 µg/mL	DMEM 15% FBS, 2 µg/mL gentamisin
5	10 nM	10 mM	50 µg/mL	DMEM 10% FBS, 2 µg/mL gentamisin

3. Results

3.1. Isolation and cultivation of hADMSCs and rADMSCs

In the present study hADMSCs and rADMSCs were cultured in tissue culture flasks until they reached 70%–80% confluency. Thereafter, these cells were subcultured at a 1:4 dilution.

No significant differences were detected in the morphologies of hADMSCs and rADMSCs. Both of these mesenchymal stem cell types were spindle and large flat shaped (Figure 1).

3.2. Osteogenic differentiation of hADMSCs and rADMSCs

hADMSCs and rADMSCs at passage 3 were cultured in six-well tissue culture plates and induced into the osteogenic lineage with five different osteogenic medium compositions as described above. Following the incubation period for osteogenesis, some morphological changes were observed in the hADMSCs that were cultivated in media 2, 3, and 5. These changes were mineralization in the extracellular matrix and more cubical shaped cells than the others, which were cultivated with control medium, medium 1, and medium 4 (Figures 2 and 3). On the other hand, rADMSCs did not display any changes with these five different osteogenic medium compositions (Figures 2 and 3).

After 28 days of osteogenic induction, the differentiation status of hADMSCs and rADMSCs was observed by Alizarin Red S and Von Kossa staining. Following Alizarin Red S staining, hADMSCs underwent calcium deposition in media 2, 3, and 5, while no calcium deposition was detected in rADMSCs cultures in the same media (Figures 4 and 5). Furthermore, similar results were obtained following Von Kossa staining (Figures 6 and 7). hADMSCs in media 1 and 4 did not differentiate into the osteogenic lineage. On the other hand, none of the rADMSCs displayed any osteogenic differentiation with these five different osteogenic medium compositions and all controls were negative for calcium deposition (Figures 4, 5, 6, and 7).

4. Discussion

MSCs derived from different adult tissues such as bone marrow and adipose tissue represent a source of multipotent cells. These cells are able to differentiate into various mesodermal lineages including bone, cartilage, and muscle (Vater et al., 2011). Therefore, they are available for cell therapy and tissue engineering applications.

ADMSCs were identified in 2001 and it was shown that they were able to differentiate into various mesodermal lineages (Zuk et al., 2001, 2002). Since then ADMSCs have been studied extensively for many years by different researchers all over the world (Seong et. al., 2010). Thus, ADMSCs became one of the most preferred MSC types because of their fast proliferation rate, abundance, high plasticity, secreted proteins (basic fibroblast growth factor, interferon-γ, and insulin-like growth factor-1), and immunomodulatory effects on variable cell types (Li et al., 2015). For example, with their capability to differentiate into osteoblasts, ADMSCs are quite promising in orthopedic clinical trials.

MSC type comparison studies in the literature are mostly about bone marrow stem cells from different species (Dosier et al., 2012). Additionally, many of them were concerned with age-related differences of MSCs (Dupont et al., 2010). On the other hand, in some studies it was pointed out that the species, as well as the age of the organism from which the MSCs were isolated, were important (Dosier et al., 2012). However, only one published study about species-specific differences of ADMSCs was found in our literature survey (Ni et al., 2009).

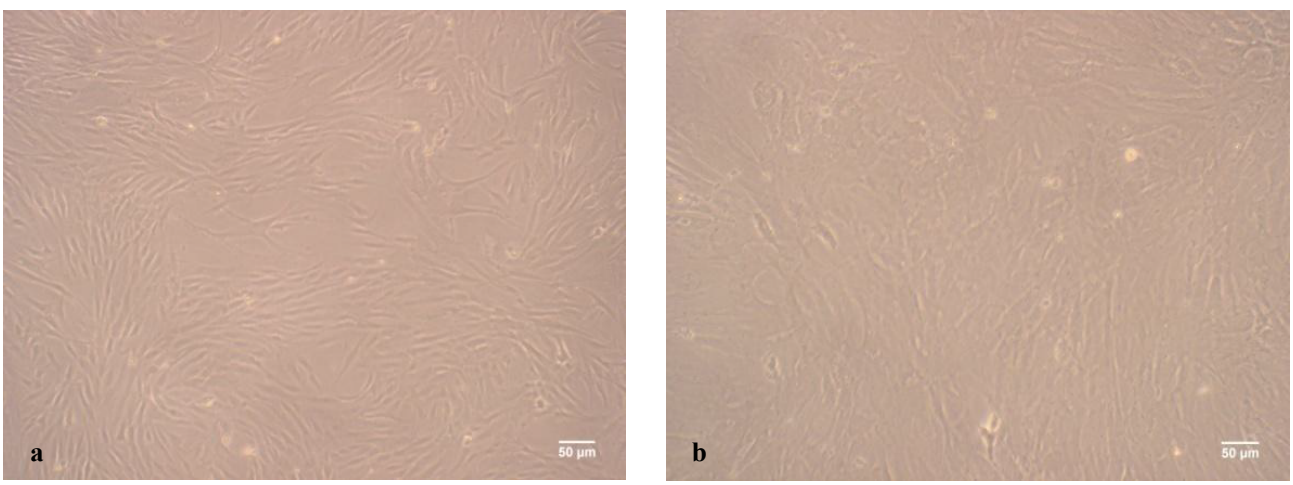


Figure 1. Inverted microscope images taken prior to osteogenic differentiation (×10 magnification) (a) hADMSC, b) rADMSC).

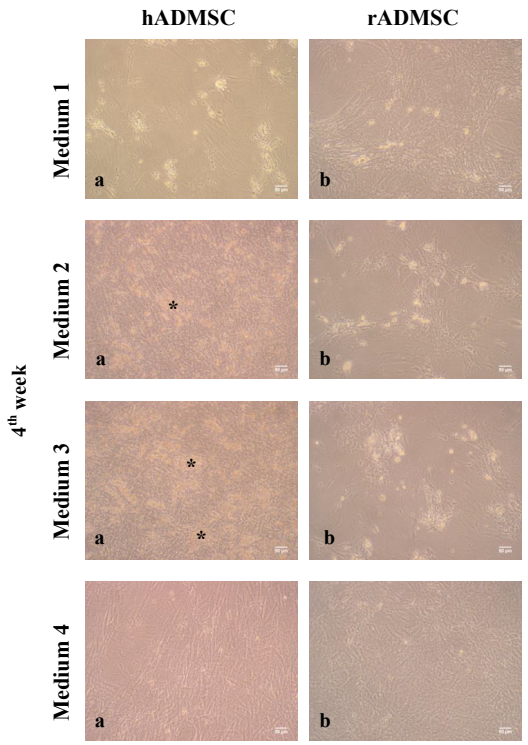


Figure 2. Inverted microscope images of week 4 cultures of hADMSCs and rADMSCs. In the hADMSCs that were cultivated with osteogenic differentiation media 2 and 3, mineralized extracellular matrix areas are shown with * signs (×10 magnification).

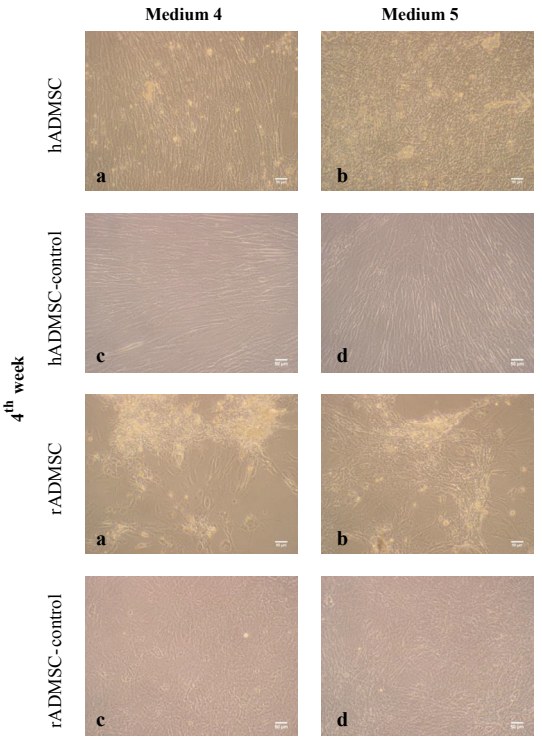


Figure 3. Inverted microscope images of week 4 cultures of hADMSCs and rADMSCs (×10 magnification).

The aim of the present study was to compare the osteogenic differentiation conditions of hADMSCs and rADMSCs; however, we did not evaluate any age-related differences of MSCs.

Differentiation experiments with five different medium compositions and two ADMSC types isolated from humans

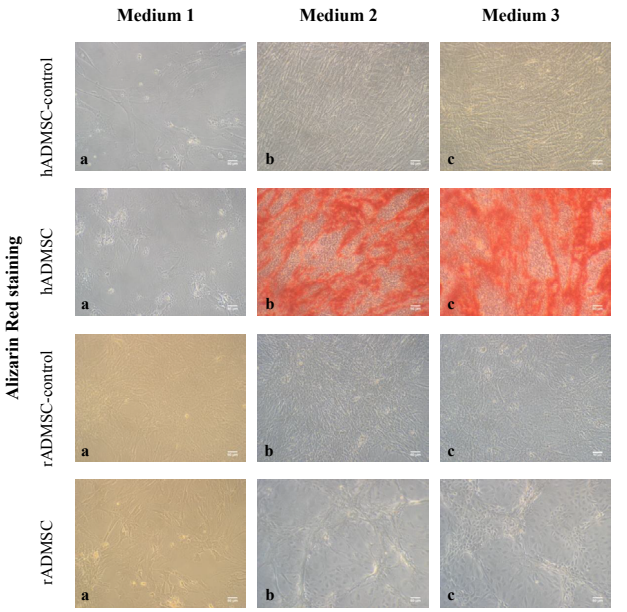


Figure 4. Alizarin Red staining of week 4 cultures of hADMSCs and rADMSCs (×10 magnification).

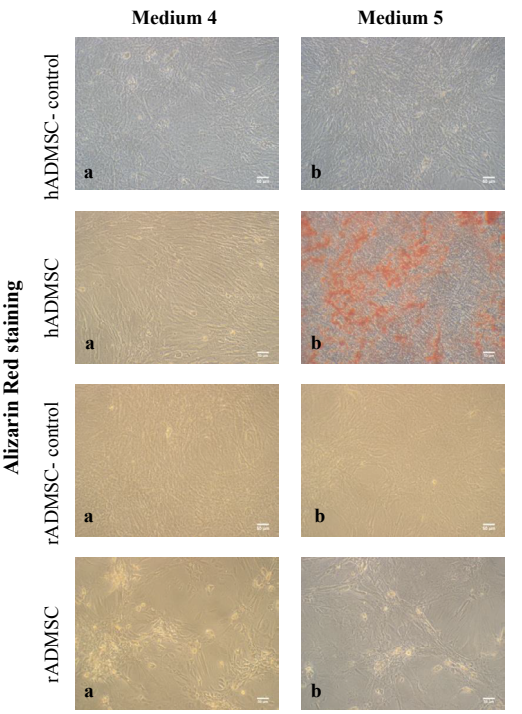


Figure 5. Alizarin Red staining of week 4 cultures of hADMSCs and rADMSCs (×10 magnification).

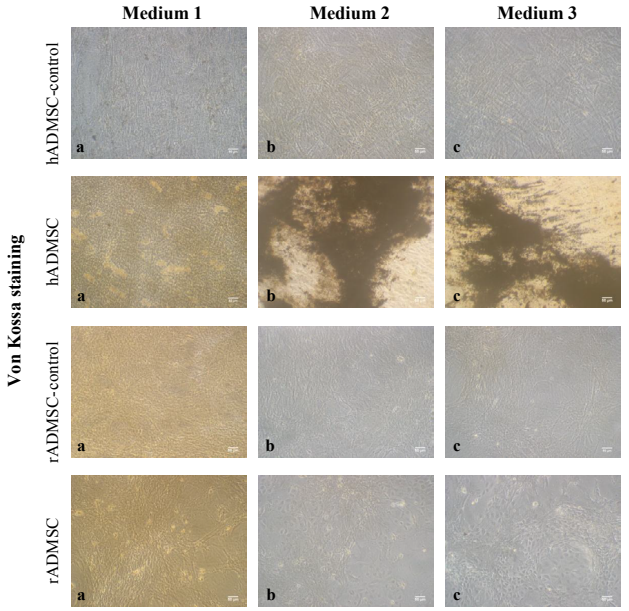


Figure 6. Von Kossa staining of week 4 cultures of hADMSCs and rADMSCs (×10 magnification).

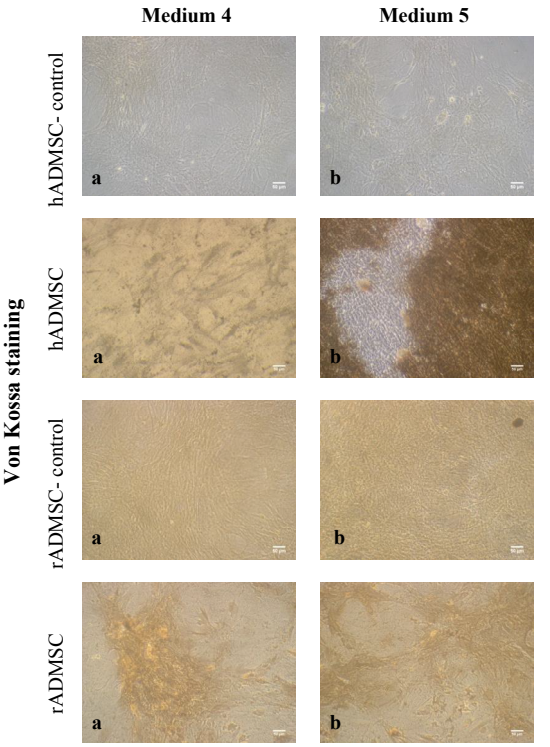


Figure 7. Von Kossa staining of week 4 cultures of hADMSCs and rADMSCs (×10 magnification).

and rats were carried out. In this way, dose- and species-dependent differences or similarities in the mineralization responses of hADMSCs and rADMSCs were demonstrated. Our results showed that hADMSCs had a better response to the osteogenic culture conditions than rADMSCs.

Thus, this study highlighted the possibility of using hADMSCs in osteogenesis protocols and generated a useful database addition to previous ADMSC research. The obtained data indicate that ADMSCs can be a good choice for bone tissue engineering applications and orthopedic clinical trials.

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References

Arrigoni E, Lopa S, Girolamo L, Stanco D, Brini AT (2009). Isolation, characterization and osteogenic differentiation of adipose-derived stem cells: from small to large animal models. Cell Tissue Res 338: 401-411.

Colter DC, Sekiya I, Prockop DJ (2001). Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. PNAS 98: 7841-7845.

Çapkın M, Çakmak S, Özdal-Kurt F, Gümüşderelioğlu M, Şen HB, Türk T, Deliloğlu-Gürhan Sİ (2012). Random/aligned electrospun PCL/PCL-collagen nanofibrous membranes: comparison of neural differentiation of rat AdMSCs and BMSCs. Biomed Mater 7: 045013.

Dosier CR, Erdmana CP, Parkb JH, Schwartza, Z, Boyana, BD, Guldberga RE (2012). Resveratrol effect on osteogenic differentiation of rat and human adipose derived stem cells in a 3-D culture environment. J Mech Behav Biomed 11: 112-122.

Dupont KM, Sharma K, Stevens HY, Boerckel JD, García AJ, Guldberg RE (2010). Human stem cell delivery for treatment of large segmental bone defects. P Natl Acad Sci USA 107: 3305-3310.

Hildebrandt C, Büth H, Thielecke H (2009). Influence of cell culture media conditions on the osteogenic differentiation of cord blood-derived mesenchymal stem cells. Ann Anat 191: 23-32.

Lee JH, Rhie JW, Oh DY, Ahn ST (2008). Osteogenic differentiation of human adipose tissue-derived stromal cells (hASCs) in a porous three-dimensional scaffold. Biochem Bioph Res Co 370: 456-460.

Li CY, Wu XY, Tong JB, Yang XX, Zhao JL, Zheng QF, Zhao GB, Ma ZJ (2015). Comparative analysis of human mesenchymal stem cells from bone marrow and adipose tissue under xeno-free conditions for cell therapy. Stem Cell Res Ther 6: 55.

Liu TM, Martina M, Hutmacher DW, Hui JHP, Lee EH, Lim B (2007). Identification of common pathways mediating differentiation of bone marrow- and adipose tissue- derived human mesenchymal stem cells into three mesenchymal lineages. Stem Cells 25: 750-760.

Ni YW, Zhou YS, Liu YS, Zeng BJ, Xu YW (2009). Comparison of biological characteristics of human, rabbit and rat adipose tissue-derived stromal cells *in vitro*. Beijing Da Xue Xue Bao 41: 95-99.

Pytlik R, Stehlik D, Soukup T, Kalbacova M, Rypacek F, Trc T, Mulinkova K, Michnova P, Kideryova L, Zivny J et al. (2009). The cultivation of human multipotent mesenchymal stromal cells in clinical grade medium for bone tissue engineering. Biomaterials 30: 3415-3427.

Seong JM, Kim BC, Park JH, Kwon IK, Mantalaris A, Hwang YS (2010). Stem cells in bone tissue engineering. Biomed Mater 5: 062001.

Song I, Kim BS, Kim CS, Im GI (2011). Effects of BMP-2 and vitamin D_3 on the osteogenic differentiation of adipose stem cells. Biochem Bioph Res Co 408: 126-131.

Vater C, Kasten P, Stiehler M (2011). Culture media for the differentiation of mesenchymal stromal cells. Acta Biomater 7: 463-477.

Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7: 211-228.

Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH (2002). Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13: 4279-4295.