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Research Article

Comparing the Osteogenic Potency of Mesenchymal Stem Cells Derived from Bone Marrow and Adipose Tissue

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Abstract

Background: The multi-potent Mesenchymal Stem Cells (MSCs) are adult stem cells that found in several tissues such as umbilical cord, bone marrow and fat tissues. These multi-potent MSCs can differentiate into multiple tissues including bone, cartilage, muscle, tendons, skin, fat cells, and connective tissue. The aim of the present study is to compare the osteogenic potential of MSCs derived from Bone Marrow (BM-MSCs) and Adipose Tissue (AD-MSCs) of C57BL/6 mice.

Materials and Methods: MSCs were isolated from bone marrow and adipose tissues of mice. Both cell types were cultured in osteogenic medium. Using reverse transcription PCR (RT-PCR), six osteogenic specific genes (Collagen I, Osteocalcin, Osterix, Runx2, Alkaline phosphatase and Osteonectin) were examined in both BM-MSCs and AD-MSCs cell types which maintained *in vitro* under osteogenic conditions along 21-day time course. Alkaline phosphatase activity and matrix mineralization were also assayed.

Results: According alkaline phosphatase activity, the osteoblastic differentiation of BM-MSCs was found to be higher than that of AD-MSCs. The amount of matrix mineralization was also higher in BM-MSCs when compared to AD-MSCs. Up on osteogenic differentiation; BM-MSCs reached higher levels of osteogenic gene up-regulation early than AD-MSCs.

Conclusion: BM-MSCs have greater capacity for osteogenic differentiation than AD-MSCs and hold promising potential for bone tissue engineering and cell therapy application.

Keywords: Mesenchymal stem cells; Bone marrow; Adipose tissue; Osteogenic; differentiation; Mice

Introduction

Stem cells are defined recently as the unspecialized cells in our body that have the capability of becoming specialized cells, each generated cell have new specialized cell functions [1]. Mesenchymal Stem Cells (MSCs) are potential cells that capable for treatment of different diseases [2]. Degenerative diseases as osteoporosis and arthritis pose significant challenges for health care professional) [3]. MSCs from both bone marrow and adipose tissues were found to possess the highest benefit stem cells for bone regeneration [4].

MSCs have the ability to regulate immune responses and change the gradual development of several inflammatory diseases [5]. Damaged tissues are often associated with inflammation mechanisms which stimulate anti-inflammatory mediators which act as chemotactic signals that directing the MSCs to damaged tissues. MSCs have the ability to regulate immune responses and change the gradual development of several inflammatory diseases. Before tissues repair themselves, MSCs have the ability to alter inflammatory process by releasing growth factors. This process has shown a relationship between MSCs and the immune system which has been utilized in many clinical applications, as in the

treatment of bone and cartilage defects, myocardial infarction and some autoimmune diseases [6].

Several researchers, based on *in vitro* results affirm that AD-MSCs have greater osteogenesis potential [7-9]. However, other studies have shown that BM-MSCs have higher capability for osteogenic differentiation compared with AD-MSCs [10-12]. This disagreement between results is due to comparisons made between MSCs from bone marrow and adipose tissues of different individuals as with different ages, with varying health status and subjected to physical, chemical and environmental different [12].

In addition, comparative results should be performed between MSCs harvested from the same individual but subjected to different *in vitro* experimental protocol, since the proliferative and differentiation potential of MSCs vary according to the culture circumstances [2,12]. Therefore, the objectives of the present study were to determined which of the two cell types have the better osteogenic potential early and might be more indicated for cell therapy as an autologous source of MSCs. For this, we compared by RT-PCR technique for six osteogenic genes (Collagen I, Osteocalcin, Osterix, Runx2, Alkaline phosphatase and Osteonectin) and Alkaline phosphatase activity as well as mineralization assay, the differentiation of BM-MSCs and AD-MScs cells toward osteogenic lineage under osteogenic permissive condition during a well controlled 21 days *in vitro* time-course study in C57BL/6 mice.

Material and Methods

Animals

A total 24 male C57BL/6 mice, aged 12-14 weeks and weighing 25-30 gm were included in this study. All mice were purchased from France (Charles River, France), breed and maintained in Medical Experimental Research Center (MERC), Mansoura University, Egypt. All the procedures used in the animal experiments were governed by Guidelines for the Care and Use of Laboratory Animals from the National Research Center, Egypt. Mice were housed at a constant environmental condition and kept in separate metal cages, they given constant food and fresh clean drinking water *ad-libtium* through specific nipple. All mice were killed by cervical dislocation. Femurs, tibia and adipose tissue were obtained for isolation of mesenchymal stem cells.

Isolation and Culture of Stem cells -Bone Marrow Mesenchymal Stem Cells (BM-MSCs)

Bone Marrow (BM) cells were collected from tibia and femurs, using a 27-gauge needle attached to a 10ml syringe filled with Dulbecco's Modified Eagle's Medium (DMEM, GIBCO/BRL) supplemented with 1% penicillin-streptomycin. The medium was inserted and flushed inside tibia and femurs and collected in 15ml tube preserved in ice. Cells suspension was filtered through a 70-mm filter mesh to remove any debris. BM cells were cultured in complete culture medium containing {DMEM + 1% penicillin-streptomycin + 10% Fetal Bovine Serum (FBS)}. BM cells were incubated at 37°C in 5% humidified CO_2 incubator. After The 1st day the nonadherant cells washed using Phosphate Buffer Saline (PBS) (Gibco/Invitrogen, Grand Island, New York, USA) and the medium was changed slowly and replaced with a fresh complete medium for further culture. Thereafter the culture medium was changed with a fresh complete medium every 3 days until the adherent cells reached 70~80% confluence. Cultured cells were washed twice with PBS and the cells passaging were performed using 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5 min at 37°C then fresh free medium was added to stop the trypsin-EDTA action. After centrifugation of the collected suspended cells it was washed with PBS and cells were resuspended in fresh complete medium, seeded in 75 cm^2 culture flasks and incubated at 37°C in 5% CO_2 incubator. The resulting cultured cells were referred to as BM-MSCs first-passage cultures [13].

Adipose Tissue Mesenchymal Stem Cells (AD-MSCs)

The Adipose Tissue (AD) was collected from inguinal groove under sterile conditions and washed with PBS containing 1% antibiotic-antimycotic solution further times until all blood vessels and connective tissues were liberated [14]. AD were minced cutted of into small segments and enzymatic digestion was performed using 0.1% Collagenase I (Serva Electrophoresis GmbH, Mannheim, Germany) with shaking for 2 hours at 37°C. Digested AD were filtered through 70mm filter mesh and centrifuged. Erythrocytes were removed by treatment with lysis buffer. The cells were transferred to tissue culture flasks with complete culture medium (DMEM + 1% penicillin-streptomycin + 10% FBS) (FBS, Gibco/BRL) and, after an attachment period of 24 hours, non-adherent cells were removed by 3 times PBS wash. Attached cells were cultured in complete culture medium, and expanded *in vitro*. Thereafter the culture medium was changed with a fresh complete culture medium every 3 days until the adherent cells reached 70~80% confluence When large colonies of AD-MSCs developed, cultures were washed twice with PBS and the cells were trypsinized with 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5 min at 37 °C. After centrifugation of the collected suspended cells, supernatant was removed and cells were resuspended in fresh complete medium in 75 cm^2 culture flask and incubated in 5% CO_2 at 37°C incubator. The resulting cultured cells were referred to as AD-MSCs first-passage cultures [15]. To ensure the MSCs identity with elonged shape, the cells were characterized morphologically by inverted microscope examination (Leica, Wetzlar, Germany).

Growth curves

BM-MSCs and AD-MSCs at passage 3 were seeded in 24-well plate with 1×10^4 cells/well in triplicate. Cells were collected from each well 1-9 days after seeding and counted microscopically to produce cell growth curves.

Cell Viability assay

Before being cultured in osteogenic medium, the BM-MSCs and AD-MSCs were tested for viability using trypan blue stain. In brief, the cells were cultured in a 75-cm^2 culture flask (1×10^4 cells/cm^2) in complete culture medium. During the test, the cells were harvested with trypsin/EDTA, centrifuged at 1400 rpm for 10 min and stained with 0.4% trypan blue. The non-viable cells will stained with blue while viable cells will still transparent both cells will quantified in a Neubauer chamber.

Flow cytometry analysis

BM-MSCs and AD-MSCs were trypsinized and centrifuged at 1400 rpm for 10 min. Cells were then resuspended at a density of 1×10^6 cells/ml in PBS. The cell aliquots were incubated with individual primary or control antibodies for 30 min at 4°C. The mesenchymal cells were washed with PBS then, incubated for 30 minutes with fluorophore-conjugated secondary antibody at 4°C. The samples were analyzed using a FACS cytometer (BD Biosciences, San Jose, CA) and the collected data were analyzed by Cell Quest software (Becton Dickinson) [16]. The following primary antibodies were tested: anti-CD44, anti-CD29, anti-CD105, anti-Sca-1 as positive markers of stem cells and anti-CD45, anti-CD34 as negative markers of stem cells.

Colony Forming Unit (CFU) assay

For determination of CFU, harvested AD-MSCs were plated at 25,000 cells/25cm^2 culture flasks, and BM-MSCs were plated at a density of 100,000 cells/25cm^2 culture flask. After plating, the cells were incubated at 37°C and 5% CO_2 for 10~14 days with media changes occurring every 48 hours. After 14 days of culture, cells were washed using PBS then colonies were fixed using 95% ethanol for 5 minutes, thereafter the cells were exposed to 0.5% crystal violet stain solution (Sigma Aldrich, USA) for 30 minutes at room temperature then washed twice with distilled water, dried and AD-MSCs and BM-MSCs colonies were then counted using an inverted microscope (Leica, Wetzlar, Germany). Cell clusters consisting of at least 20 cells were classified as a CFU, colony.

Osteogenic differentiation

For osteogenic induction, mesenchymal stem cells after passage three were counted and seeded density of at 5×10^4cells/well in 6 well plates. When they reach 80 - 90% confluence, BM-MSCs and AD-MSCs were induced to osteogenic differentiation with osteogenic culture medium, {DMEM + 10% FBS, 600 µg/L amphotericin B, 100U/ml penicillin, 100µg/ml streptomycin, 10 mM β-glycerophosphate, 50 µg/mL ascorbic acid (Merck) and 10 nM dexamethasone (Sigma)}. The cells were grown at 37°C in 5% CO_2 incubator for 3 weeks and the medium was changed with fresh medium twice a week. Finally, the number and size of mineralization were maximized.

Mineralization assay

At differentiation days 5, 15, and 21 Alizarin red stain was used to estimate mineralization of extracellular matrix as previously described. Briefly, differentiated cells were washed using PBS twice then, fixed with 70% ethanol at room temperature for 30 minutes. Cells were washed by deionized water then stained using 0.2% Alizarin-red solution at room temperature for 30 minutes then washed with PBS three times. Cells culture plates were dried and evaluated microscopically using an inverted microscope. Alizarin red staining was quantified by colorimetric assay, alizarin red solution bounded to calcium deposit was extracted using overnight incubation with guanidine-HCL (Sigma Aldrich, USA) at room temperature to elute all calcium-bound stain. Supernatants were collected and Optical Density (OD) was determined at 450 nm.

Alkaline phosphatase activity

Alkaline phosphatase activity was determined by biochemical colorimetric assay using alkaline phosphatase kit (Biosystems, Barcelona, Spain). According to manufacture instruction, approximately 10^4 cells (After 5, 15 and 21 days of osteogenic differentiation) were washed with PBS and then 0.5 ml 0.2% Triton X-100 in distilled water was added and shaking for 20 min at room temperature to obtain cell lysate. Alkaline phosphatase enzyme activity in cell lysates was determined by measuring level of ρ-nitrophenol formed during hydrolysis of ρ-nitrophenylphosphatae substrate. The reaction was stopped by adding 1ml of 0.05 N NaOH and the OD was read by spectrophotometer at 420 nm wavelength.

RNA isolation and RT- PCR analysis

Total RNA was extracted from differentiated cells using RNeasy Mini RNA isolation kit (GF-TR-50, Vivantis, Malysia). Total RNA was eluted from the mini columns with 50 µl RNase-free water. The amount of RNA isolated was quantified by optical density at 260 nm using nanophotometer P-Class (Impen, USA). RNase treatment was used to clear residual RNA. Starting with 1 µg total RNA, 20 µl cDNA were synthesized using Maxima First Strand cDNA synthesis kit (Thermo Science, USA). Reverse transcription was performed for 1h at 42°C, followed by incubation at 75°C for 5 min to inactivate reverse transcriptase. The cDNA samples were amplified by 40 cycles of denaturation (1 min at 94°C) and elongation (30 secs, 72°C) followed by final step at 72°C for 5 min. The specific primers which were selected for the genes examined were designed based on their GenBank sequence. Primers sequence, PCR condition and PCR product size for β-Actin, Osterix, Runx2, Alkaline phosphatase, Collagen I, Osteocalcin and Osteonectin were listed in Table 1 [17].

Agarose gel electrophoresis

All PCR products were applied for electrophoresis on 2% agarose

gel with a size marker and were UV visualized by ethidium bromide staining.

Gel documentation

The products bands were photographed and analyzed by Gel documentation software (Bio Rad, USA). The bands were detected and converted to peaks. Area under each peak were calculated in square pixels and used in quantification. Gene expression levels were determined by calculating the ratio between the square pixel values of the target genes in relation to the control gene (β-Actin).

Product size (bp)	Annealing Temperature	Primer sequence	Gene
485	54	F:5CAGGATTCCATACCCAAGAAG-3 R: 5-AACCCTAAGGCCAACCGTG-3	β-Actin
218	52	F: 5-AAACATGGCAAGGTGTGTGA-3 R:5-TGCATGGTCCGATGTAGTC-3	Osteonectin
257	56	F:5-CTCACTCTGCTGGCCCTG-3 R:5-F:CCGTAGATGCGTTTGTAGGC-3	Osteocalcin
125	54	F:5-TGAGCGACACGGACAAGA-3 R:5-GGCCTGGTAGTTGTTGTGAG-3	Alkaline phosphatase
312	52	F:5-GAAGTCAGCTGCATACAC-3 R:5-AGGAAGTCCAGGCTGTCC-3	Collagen I
238	56	F:ACTCATCCCTATGGCTCGTG-5 R:GGTAGGGAGCTGGGTTAAGG-3	Osterix
289	59	F:5-CCGCACGACAACCGCACCAT-3 R:5-CGCTCCGGCCCACAAATCTC-3	Runx 2

Table 1: Primer sequence, PCR condition and product size for investigated genes**.**

Statistical analysis

Data were expressed as mean ± standard error of the mean. Mann-Whitney U-test was performed to compare between means of the osteogenic parameters including activity of alkaline phosphatase, calcium content and mRNA levels in the two MSCs sources. Results were considered significant at $p< 0.05$. Statistical analysis was performed using SPSS 16.0 (Statistical Package for Scientific Studies Inc., Chicago, IL, USA) for Windows.

Results

Stemness Phenotype

Cell culture: On the first day of cells isolation from the two sources, the population consisted of round-shaped erythrocytes and non-adherent cells. By day 2-3, cells adhered to the culture flask, exhibiting a stretched out fibroblast-like shape, and non-adherent cells were removed by medium exchange (Figure 1A, 1B). On day 10 of culture, the confluent of cells was 60-80%, organized in colonies, all large colonies consisted of small spindle-shaped cells. In contrast, the small colonies contained cells with a large polygonal shape (Figure 1C, 1D).

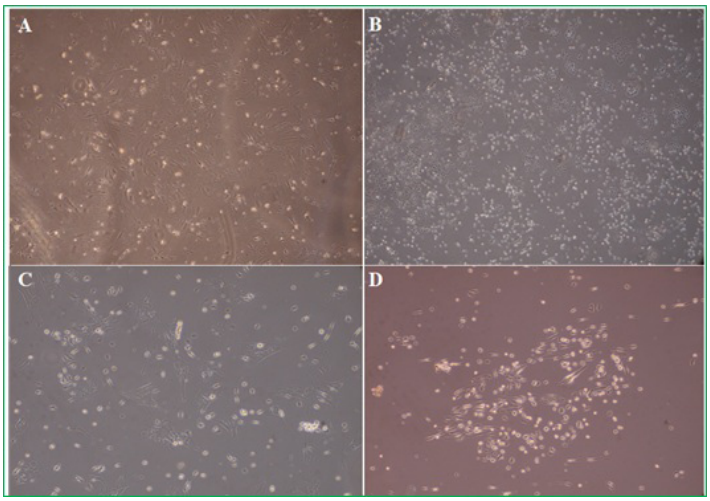

Figure 1: Primary in vitro culture of BM-MSCs and AD-MSCs. Adherent mesenchymal stem cells after 3 days of culture for BM-MSCs (A) and AD-MSCs (B), Magnification is ×100. Adherent mesenchymal stem cells after 10 days of culture for BM-MSCs (C) with 60% confluent and AD-MSCs (D) with confluent 80%, Magnification is ×200.

Colony forming unit (CFU) assay: CFU assay is used for evaluating the proliferation capacity of the cells. From 25,000 AD-MSCs plated 300 (1.5%) colonies were formed, and from 100,000 BM-MSCs plated 90 (0.09%) colonies were formed (Figure 2).

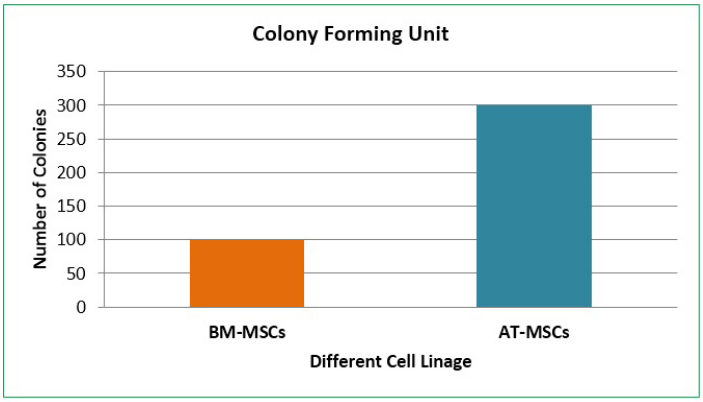

Figure 2: Number of CFU assay for BM-MSCs and AD-MSCs.

Growth curve: According to the growth curve, the BM-MSCs took several days longer to reach confluence than the AD-MSCs (Figure 3).

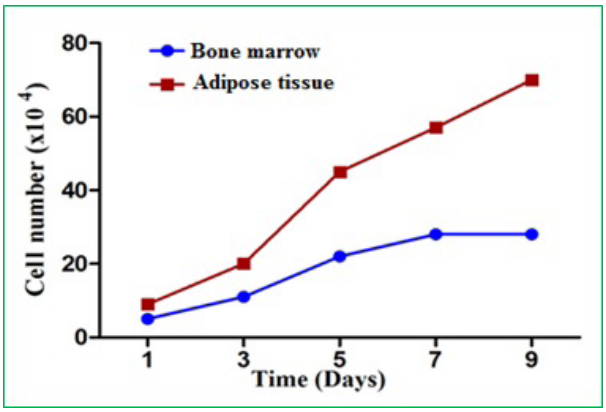

Figure 3: Growth curve of BM-MSCs and AD-MSCs**.**

Immunophenotypic characterization: For characterization of MSCs, surface proteins expression was examined by flow cytometry. The analysis revealed a positive expression of the markers related to MSCs including, CD29, CD105, CD44, and Sca-1 with average percentage of 66 ± 3.2%, 41 ± 2.4%, 35 ± 7.1% and 39 ± 5.2% respectively. In addition, cell surface markers that were negative were related to hematopoietic stem cells and include, CD34 and CD45 with percentage 4 ± 1.2% and 3 ± 0.98 respectively (Table 2 and Figure 4).

Antibody	Conjugate	Mean ± SE
CD29	PCY	66 ± 3.2
CD105	PE	41 ± 2.4
CD44	FITC	35 ± 7.1
Sca	PE	39 ± 5.2
CD34	FITC	4 ± 1.2
CD45	FITC	3 ± 0.98

Table 2: Immunophenotypic characterization of mesenchymal stem cells.

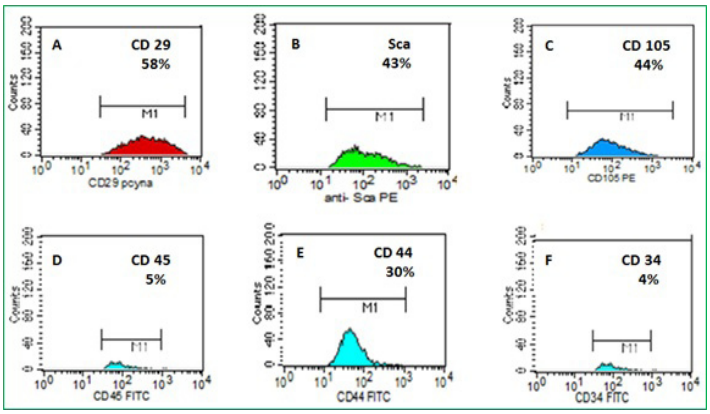

Figure 4: Flow cytometry analysis of mesenchymal stem cells at passage 4. Cells were detached and labeled with anti-CD29 (A), anti-Sca (B), anti CD105 (C), anti-CD45 (D), anti-CD44 (E) and anti-CD34 (F).

Osteogenic Differentiation

Mineralization of stem cells: Mineralization assay used as a marker in osteoblastic differentiation of stem cells was also qualified and quantified. After 5 days in osteogenic medium, all two cell types developed dense cellular aggregations; these were larger, denser and more abundant in BM-MSCs cultures. With alizarin red application, the cellular aggregates in the bone marrow cultures showed intense label up-take (Figure 5A). Alizarin Red staining of osteogenic AD-MSCs cultures was also limited to the locations of cellular aggregation but was noticeably less intense (Figure 5B).

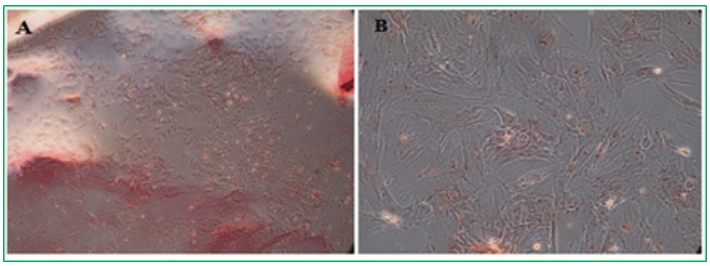

Figure 5: Alizarin Red stain showed cellular aggregates in the BM-MSCs cultures (A) with intense label up-take while, AD-MSCs cultures (B) was showed limited cellular aggregation was noticeably less intense.

The results of stain quantification were expressed as optical density were consistent with those of the alizarin red staining visualized by microscope where the calcium content was increased continuously during osteogenic differentiation. On day 5, similar deposition was found for AD-MSCs and BM-MSCs. On day 15 and 21, higher and significant mineralization and calcium content was measured for BM-MSC compared to AD-MSCs (Figure 6).

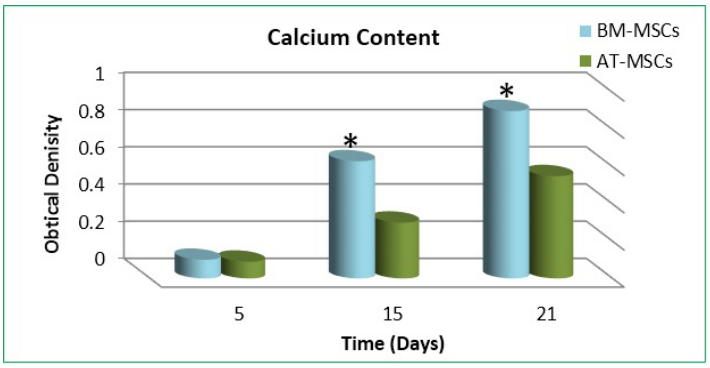

Figure 6: Calcium content of stem cells during osteogenic differentiation, asterisk shows significant difference between BM-MSCs and AD-MSCs at $p < 0.05$.

Alkaline phosphates activity: The results of bulk Alkaline Phosphatase (ALP) enzymatic activity, as a marker of osteogenesis in stem cells was measured and expressed in mmol/L p-nitrophenol during induction of osteogenesis. ALP activity in two cell types was similar including a peak during osteogenic differentiation (Figure 7). That results were somewhat different where BM-MSCs exhibited significant and highest alkaline phosphatase at all-time compared to AD-MSCs with a peak on day 15. The maximum enzymatic activity was peaked on day 21 for AD-MSCs.

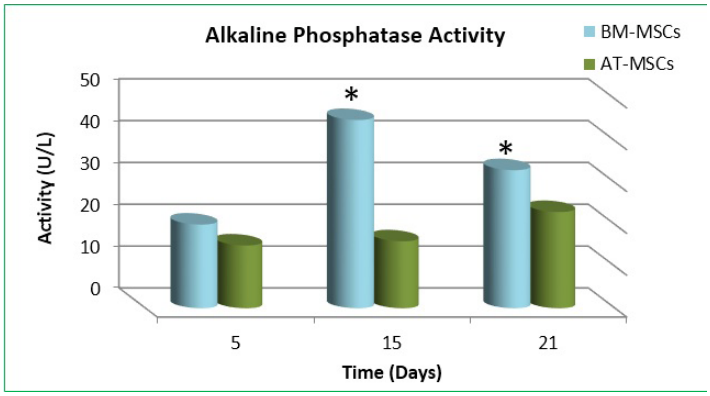

Figure 7: Alkaline phosphatase activity of stem cells during osteogenic differentiation, asterisk shows significant difference between BM-MSCs and AD-MSCs at $p < 0.05$.

Gene transcripts of osteogenic differentiation: The relative expressions of six important bone-related genes were investigated during osteogenic differentiation of the two stem cells. They were: Alkaline phosphatase, Collagen I, Osteocalcin and Osteonectin are secreted proteins expressed by cells in the osteogenic lineage, while Runx 2 and Osterix are transcription factors required for osteogenic differentiation. The transcriptional analyses of ALP mRNA expression were consistent with the enzymatic activity findings. In BM-MSCs the mRNA level of ALP reached a peak and up-regulated on day 5 on contrary, it peaked on day 21 for AD-MSCs (Figures 8A, 9A).

The relative expression of Collagen I gene was initially detected in both BM-MSCs and AD-MSCs and that expression reach peaks during osteogenic differentiation on day 5 and day 21, respectively. A dramatic down-regulation for mRNA level at BM-MSCs on day 15 followed by a slight up-regulation on day 21 (Figures 8B, 9B). An increasing in Runx 2 mRNA during induction time it peaked early on day 5 for BM-MSCs and then down-regulated at second week followed with dramatically up-regulation on day 21. On other hand in AD-MSCs it was peaked on day 15 followed by down regulation at the third week (Figures 8C, 9C). There was a significant and early up-regulation of Osterix mRNA in BM-MSCs compared to AD-MSCs on day 5. In second week, it was down-regulated followed by dramatically up-regulation for both cell types in the third week (Figures 8D, 9D).

Osteonectin mRNA pattern was similar in both BM-MSCs and AD-MSCs where it up-regulated on day 5 followed by down-regulation on day 15 for both cell types. On day 21, the two cell

type were differing where this gene was slightly up-regulated in AD-MSCs while it still down-regulated in BM-MSCs (Figures 8E, 9E). Osteocalcin mRNA level was peaked early on day 5 for BM-MSCs. while this gene was peaked on day 21 for AD-MSCs (Figures 8F, 9F).

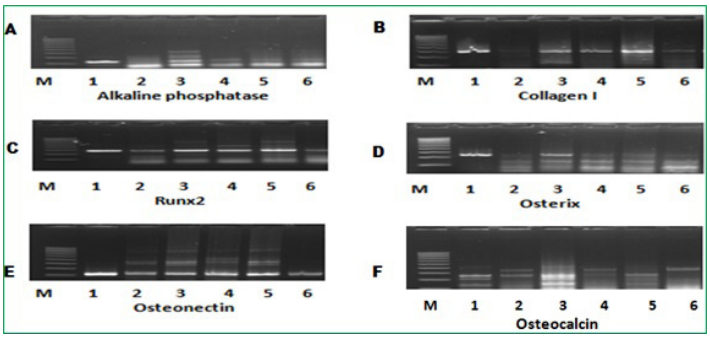

Figure 8: An agarose gel electrophoresis representing the PCR products of Alkaline phosphatase (A), Collagen I (B), Runx 2 (C), Osterix (D), Osteonectin (E) and Osteocalcin (F) genes in bone marrow and adipose tissue derived stem cell. (M): DNA Ladder; Lane (1): Bone marrow after 5 days of osteogenic induction Lane (2): Bone marrow after 15 days of osteogenic induction Lane (3): Bone marrow after 21 days of osteogenic induction Lane (4): Adipose tissue after 5 days of osteogenic induction Lane (5): Adipose tissue after 15 days of osteogenic induction Lane (6) Adipose tissue after 21 days of osteogenic induction.

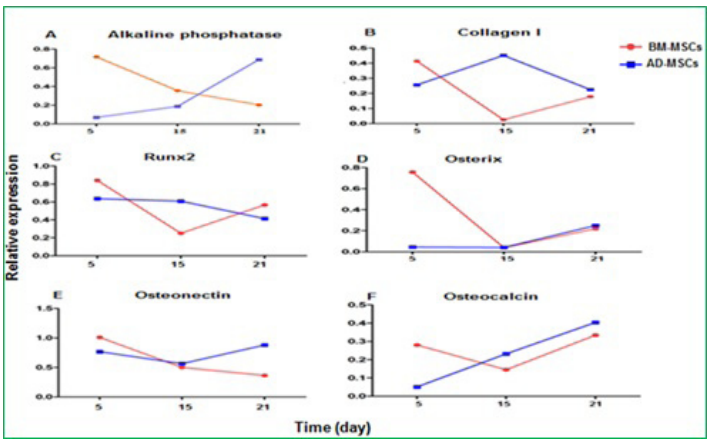

Figure 9: Relative expression expressed as normalized to β-actin of alkaline phosphates (A), Collagen I (B), Runx 2 (C), Osteric (D), Osteonectin (E) and Osteocalcin (F) on days 5, 15 and 21 in stem cells during osteogenic differentiation.

Discussion

The use of stem cells in bone regeneration has a great future and has also been demonstrated by many clinical trials [18]. Both bone marrow and adipose tissue are the most important cells that can supply MSCs because they are spread and can be harvested [19]. However, it is not yet clear whether AD-MSCs or BM-MSCs are better in obtaining viable MSCs in bone regeneration. In the present study, we compared the rapid osteogenic abilities of AD-MSCs and BM-MSCs by conducting in vitro assays. Our observations could provide some benefit experimental evidences on choosing a suitable cell source for a particular therapeutic purpose**.**

In this study, stem cells obtained from both bone marrow and adipose tissues of mice were successfully attached to tissue culture dishes and form in vitro adherent colonies. The number of CFU in AD-MSCs was much higher (1.5%) than the number of CFU in BM-MSCs (0.09%). Previous studies on human obtained 0.05% CFU of AD-MSCs and 0.008% CFU of BM-MSCs [20], or no significant differences in number of colonies between both cells [21]. The large number of CFU that we obtained in both cells types, may be due to species differences. Furthermore, in the present study the cells were recovered from the femur of the animals and not aspirate from the iliac crest as it was done in human study.

The morphological characteristics and expression of surface markers for phenotypic characterization were similar between MSCs obtained from both adipose tissue and bone marrow. These results are in agreement with other studies reported similar characteristics between these two MSCs cell types [22-24].

In this study, six bone-related genes were selected and their expression was investigated to better compare and understand the behavior of BM-MSCs and AD-MSCs under inductive culture in addition to mineralization assay for detecting calcium deposits within the differentiated osteogenic cells. Accepting that matrix mineralization and ALP activity up-regulation are the cardinal indices of osteogenic differentiation, changes in mRNA levels of several genes linked to the osteogenic phenotype were also investigated.

Choosing the better cell type was depending mainly on the early osteogenic differentiation to avoid contamination of culture media which correlated with incubation time. Here in this study, stem cells showed different patterns and amounts of alkaline phosphatase activity during differentiation into osteoblasts. Alkaline phosphatase assay is a biochemical marker for osteogenesis activity [25]. Compared to AD-MSCs, superior osteogenic of BM-MSCs can be predicted from the higher alkaline phosphatase activity in all time points.

In AD-MSCs, alkaline phosphatase mRNA expression peaked on day 21 while for BM-MSC it was peaked early at day 5. Furthermore, the delay in protein expression of alkaline phosphatase can be explained by the interval time required for translation of alkaline phosphatase mRNA into its protein.

This showed that prior to the translation of alkaline phosphatase gene, a dexamethasone-induced increase in gene transcription occurred transiently during induction [26].

Furthermore, our results of mineralization confirmed the patterns of alkaline phosphatase activity in both BM-MSCs and AD-MSCs. During osteogenic induction, increasing Calcium content of the two type of stem cells as a result of alkaline phosphatase activity, crystal nucleation and precipitation [11]**.** Moreover, calcium deposition increased in BM-MSCs compared to AD-MSCs. The higher of BM-MSCs can be explained by higher expression of osteocalcin and osteonectin based on their role in initial crystal growth on stem cells during osteogenic differentiation [27].

Also, the higher mineralization matrix synthesis in the cultures media of BM-MSCs may be due to the increased expression of, osterix and Runx 2, because these proteins perform a significant function during osteogenic differentiation [8,28]. These results possibly justify the higher osteogenic potential observed in BM-MSCs demonstrated the higher percentage of calcium deposition, since the matrix mineralization is an important markers of end-stage osteogenic differentiation [29].

In our study, gene expression of collagen I was expressed early at day 5 for BM-MSCs compared to AD-MSCs in which it peaked at the 15th day. On other hand, mRNA of collagen I was down-regulated through osteogenic differentiation. The down-regulation of collagen type I mRNA was mediated by destabilization and inhibition of transcription of its mRNA by glucocorticoids. Although collagen I was down-regulated during osteogenic induction its transcription still remained in an adequate level for synthesis of protein [30].

The higher expression of collagen I by BM-MSCs was responsible for the increased synthesis of mineralized matrix, since collagen I representing about 90% of the proteins that composed of the bone matrix. In addition, collagen I showed significant role in the extracellular matrix formation and it is composed of two α1 and α2 chain and their fibrils are organized in layers, with crystals of hydroxyapatite which deposited in them to form the mineralized matrix [31,32]. Thus collagen associated with osteocalcin, osteonectin, osterix and Runx 2 may have contributed significantly to the increased formation of mineralization nodules in BM-MSCs in this study.

Dexamethasone, β-glycerophosphate (β-GP) and ascorbic acid, are acting as chemical inducers that promote MSCs differentiation into osteoblasts [33]. Dexamethasone along with osteocalcin is working to increase cAMP level in MSCs in response to parathyroid hormone and prostaglandin E2 this is in turn increases MSCs differentiation. These chemical inducers are capable of stimulating osteoblast-like growth factor secretion, Collagen synthesis and ALP activity. β-GP, as an ALP substrate, provides ions, increases the activity of ALP enzyme, and accelerates the formation of mineralized extracellular matrix, as well as mineral deposits [34].

In this study an increase in calcium deposition and ALP activity was detected when both types of MSCs were incubated in an induction medium consisting of dexamethasone, ascorbic acid and β-GP. These results strongly suggest that BM-MSCs have the better capability to differentiate into osteogenic cells.

Since Runx 2 is considered as an early marker for osteogenic differentiation, early up-regulation and higher levels on day 5 in BM-MSCs compared to AD-MSCs was observed. We also studied the gene expression of osterix as a growth factor secreted by mature osteoblast. The mRNA of that gene was expressed on 5th day in BM-MSCs which showed a high level of transcripts compared to AD-MSCs. This result could estimate the high capacity for osteogenic differentiation of BM-MSCs along with the results of ALP activity and calcium content [35].

Although it is important to estimate the osteogenic differentiation capacity to select the suitable choices for bone therapies, the rate of proliferation and senescence-associated characteristics should be considered in the harvest, expansion and selection process.

Our results show that both BM-MSCs and AD-MSCs have the ability for proliferation. Furthermore, our study showed that AD-MSCs are growing more rapidly than BM-MSCs. the overall results of the present study is conflicting with the results of some previous studies in which equal capacity between BM-MSCs and AD-MSCs for osteogenesis has been suggested [36]. While, our results are in agreement with the studies which confirm that BM-MSCs are more efficient for osteogenesis more than AD-MSCs [37-41].

Conclusion

A comparative analysis was performed between the proliferation and osteogenic differentiation of BM-MSCs and AD-MSCs. Despite both types of stem cells showed some similarities and both BM-MSCs and AD-MSCs have the potential to differentiate into osteogenic lineage. We confirm that BM-MSCs have a greater osteogenesis potential.

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