International Association of Scientists
www.iasnetedu.com

Available online at www.jobiost.com
IJBLS 2022; 1(3):193-200

International Journal of BioLife Sciences

Original paper

Isolation and Characterization of Human Adipose Tissue Derived Mesenchymal Stem Cells

Sona Zare[1], Seyedeh Nasim Mirbahari[2,3]*

[1] Skin and Stem Cell Research Center, Tehran University of Medical Sciences, Tehran, Iran
[2] Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
[3] Research Institute for Gastroenterology and Liver, Shahid Beheshti University of Medical Sciences

***Received**: 28 September 2022* ***Revised**: 2 October 2022* ***Accepted**: 9 October 2022*

Abstract

Background and aim: Mesenchymal stem cells are a group of stem cells that have attracted a great deal of attention from researchers for the treatment of malignant diseases due to their inherent ability to differentiate into different cell lines. There have been numerous studies on the biological properties of Mesenchymal stem cells, but studies on the biological properties of Adipose-derived Mesenchymal stem cells are limited, particularly at the cellular and molecular level. In this study, we investigate the biological properties of Adipose tissue derived Mesenchymal stem cells at the cellular and molecular levels.

Materials and methods: In this experimental in vitro study, Adipose tissue was obtained from 10 patients and transferred to a cell culture laboratory and kept under standard conditions. Adipose cells were cultured in high glucose DMEM supplemented with 10% FBS culture medium. Finally, with the preparation of cells, their biological properties, such as surface cell markers, were analyzed by flow cytometry and finally the data were analyzed by statistical methods.

Results: In these cells, CD105-PE, CD73-PE, CD90-PE expression was over 95% and CD105-PE expression was less than 2% which follows the pattern consistent with the expression of CDs in MSCs. Adipose-derived cells had also the potential of adipogenic and osteogenic differentiation.

Conclusion: Morphology, cell cycle, expression of cell surface markers and differential potential in adipose-derived cells were similar to MSCs.

Keywords: *Mesenchymal stem cells, Adipose tissue, cell surface markers (CD markers), Apoptosis*

***Corresponding author:** Seyedeh Nasim Mirbahari, Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran; Research Institute for Gastroenterology and Liver, Shahid Beheshti University of Medical Sciences.

E-mail address: nasim.mirbahari@yahoo.com

Introduction

Mesenchymal stem cells are multipotent stromal cells that have the ability to differentiate into several types of cells, including bone cells, muscle cells, and cartilage cells. Mesenchymal stem cells can be found in bone marrow, umbilical cord cells, fat tissue and amniotic fluid. Mesenchymal stem cells have a regulatory role in the immune system, and their differentiation ability and antimicrobial properties can be mentioned among their other functions [1]. Mesenchymal stem cells have specific biological features such as surface markers (CD markers), differentiation and apoptotic features. Biomarkers are actually a way to detect and differentiate cells from one another. Physiologically, biomarkers play the role of receptor and ligand. Other roles of biomarkers include their role in signaling and cell-to-cell adhesion. It should be noted that some biomarkers are not involved in cell signaling [2]. Apoptosis or programmed cell death is one of the most important physiological processes that occurs in multicellular organisms. In fact, it can be said that biochemical events change the morphological characteristics of the cell and finally its death. Apoptosis plays an important role in body homeostasis. Programmed cell death is triggered through different pathways. Some of these pathways are triggered by the binding of ligands to cell surface receptors, and some of these pathways are triggered by the lack of some growth factors [3]. Research has shown that mature mesenchymal stem cells have surface markers such as CD 105^{+}, $CD73^{+}$, $CD90^{+}$, $CD34^{-}$, $CD45^{-}$, $CD11b^{-}$ [4]. Also, researches have shown that MSCs have various biomarkers, including microRNA451 and microRNA21 biomarkers [5]. Due to the increasing progress of medical science and the significant increase in the applications of mesenchymal stem cells in research, clinical and therapeutic fields, as well as the ability of mesenchymal stem cells to repair tissues of mesenchymal origin such as bone, cartilage, muscle, tendon and fat, in this study we decided to investigate the biological characteristics of adipose tissue-derived mesenchymal stem cells (ADSCs) such as surface markers, cell cycle and their differentiation potential.

Material and Methods

Isolation of MSCs

MSCs were isolated from human adipose tissue according to previous studies [6]. Informed written consent for sample collection was obtained from 10 patients (aged 30 to 58 years) undergoing lipoabdominoplasty. Adipose tissue was obtained using liposuction technique and transferred a sterile tube. PBS and antibiotics (400 unit/ml penicillin and 200 /lg/ml streptomycin (GIBCO (USA)) were added. The solution was centrifuged (1200 RPM, 5min). The supernatant was discarded. Collagenase I (0.1mg/ml) (Sigma-Aldrich (USA)), plus 0.5x CnT-GAB10 antibiotic was added to cell pellet and the solution incubated for 2 hours. The enzyme activity was neutralized by adding an equal volume of 10% culture medium (DMEM) (GIBCO (USA)) and the solution was centrifuged (1200RPM and 5min). The supernatant was drained and high glucose DMEM medium containing 10% serum (FBS) (GIBCO (USA)) was added to the cell pellet and the cells were transferred to a culture flask.

Cell Apoptosis and Viability Assay

To study the viability of the cells, Annexin-V and 7-AAD (BD Biosciences) were used according to the manufacturer's instructions. Briefly, cells were cultured, detached and washed and incubated with PE-conjugated Annexin-V and 7-AAD in Annexin-V-binding buffer at r/t for 15 min. The percentage of live and dead cells were analyzed in a Beckman Coulter Navios flow cytometer. Cell apoptosis was analysed by AO/PI staining or an annexin V-FITC/PI apoptosis detection kit (BD) with a FACSCalibur flow cytometer (BD). MSCs cells were seeded in 6-well plates for 48 h. The

cells were harvested and stained with AO/PI for 5 min or fixed with 1× binding buffer following incubation with 5 µL FITC-Annexin V for 15 min and 5 µL PI for 5min. Apoptotic cells were detected by microscopy or FACS.

Cell Cycle Analysis

After treatment, the harvested cells were detached for the cell cycle analysis with 0.25% trypsin-EDTA, washed with PBS and then fixed with ice-cold 70% ethanol for 2 h. Cells were rewashed with PBS and treated with 50 µg/ml RNase A for 30 min. Then, cells were incubated with Propidium Iodide (PI; Sigma-Aldrich, MO, USA). Flow cytometry analysis was performed using the BD FACS Calibur cytometer.

Evaluation of ADSCs Surface Markers

The expression of CD105-PE, CD73-PE, CD90-PE and CD14-PE antibodies was evaluated to determine the identity and prove the stemness of cells isolated from adipose tissue. For this purpose, the cells were trypsinized and suspended in 1 ml of serum-free culture medium and transferred to a microtube. 70 microliters of the microtube containing the cells was poured into each of the tubes. 5 tubes were mixed by vortex and aluminum foil was wrapped around it and placed in the refrigerator for 30 minutes, and then the expression level of surface markers and the percentage of cell populations expressing these markers were read by flow cytometry. Flow cytometric analyses were performed using Fortessa LSR Flow Cytometer (BD Biosciences) or BD Accuri C6 Plus (BD Bioscience). FlowJo software v.10 (FlowJo) was used for the data analysis.

Adipogenic Differentiation Assay

At P3, in vitro differentiation of MSCs was performed into adipocytes and osteocytes. For the adipogenic differentiation assay, 2.5×10^4 cells/well were seeded in 24-well plates for confluence. Cells were provided with an adipogenic induction medium that was LG-DMEM supplemented with 10% FBS, 0.1 mM IBMX (Sigma-Aldrich, USA), 10 µM rosiglitazone, 0.3 mM dexamethasone, 5 µg/mL insulin (Nova Nordisk, Denmark), and 1% penicillin-streptomycin, and amphotericin-B. After 48 h, the adipogenic induction medium was replaced with adipogenic maintenance medium, which composed of LG-DMEM supplemented with 1% Ex-cyte (Millipore, USA), 5 µg/mL insulin, 1% penicillin-streptomycin, and amphotericin-B for 7 days with medium change after every 48 h. Before staining with oil-red-O (ORO), the cells were washed with $DPBS^{-/-}$ and fixed with 4% buffered formalin (Sigma-Aldrich, USA) at room temperature for 30 min and rinsed twice with $DPBS^{-/-}$ to remove the traces of formalin. Subsequently, the working solution of ORO (6:4) was poured into all the wells and incubated in the dark at room temperature for 30 min. Cells were rinsed with $DPBS^{-/-}$ and visualized under an inverted light microscope. The ORO stain was eluted from the cells using anhydrous isopropanol, the absorbance measured at 490 nm and normalized as follow:

$$\text{ORO Quantification} = \text{ORO Concentration} / \text{Cell Number} \times 100$$

Osteogenic Differentiation Assay

For osteogenic differentiation, 2.5×10^4 cells/well were seeded in 24-well plates. After reaching confluence, the cells were provided with an osteogenic medium consisting of α-MEM supplemented with 10% FBS, 10 mM β-glycerophosphate (Sigma-Aldrich, USA), 50 µM ascorbate-2-phosphate, 100 nM dexamethasone, 0.75 nM vitamin-D3, 1% penicillin-streptomycin and amphotericin-B. After 21 days of incubation, the cells were stained with Alizarin Red Stain (ARS; Sigma-Aldrich, USA) to assess the degree of mineralization. Briefly, the medium was removed, and the wells were washed twice with $DPBS^{-/-}$. The cells were fixed with 4% formalin for 30 min, and washed again with $DPBS^{-/-}$. Then, ARS working solution (40 mM) was added and cells were incubated for 45 min in the dark. The dye was removed, cells were washed thrice with

DPBS$^{-/-}$ and observed under an inverted light microscope.

Statistical Analysis

The data sets presented in this manuscript were statistically analyzed and graphs were plotted by using Sigma Plot 12.0 software (Systat Software Inc., San Jose, CA, USA). An ANOVA was applied for the comparison of three or more groups. The analysis of the combined effects was performed with CalcuSyn software 2.0 (Biosoft). Data are expressed as mean ± SD. Statistical analysis was performed with IBM SPSS Statistics software version 20 (SPSS Inc., Chicago, IL, USA). Statistical significance was prescribed at $P < 0.05$.

Results

The Morphology of ADSCs

Stem cells were successfully isolated from adipose tissue sources. MSCs showed a typical fibroblast-like morphology (Figure 1). All the separated MSCs adhere to the plastic surface.

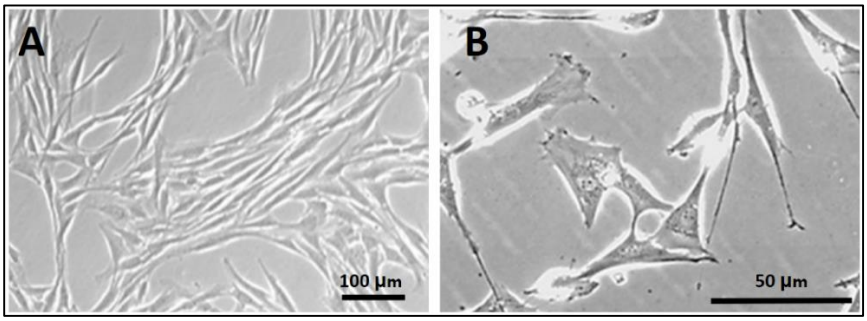

Figure 1. The phenotype of adipose tissue derived MSCs at passage 3 Panel A. (objective 20x) B. (objective 40x).

Cell Apoptosis and Viability Assay

Cell viability was measured by Annexin V/Propidium Iodide assay. It was shown that the viability of ADSCs was 98.06%.

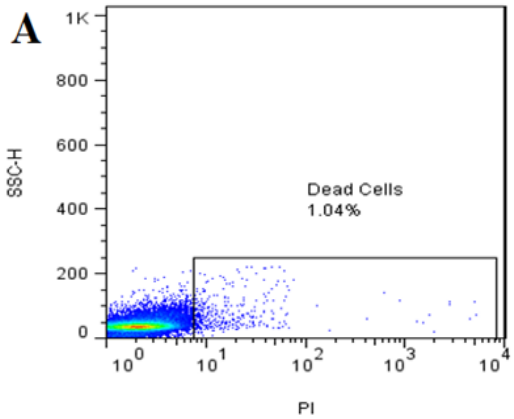

Figure 2. Indicates the viability of MSCs on the day of isolation

Cell Cycle Analysis

The accelerated cell cycle represents an increase in proliferation in ADSCs. The cell cycle consists of phase G1, phase S, phase G2, and phase M. During step S, DNA is duplicated, and cell growth

occurs in phases G1 and G2. In the ADSCs, the largest proportion of cells belonged to phase G1 at around 63%. The figure for phase S stood at 22.46%, while that of phase G2 was lower at nearly 5%.

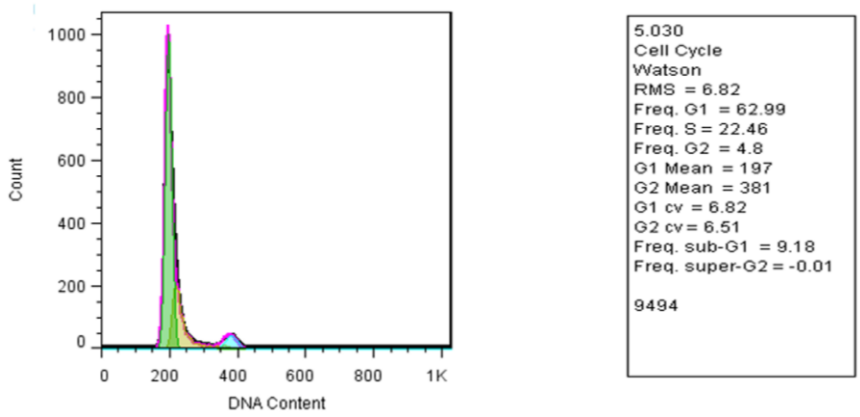

Figure 3. Cell cycle in ADSCs

Evaluation of ADSCs Surface Markers

At P3, the cell surface antigen expression of MSCs was evaluated by flow cytometry. It was indicated that the expression of CD105-PE, CD73-PE, CD90-PE were more than 95%. And the expression of CD14-PE was lower than 2%.

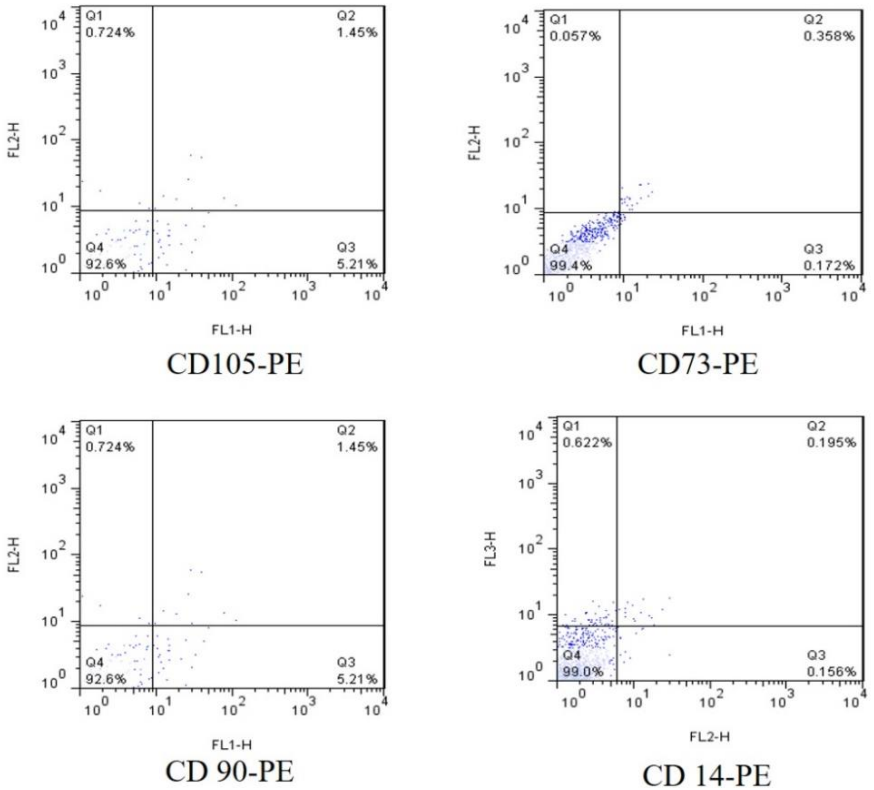

Figure 4. Flow cytometric analysis of ADSCs of the third passage-reaction of cells with anti- CD105-PE, CD73-PE, CD90-PE and CD14-PE.

Adipogenesis

To confirm adipogenesis, cells were stained with ORO for the identification of lipid droplets. The stained lipid droplets appeared red under in an inverted light microscope (objective 40×) as shown in figure. For quantitative analysis of ORO, the dye was eluted with anhydrous isopropanol and the absorbance was measured spectrophotometrically at 490nm. Adipose-derived MSCs showed a significant difference ($P<0.001$) in the concentration of ORO during adipocyte differentiation.

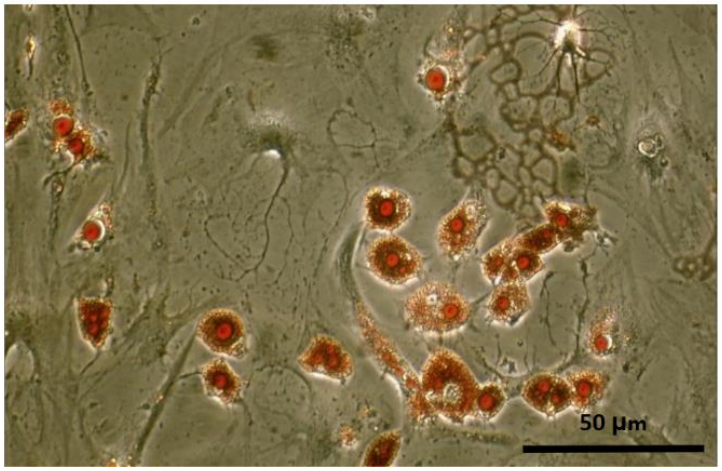

Figure 5. After 7days of adipogenesis in the adipogenic media the cells were stained with Oil-Red-O (Objective 20×). Scale bar=20µm

Osteogenesis

The osteogenic abilities of Adipose-derived MSCs were evaluated by treating them with osteogenic growth media for a period of 21days and staining with Alizarin Red Stain. The differentiated cells showed positive staining of the extracellular mineral matrix, as shown in figure 3.

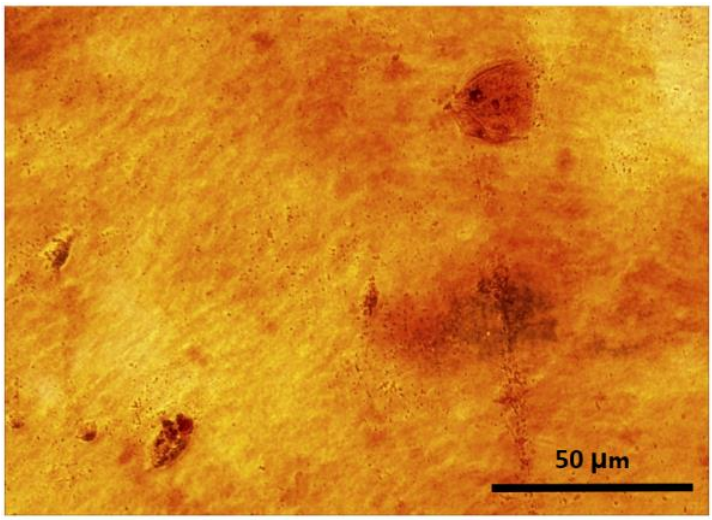

Figure 6. Alizarin red staining of adipose tissue derived MSCs in osteogenic medium after 21days. Osteogenic cells show deposition of hydroxyapatite mineral stained red with Alizarin red stain (objective 20×). Scale Bar=50µm.

Discussion

Mesenchymal stem cells were first discovered in bone marrow in 1976 by a researcher named Friedenstein. Today, mesenchymal stem cells play an important role in almost all fields of medicine, especially regenerative medicine, due to their ability to repair tissue and wound healing [7]. Currently, many researches have pointed out the use of mesenchymal cells in diseases such as multiple sclerosis (MS), stroke, acute leukemia, and joint and bone problem [8]. In general, stem cells are also used in cell therapy, but it should be noted that cell therapy may be dangerous, and in some cases, the death of patients who have been treated has been reported. Patients undergoing cell therapy may experience severe and even fatal allergic reactions. For this reason, investigating the effect of stem cells for the treatment of diseases is controversial. Mesenchymal stem cells can be extracted from bone marrow, umbilical cord, placenta tissue, amniotic fluid and fat tissue. In this research, we investigated the biological characteristics of mesenchymal stem cells derived from adipose tissue. As stated in the introduction, surface markers are one of the biological characteristics of mesenchymal stem cell. Scientists have shown that $CD105^{+}$, $CD73^{+}$, $CD90^{+}$, $CD166^{+}$, $CD31^{-}$, $CD34^{-}$, $CD45^{-}$ and HLA-DR are expressing on mesenchymal stem cells 15. The variable expression levels of these indicators are probably due to differences in species, tissue source and cultivation conditions [9]. In this study, the apoptosis of ADSCs cells has been investigated as a biological feature. Studies have shown that mesenchymal stem cells derived from adipose tissue induce apoptosis by activating caspase [10]. This study has been carried out in the field of biological characteristics of mesenchymal cells derived from adipose tissue. Due to the limited financial support, advanced cellular and molecular analysis was not available.

Conclusion

The present study showed that cells isolated from adipose tissue have the same morphology as mesenchymal stem cells. In these cells, CD105-PE, CD73-PE, CD90-PE expression was over 95% and CD105-PE expression was less than 2%. which follows the pattern consistent with the expression of CDs in MSCs. Adipose-derived cells had also the potential of adipogenic and osteogenic differentiation.

Acknowledgment

We thank our colleagues who provided insight and expertise that greatly assisted the research.

Conflict of interests

The authors declare that there are no competing interests.

Reference

[1]. Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. NPJ Regenerative medicine. 2019;4(1):1-15.

[2]. Vosoughi T, Bagheri M, Hosseinzadeh M, Ehsanpour A, Davari N, Saki N. CD markers variations in chronic lymphocytic leukemia: new insights into prognosis. Journal of Cellular Physiology. 2019;234(11):19420-39.

[3]. D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell biology international. 2019;43(6):582-92.

[4]. Guo KT, SchÄfer R, Paul A, Gerber A, Ziemer G, Wendel HP. A new technique for the isolation and surface immobilization of mesenchymal stem cells from whole bone marrow using

high-specific DNA aptamers. Stem cells. 2006;24(10):2220-31.
[5]. Huang JY, Zhang K, Chen DQ, Chen J, Feng B, Song H, Chen Y, Zhu Z, Lu L, De W, Wang R. MicroRNA-451: epithelial-mesenchymal transition inhibitor and prognostic biomarker of hepatocelluar carcinoma. Oncotarget. 2015;6(21):18613.
[6]. Mohamed-Ahmed S, Fristad I, Lie SA, Suliman S, Mustafa K, Vindenes H, Idris SB. Adipose-derived and bone marrow mesenchymal stem cells: a donor-matched comparison. Stem cell research & therapy. 2018;9(1):1-5.
[7]. Berebichez-Fridman R, Montero-Olvera PR. Sources and clinical applications of mesenchymal stem cells: state-of-the-art review. Sultan Qaboos University Medical Journal. 2018;18(3):e264.
[8]. Ma T. Mesenchymal stem cells: from bench to bedside. World journal of stem cells. 2010;2(2):13.
[9]. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nature reviews immunology. 2008;8(9):726-36.
[10]. Grisendi G, Bussolari R, Cafarelli L, Petak I, Rasini V, Veronesi E, De Santis G, Spano C, Tagliazzucchi M, Barti-Juhasz H, Scarabelli L. Adipose-Derived Mesenchymal Stem Cells as Stable Source of Tumor Necrosis Factor–Related Apoptosis-Inducing Ligand Delivery for Cancer TherapyAdipose Stromal/Stem Cells as TRAIL Vehicles. Cancer research. 2010;70(9):3718-29.