Isolation, Cultivation, Characterization and Expansion of Human Adipose-Derived Mesenchymal Stem Cell for Use in Regenerative Medicine

Ali M. Sharifi,[1, 2, 3, 4] Roshanak Ghazanfari,[4] Neda Tekiyehmaroof,[1] Mohammad A. Sharifi[1]

[1]Razi Institute for Drug Research, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran.
[3]Endocrinology and Metabolism Research Center (EMRC), Shariati hospital, Tehran, Iran.
[4]Cellular and Molecular Research Center, Tehran University of Medical Sciences, Tehran, Iran.

Sponsorship: This work was supported by a grant from Iranian stem cell network, Tehran, Iran.

Corresponding author: Dr. Ali M. Sharifi, PhD
Department of Pharmacology, School of Medicine,
Tehran University of Medical Sciences,
P.O.Box: 14155-6183
Tehran, Iran.
TeleFax: +98 21 88622523
E-mail: sharifal@yahoo.com, or amsharifi@tums.ac.ir

Abstract

Introduction: Stromal cells having preadipocyte characteristics can be isolated from adipose tissue, propagated in vitro and induced to differentiate in vitro toward the osteogenic, adipogenic, myogenic and chondrogenic lineages when treated with established lineage-specific factors.
In this research we isolated stromal cells from human adipose tissue and cultured and expanded and examined their stemness by determining their surface CD markers and their ability to differentiate into adipocyte lineage.
Material and methods: For isolating ASCs, raw lipoaspirates were washed with sterile phosphate-buffered saline (PBS) containing 5% penicillin/streptomycin. To digest the adipose tissue, aspirates were treated with 0.075% collagenase for 1 h. To differentiate the cell to adipocyte, confluent cells were exposed to adipogenic medium containing α-MEM, FBS, dexamethasone, indomethacin, IBMX, L-glutamin, and penicillin/streptomycin.
Results: Adhered cells were cultured for 2-3 weeks with replacing the media every 3-4 days and the ADSC were isolated, cultured and expanded. To examine the differentiation potential of the isolated cell, they were differentiated in specific medium and lipid droplets were appeared within the cells within 2-3 weeks. To confirm the lipid droplet, Oil red O lipid staining was used.
Conclusion: In conclusion, it could be taken to serious consideration that, besides, other sourses of mesenchymal stem cell, adipose derived are one the best and promising source being easily accessible and available by noninvasive method, as well as potential of being used in autologous cell transplantation in wide variety of disorders from nerve to cardiac injury from one side and musculoskeletal problem from other side.

Keywords: Adipose derived Mesenchymal stem cell, Regenerative medicine.

Introduction

The properties of adult stem cells that make them suitable for clinical uses are: ease of harvest, high expansion rate in vitro and multilineage differentiation capacity. Bone marrow derived mesenchymal stem cells (MSCs) have been the subject of considerable research. But some problems like donor site morbidity limit the amount of marrow that could be obtained. By contrast, a desired volume of adipose tissue, as a source of stem cells, can be easily harvested by liposuction. In this study, the stromal cells from human adipose tissue were isolated and examined their stemness by determining their surface CD markers and their ability to differentiate into adipocyte lineage.
Stem cells are a population of cells possessing self-renewal capacity, long-term viability and multilineage potential in differentiating to various

tissues. Because of ethical issues regarding the use of embryonic stem cells, adult stem cells have been proposed as an alternative source.(1)
Similar to bone marrow, adipose tissue is a mesodermally derived organ that contains a population of stem cells that can be enzymatically derived out of adipose tissue and make a homogeneous population in culture under suitable conditions for mesenchymal cells growth and exhibits stable growth and proliferation kinetics in culture.(2)
Stromal cells that have preadipocyte characteristics can be isolated from adipose tissue of adult subjects, propagated in vitro and induced to differentiate in vitro toward the osteogenic, adipogenic, myogenic and chondrogenic lineages when treated with established lineage-specific factors.(3) A variety of names have been used to describe the plastic adherent cell population isolated from collagenase digests of adipose tissue. Among them, the International Fat Applied Technology Society reached a consensus to adopt the term "adipose-derived stem cells" (ADSCs) to identify the isolated, plastic-adherent, multipotent cell population.(4)
Adipocytes develop from mesenchymal cells via a complex cascade of transcriptional and non-transcriptional events that occurs throughout human life.(5) Adipocyte differentiation is a complex process accompanied by coordinated changes in cell morphology, hormone sensitivity and gene expression that have been studied primarily in murine preadipocyte cell lines rather than in human preadipocytes.(6)
In this study, we isolated ADSCs from human adipose tissue and to confirm whether human derived ADSCs represent stem cell population features, we studied the expression of several CD marker antigens that are specific to mesenchymal stem cells. Besides, we induced human derived ADSCs to differentiate into adipocytes in order to examine their differentiation potential.

Material and methods

Isolation of mesenchymal stem cells from adipose tissue: The initial methods to isolate cells from adipose tissue were pioneered by Rodbell and colleagues in the 1960s.(7) Subsequently, this procedure has been modified for the isolation of cells from human adipose tissue specimens.(8) Initially, fragments of human tissue were minced by hand; however, with the development liposuction surgery, this procedure has been simplified. During liposuction, plastic surgeons infuse the subcutaneous tissues with a saline solution containing anesthetic via a cannula and then remove both the liquid and tissue under suction.(9) The procedure generates finely minced tissue fragments whose size depends on the cannula's dimensions. The adipose sample can be kept at room temperature for no more than 24 h prior to use. For isolating ADSCs from adipose tissue, raw lipoaspirates were washed extensively with sterile phosphate-buffered saline (PBS) containing 5% penicillin/streptomycin to remove contaminating debris and red blood cells. In order to tissue digestion, washed aspirates were treated with 0.075% collagenase Type I prepared in PBS containing 2% penicillin/streptomycin for 30 min at 37˚C with gentle agitation. The collagenase was inactivated with an equal volume of α-MEM containing 10% fetal bovine serum (FBS). The samples were centrifuged at 2000 rpm for 5 min and then were shaken vigorously to thoroughly disrupt the pellet and to mix the cells. The centrifugation step was repeated. After spinning, all the collagenase solution above the pellet was aspirated without disturbing the cells. The pellet was resuspended in 1 ml of lysis buffer, incubated for 10 min on ice, washed with 20 ml of PBS and centrifuged at 2000 rpm for 5 min. The cell pellet was resuspended in 2 ml of α-MEM supplemented with 20% FBS, 1% L-glutamine and 1% penicillin/streptomycin. The cell suspension was filtered through a 100µm mesh filter to remove debris. The filtrated sample was plated onto tissue culture flasks and incubated at 37˚C, 5% CO_2.
Culture and expansion of ASCs: Seventy-two hours after plating, the entire medium was aspirated from the flasks. The cells were washed with pre-warmed PBS several times in order to clean the cells thoroughly from any tissue fragments and blood cells. In the presence of fresh medium, cells were maintained in an incubator at 37˚C with 5% CO_2.

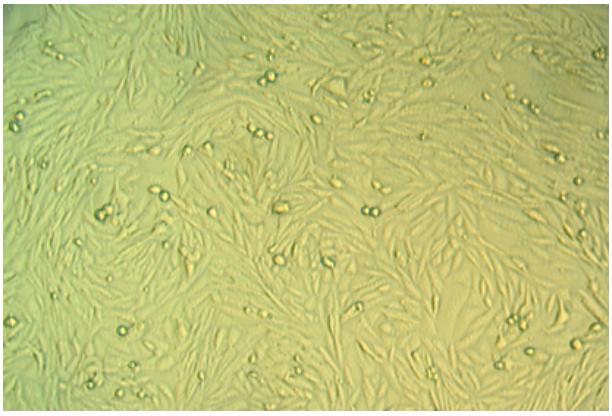

Figure- 1: Cells which were isolated and cultured until the cells reached 80-90% confluency.

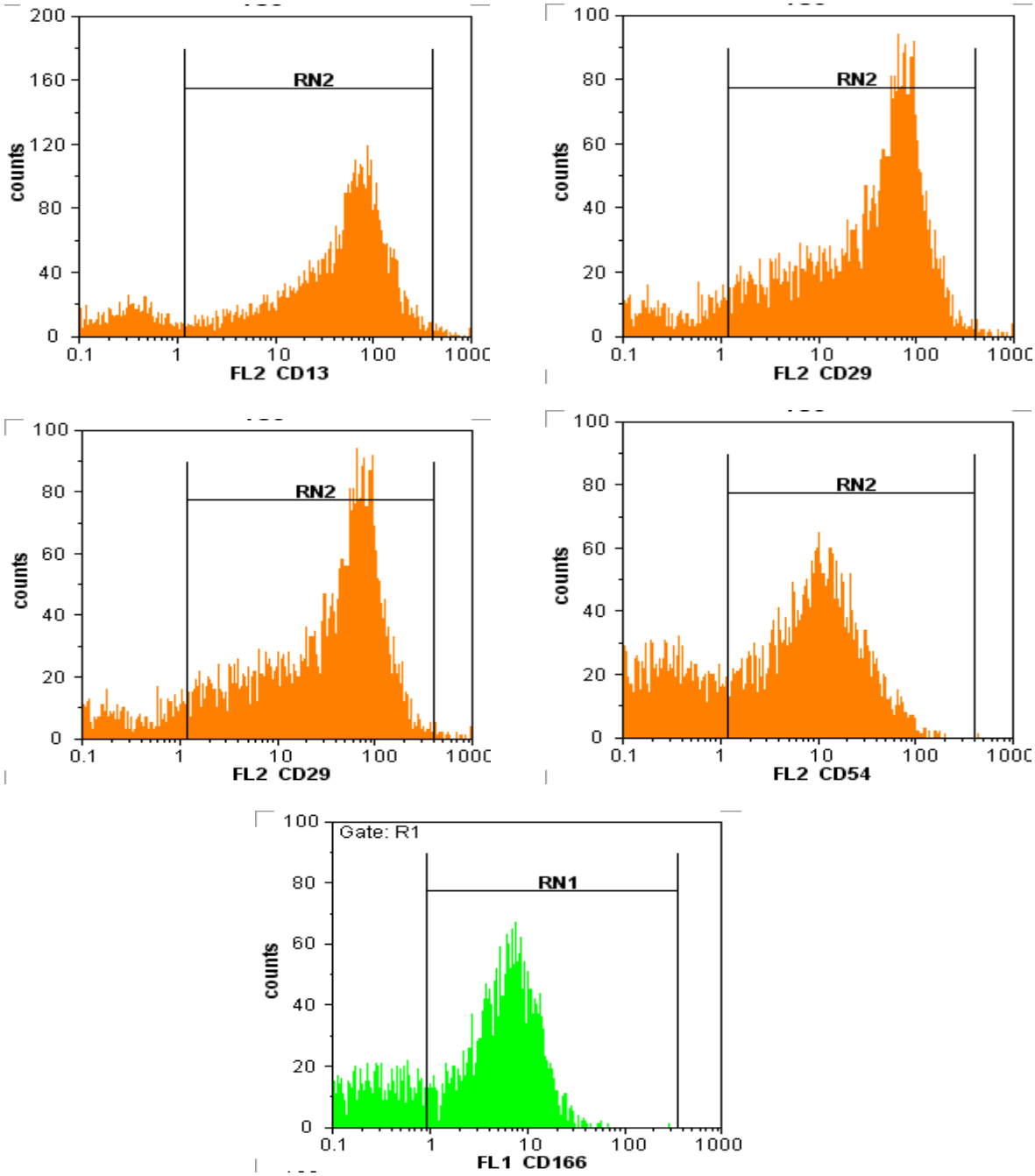

Figure- 2A. The cell surface phenotype of ADSCs was studied using flow cytometric analysis. The cells displayed CD13, CD29, CD44, CD54, CD166

The medium was changed every second day until the cells reached 80-90% confluency (figure-1).

Adipogenic differentiation: ADSCs were seeded in a 6-well plate at a density of 50,000 cells per well and cultured in complete growth medium containing α-MEM, 10% FBS, 2.0 mM L-glutamine and 1% penicillin/streptomycin.

When cells became fully confluent, adipogenic medium containing α-MEM, 10% FBS, 1.0 µM dexamethasone, 10 µg/ml insulin, 100 µM indomethacin, 500 µM IBMX, 2.0 mM L-glutamin, 1% penicillin/streptomycin was added to the wells.

The adipose differentiated cells would be obtained within 2-3 weeks.

Results

Cell Surface Characterization: The cell surface phenotype of ADSCs was studied using flow cytometric analysis. The cells displayed CD13, CD29, CD44, CD54, CD166 (Figure- 2A) and lack the expression of CD31, CD34, CD45 and CD 106 and CD117. (Figure- 2B)

Cells were cultured for 2-3 weeks with replacing the media every 3-4 days, and lipid droplets were appeared within the cells (Figure- 3A). In order to confirm the lipid droplet, Oil red O lipid staining was used. The cells were fixed in buffered formalin and then were stained for 3 minutes and droplet were appeared as cherry red spheres within individual cells (Figure- 3B).

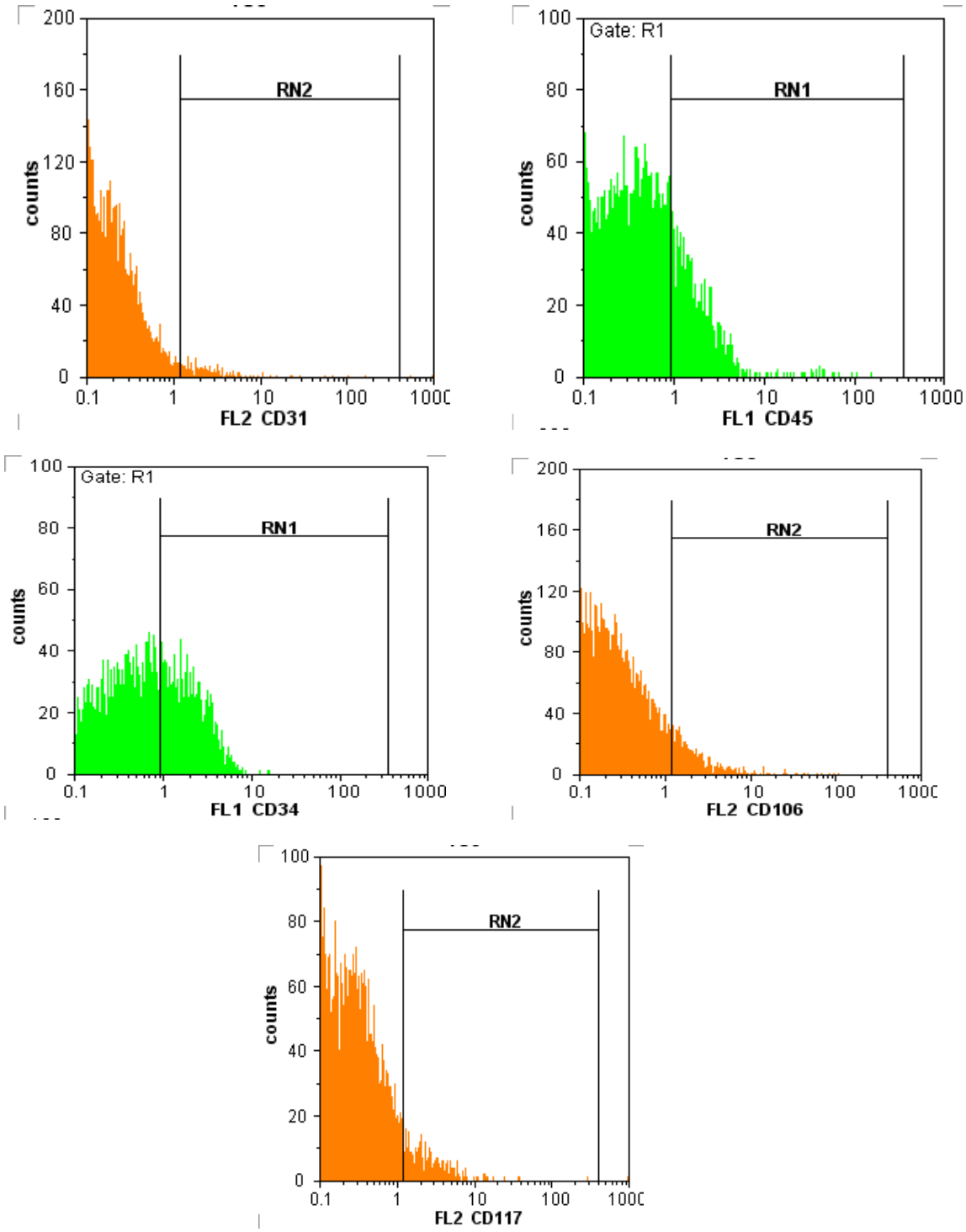

Figure- 2B. The cell surface phenotype of ADSCs was studied using flow cytometric analysis. The cells displayed lack the expression of CD31, CD34, CD45 and CD 106 and CD117.

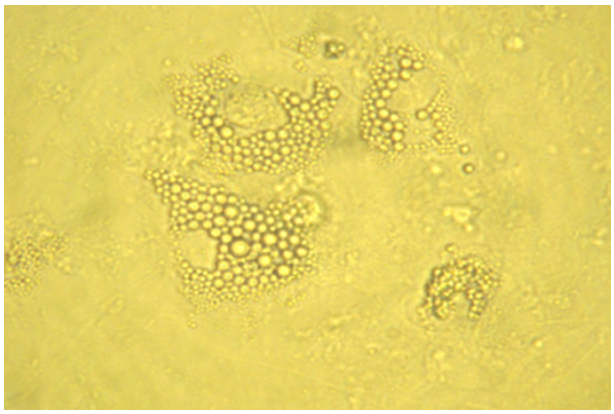

Figure- 3A. Mesenchymal stem cells differentiated into adipose cell, appearing in form of lipid droplets.

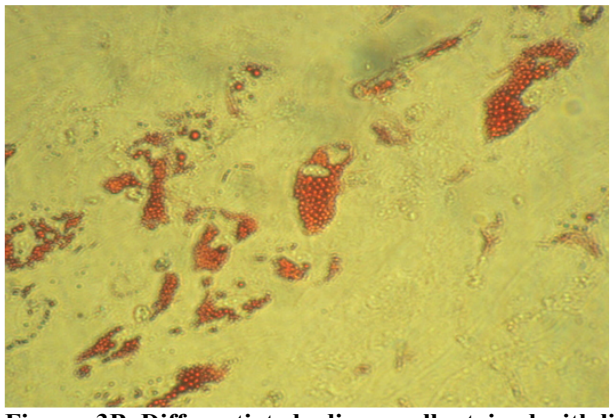

Figure- 3B. Differentiated adipose cells stained with lipid specific dye, Oil red O confirming lipid droplets.

Conclusion

Much of the work conducted on adult stem cells has focused on mesenchymal stem cells (MSCs) found within bone marrow. However, the clinical use of MSCs has presented problems, such as pain, morbidity and low cell number upon harvest.(10) This has led many researchers to find alternate sources for MSCs. Adipose tissue, like bone marrow, is derived from mesenchyme and contains a source of stem cells that could be easily isolated.

The proposed uses for ADSCs in tissue repair are so impressive. With its mesodermal origin, ADSCs are considered as a valuable tool for repairing bone and cartilage defects. However, the use of ADSCs is expanding to both the ectodermal and endodermal lineages, such as: stimulation of peripheral nerve repair,(11) functional recovery in spinal cord damage,(12) liver injury repair,(13) and treatment of diabetes.(14) Besides, in the field of surgery, ADSCs can be used widely as filler in plastic and cosmetic surgery.(15)

In conclusion, it could be taken to serious consideration that, besides, other sourses of mesenchymal stem cell, adipose derived mesenchymal stem cell are one the best and promising source being easily accessible and available by noninvasive method, easily expandable to millions of cells without significant changes in phenotype and genotype, as well as potential of being used in autologous cell transplantation in wide variety of disorders from nerve to cardiac injury from one side and musculoskeletal problem and also from other side.

References

1. Undale AH, Westendorf JJ, Yaszemski MJ, et al. Mesenchymal Stem Cells for Bone Repair and Metabolic Bone Diseases. Mayo Clin Proc. 2009; 84(10): 893-902.
2. Mvula B, Moore TJ, Abrahamse H. Effect of Low-Level Laser Irradiation and Epidermal Growth Factor on Adult Human Adipose-derived Stem Cells. Lasers Med Sci. 2010; 25(1): 33-39.
3. Zuk PA, Zhu M, Ashjian P, et al. Human Adipose Tissue Is a Source of Multipotent Stem Cells. Molecular Biology of the Cell. 2002; 13: 4279- 4295.
4. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell W, Katz AJ, Benhaim P, Lorenz HP, and Hedrick MH. Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies. Tissue Engineering. 2001; 7(2): 211- 228.
5. Niemelä S, Miettinen S, Sarkanen JR, et al. Adipose Tissue and Adipocyte Differentiation: Molecular and Cellular Aspects and Tissue Engineering Applications. Topics in Tissue Engineering. Vol. 4. Eds. N Ashammakhi, R Reis, & F Chiellini© 2008.
6. Gimble GM, Katz AJ, Bunnell BA. Adipose-Derived Stem Cells for Regenerative Medicine. Circ Res. 2007; 100: 1249- 1260
7. Heilbronn L, Smith SR, Ravussin E. Failure of Fat Cell Proliferation, Mitochondrial Function and Fat Oxidation Results in Ectopic Fat Storage, Insulin Resistance and Type II Diabetes Mellitus. Intern J of Obesity 2004; 28: S12- S21.
8. Bunnella BA, Mette Flaata M, Gagliardia C, et al. Adipose-derived Stem Cells: Isolation, Expansion and Differentiation. Methods in Stem CellR esearch, 2008; 45(2): 115- 120.
9. Zuk PA. The Adipose-derived Stem Cell: Looking Back and Looking Ahead. Mol Biol Cel. 2010; 21(11): 1783- 1787.
10. Moseley TA, Zhu M, Hedrick MH. Adipose-derived Stem and Progenitor Cells as Fillers in Plastic and Reconstructive Surgery. Plast Reconstr Surg. 2006; 118(3 Suppl): 121S- 128S.
11. Rochelle Nataloni. Adipose Stem Cell Developments Overseas Open New Doors for Cosmetic Surgery. Aug 2010.
12. van de Ven C, Collins D, Bradley M.B, et al. The Potential of Umbilical Cord Blood Multipotent Stem Cells for non-Hematopoietic Tissue and Cell Regeneration. Experimental Hematology. 2007; 35: 1753- 1765.
13. Strem B.M, Hicok KC, Zhu M, et al. Multipotential Differentiation of Adipose Tissue-derived Stem Cells. Keio J Med. 2005; 54 (3): 132-141.
14. Tuan R.S, Boland G, Tuli R. Adult Mesenchymal Stem Cells and Cell-based Tissue Engineering. Arthritis Res Ther. 2003; 5(1): 32- 45.
15. Otto T.C, Lane M.D. Adipose Development: from Stem Cell to Adipocyte. Crit Rev Biochem Mol Biol.2005; 40(4): 229 - 242.
16. Gregorie FM, Smas CM, Sul HS. Understanding Adipocyte Differentiation. Phsiol Rev. 1998; 78(3): 783- 809.
17. Gimble JM. Adipose Tissue-derived Therapeutics. Expert Opin Biol Ther. 2003 Aug; 3(5): 705- 713.
18. Chatterjea A, Meijer G, van Blitterswijk C, et al. Clinical Application of Human Mesenchymal Stromal Cells for Bone Tissue Engineering. Stem Cells International. 2010; 11; 215625.
19. Stevenson K, McGlynn L, Shiels PG. Stem Cells: Outstanding Potential and Outstanding Questions. Scott Med J. 2009; 54(4): 35- 7.