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IN VITRO REGULATION OF ADIPOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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ABSTRACT

Human adipose tissue acts as a practical supply of mesenchymal stem cells (hAMSCs) compared to other donor sites. So our attention was directed to study the adipogenic differentiation capacity of mesenchymal stem cells derived from human adipose tissue. Extraction of MSCs from human adipose tissue were done. After that we were studied the *in vitro* characterization of MSCs clustar differentiation(CD) surface marker. Moreover, we tested the ability of it to form fibroblast-like colony and differentiated into adipogenic tissues by special identification markers. Some identification tests have been done such as: viability test, Fibroblast-Like Colony- Forming Unit Assay and CD surface marker to assure that we have correctly obtained mesenchymal stem cells. These hAMSCs expressed CD29, CD 90, CD 105 and CD13 but not CD14 and CD34. RT-PCR of adipogenic differentiated hAMSCs expressed Lipoprotein Lipase, Leptin , Adiponectin, peroxisome proliferator-activated receptor-γ and a transcription factor known to be involved in control of adipocytic differentiation at different time intervals after 4, 7, 14 and 21 days. HAMSCs have the ability to proliferate into monolayer culture and multilineage adipogenic differentiation as a result of treating with inductive conditions and thus have potential clinical applications in regenerative medicine.

KEYWORDS: Human Adipose Tissue, Mesenchymal Stem Cells, Surface Marker, Differentiation, Adipogenesis and Gene Expression.

1. INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent cells, that have intrinsic capability of self renewal[1] and differentiation into several tissue type.[2] The primary source of MSCs was bone marrow.[3] MSCs have been extracted from a number of tissues especially from adipose tissue with 100% efficiency.[4,5] Adipose tissue is an abundant source of MSCs about >100-fold more than bone marrow.[6] Also, it can be extracted with less aggressive procedures and without hurt to the donor, compared to other tissues. As a result, adipose tissue progress advantages in terms of its accessibility, abundance and regenerative capacity, as well as the willingness of many individuals to consent to its donation for tissue engineering and regenerative medical applications.[7] Recently, Adipose-derived mesenchymal stem cells (ADSCs) are able to differentiate into adipogenic, chondrogenic, myogenic and osteogenic cells.[8] Thus, MSC become of potential interest due to their applications in tissue engineering.[9] As a result, it is an easily harvested source of stem cells and can be reached in an ethically conventional manner, which is necessary for regenerative medicine. Human mesenchymal stem cells (HMSCs) are fusiform, fibroblast-like cells. They can also form colonies called in analogy with (HSC colony forming unit-fibroblasts [CFU-f]) through their initial *in vitro* growth.[10] Differences in culture procedures or differentiation step of the cells may be the reasons of causing differences between the previous works in the surface marker characteristics. Adipogenesis process is a method of cell differentiation by which preadipocytes become adipocytes**.**[11] Thus, the term of adipocyte differentiation is defined as a process that required activation of some transcription factors like (PPAR-ɣ)[12], CCAAT/enhancer-binding protein and (C/EBP) gene family.[13] Also, insulin/insulin-like growth factor are factors that enhance adipogenesis process.[14] Beside the previous activations of the differentiation process, extra cellular matrix proteins have also an significant role in regulation.[15] Adipogenic differentiation process also improved by the addition of inducing agents that including: dexamethasone that motivates the glucocorticoid receptor pathway and (1-methyl-3-isobutylxanthine, MIX) or (3-isobutyl-1-methylxantine (IBMX)) that is used the cAMP-dependent protein kinase pathway. In combination with these inducing agents also high concentrations of insulin have been used.[16] The participation of glucocorticoid, cAMP signaling pathways and insulin/insulin-like growth factor 1 (IGF-1) have been assured in the adipocyte differentiation process.[17]

In the present study we used PLA cells. These cells were took from fresh human subcutaneous adipose lipoaspirate. By studing the heterogeneity and growth kinetics of these

cells we confirmed that it may have multi-lineage potential.[18] The main aim of this study was two folds: to approve the occurance of stem cells in human adipose tissue and to test the differentiation capacity of a well characterized stem cell population (hMSCs) into adipocytes.

2. MATERIALS AND METHODS

2. 1- Isolation of human adipose tissue stem cells

Human adipose tissue was obtained from plastic surgery of twenty five patients of abdominal liposuction. The permission forms were accepted by the Institutional Review Board for Human Investigation of Mansoura University Hospital. We obtained written approval from all patients. The adipose tissue was already harvested and put in a sterile specimen container. Preservation of the harvested sample must be at room temperature and deal with it within 8 hours of operation. Avoid refrigeration as lipids solidifies at low temperatures. Processed about 60 gm of adipose tissue via ten 15ml disposable centrifuge tubes.

2. 1.1- Isolation of MSCs from human lipoaspirates

Processed lipoaspirate (PLA) cells were extracted from fresh human subcutaneous adipose lipoaspirate according to issued procedures with some slight modifications.[19] Briefly, erythrocytes and contaminating debris were removed by addition of sterile phosphate buffered saline (PBS) for washing raw lipoaspirates. The washed aspirates were treated with 0.075% type IA collagenase with PBS at 37°C for 30 min with gentle agitation for digestion. The collagenase activity was neutralized by the addition of DMEM with 10% FBS, because over digestion may cause destruction of the cells and decrease the final yield. The prelast step was to Isolate the stromal vascular fraction (SVF), this was done by filteration of the formed cell suspension using a 100-mm cell strainer then centrifuged at 2,000 rpm for 5 minutes. After that, resuspension of cell pellets in DMEM with 10% FBS, L-glutamine (1%) and penicillin/streptomycin then incubated for 24 hours at 5% CO2, 37°C. After incubation for 24 hours debris and unattached cells were detached by aspiration and the adherent cells was treated with fresh medium. During that the medium was changed two times a week till reaching 80% confluence.

2. 1.2- Harvesting and culturing of MSCs

The hAMSCs are usually cultured in CO2 incubator (36°±1°C) containing 5% regular media (DMEM, 10% FBS and 1% penicillin/streptomycin, Sigma). Freshly prepared media was carried out every 3 days to get rid of non-sticky cells twice a week afterwards at least 3 weeks. Cells were trypsinized using (0.25% trypsin and 0.1% EDTA) until achieved 90%

confluence and prolonged until passage four and so they were analyzed. At this point, cultures were established for surface marker characterization, and to test differentiation capacity.

2. 2- *In Vitro* Assessment of Stem Cells

2. 2.1- Viability Test

Consistent with Maclimans method Trypan blue exclusion was used for testing stem cells viability.[20] Mix equal volume of both 0.04% trypan blue and sample then incubate for 10 minutes at 37°C. By using a haemocytometer check the number of viable cells (unstained) by light microscope. The number of viable stem cells/ml was then determined by the next calculation.

$$\text{Viable cells (\%)} = \frac{\text{Number of viable cells / ml}}{\text{Total number of cells / ml}} \times 100$$

2.2.2- Fibroblast Like Colony Forming Unit Assay

Firstly, cells were seeded on tissue culture six well dishes, fibroblast like colony growth was assessed. Thus, at the density of (25×10^6) cells/well total hAMSCs were plated. The ability of hAMSCs in forming fibroblast like colonies was evaluated after 7 days. Then, images that appear hAMSCs morphology were taken by contrast phase microscope.

2.3- Cell surface antigens expression

For immunophenotypic characterization hAMSCs cells at third passage were trypsinized for 3min at 37°C, collected and washed then resuspended in PBS. Then, cells preserved at room tempreture with the isotype control mAbs or with the specific anti-human antibodies such as anti -CD29-PE, -CD90-PE, -CD34-FITC, -CD105-PE, -CD14-PE and -CD13-PE were done for 30min. The cells washed twice in PBS then fixed in fix as recommended. Afterwards, the definite fluorescent labeling was examined by FACS Calibur flow cytometer by using the Cell Quest software.

2.4- Adipogenesis assay

It was accomplished in monolayer culture in six well dishes.[2,21] Briefly, about 15,000 cells were cultured per well till reach confluency. Afterwards, the cells were cultured with the adipogenic induction medium (AIM) for 72h. Then cultured with adipogenic maintenance medium (AMM) for 24h. These steps were repeated four times. After that, the cells were grown in AMM for another week. While, the negative control cells were treated with base

medium which including 10% FBS. Finally, adipogenic differentiated stem cell samples were taken for examination by light microscopy and RT-PCR study at different time intervals after 4 days, 7 days, 14 days and 21 days. **AIM:** involving DMEM as base medium with 10% FBS in addition to streptomycin/penicillin solution (100 U/ml), (0.2 mM) Indomethacin, (1 µM) dexamethasone, (0.1 mg/ml) insulin and (1 mM) (IBMX). **AMM:** involving of DMEM as base medium with 10% FBS beside penicillin (100 U/ml), streptomycin (100 µg/ml) and (0.1 mg/ml) insulin.

2.4.1- Oil Red-O staining

After adipogenic differentiation cells were grown in petri dishes for various time intervals at 4, 7, 14 and 21 days in medium consisting of [DMEM, (200 µM) indomethacin, (10 µM) insulin, (1 µM) dexamethasone and (0.5 mM) isobutyl-methylxanthine].[22] After that, the medium was removed, the cells were washed in PBS and fixed for 30 min in 3.7% formaldehyde, then remove the fixative and add water until performing the dye-staining. Staining with Oil Red O for 30 min were done after fixation. Then, plates were washed two times in water and photographed using microscope.[23] Afterwards, by counting adipocytes at magnification (100) in five different visual fields adipogenic differentiation were quantified for every sample (n=5). Finally, determining the percentage of adipogenic cells was occured.

percentage of adipogenic cells = (number of cells with oil red O-positive intracellular vesicles/total number of cells).

Oil Red O preparation: diluting a stock solution which involving of 0.5 g of Oil Red O in 100 ml of isopropanol with water (6:4) and then filtered.

2.4.2- Gene expression by RT-PCR

Extraction of total RNA from hAMSCs was occured and reverse transcriptase PCR was achieved. For detecting adipogenic expressed gene adipogenic differentiated samples were taken at different time intervals after 4, 7, 14, and 21 days. The primer sequences as follows for adiponectin were used forward primer: GGGATTACTGCAACCGAAGG and reverse primer: CCATCCAACCTGCACAAGTTT, For leptin forward primer: TTCACACACGCAGTCGGTATC and reverse primer: GTGAAGCCCGGGAATGAAG, for lipoprotein lipase forward primer: GTACAGTCTTGGAGCCCATGC, and reverse primer: GCCAGTAATTCTATTGACCTTCTTGTT, peroxisome proliferator-activated receptor-γ forward primer: CATACATAAAGTCCTTCCCGCTG and reverse primer:

TTGTCTGTTGTCTTTCCTGTCAAGA and glyceraldehyde-3-phosphate dehydrogenase forward primer: ACAAGATGGTGAAGGTCGGTG and reverse primer: AGAAGGCAGCCCTGGTAACC. Afterwards, the procedure of RT-PCR was achieved. First step for reverse transcription which occurred at 50°C for 30min then 95°C for 15min. Second step consisting of 35 cycles. Every cycle involving denaturion at 94°C, annealing at 57°C, elongation at 72°C for 1min of each and the final extension at 72°C for 10min. Finally, the amplified DNA fragments were visualized over 2% agarose gel electrophoreses and photographed under UV light.[24] GAPDH acts as an internal standard.

2.5- Statistical analysis

SPSS version 16 was used for calculating of statistical analysis. Then, data were resulted as mean ± SD. To compare the groups we used One-way ANOVA and Duncan's post-hoc test. $P<0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

MSCs are clonogenic cells that able to multilineage differentiation into mesodermal cells like osteoblasts, adipocytes and chondrocytes.[25] Currently, MSCs presented a great effort in various clinical medicial applications and through different ways of administration. Many of these applications have been confirmed the safety and efficiency of MSCs. This all is because of their easily isolation and their differentiation potential.

3.1. Morphological character of the cultured MSCs

Mesenchymal stem cells were obtained from human adipose tissue after purification. By growing as a minimum of two passages in culture medium hAMSCs were then generated. Afterwards, debris and contaminating hematopoietic cells were removed through passage one. HAMSCs were featured by a fibroblast like appearance in morphology (Fig.1A). In addition to a fibroblast-like appearance of (hAMSCs) also have the ability to form fibroblast like colonies. After (3–5) days in culture we noticed colony formation (Fig.1B). The colonies became confluent after (6–7) days in culture and were passaged for the first time. These results are similar to those described inprevious studies.[26,27]

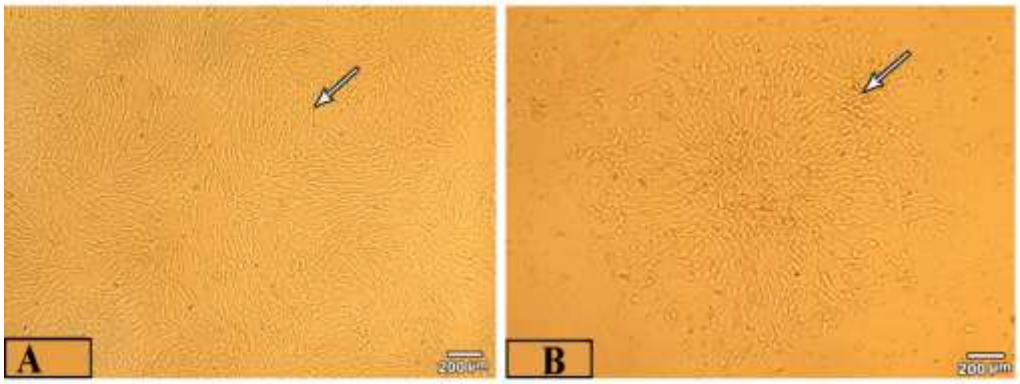

Fig. 1 (A): Photomicrograph showing cultured human adipose mesenchymal stem cells under an inverse microscopy at passage 3 which were featured by a fibroblast like appearance in morphology. B) Photomicrograph showing colonies formation of hAMSCs after one week of grewing in culture that had heterogeneous small spindle-shaped fibroblastoid cells and more rounded cells. (Scale bar = 200 µm).

3.2. *In Vitro* Assessment of Stem Cells.

After harvesting and culturing of MSCs from human adipose tissue "*In Vitro* Assessment of Stem Cells" should be made to assure that we have correctly obtained viable mesenchymal stem cells, some identification tests have been done such as: viability test and Fibroblast-Like Colony- Forming Unit Assay.

3.2.1. Viability Test

Since cells are very selective in the compounds that pass through the membrane and they lose this selectivity when they are dead. Our results showed that all hMSCs obtained were viable and did not accept the stain, as the viability percentage was 100% (Fig.3A, B).

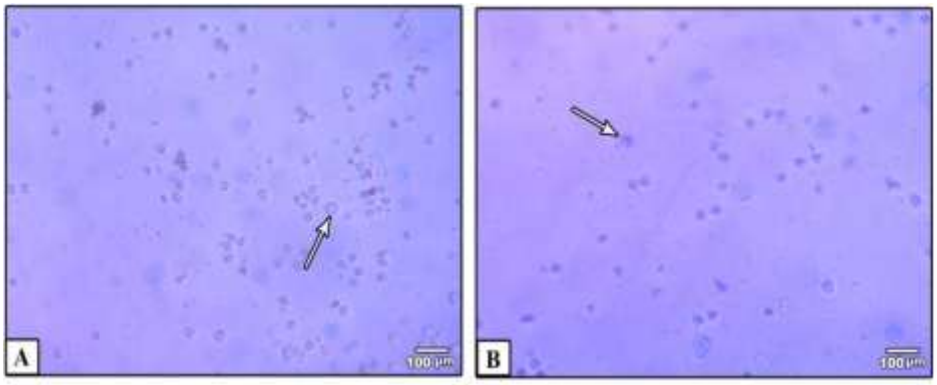

Fig. 2 (A): Light photograph of viable hAMSCs (arrow). hAMSCs didn't accepted trypan blue stain.(Scale bar = 100 µm). (B) Light photograph of non-viable hAMSCs (arrow). hAMSCs accepted trypan blue stain. (Scale bar = 100 µm).

At this point of the current study investigation of cell surface markers occured. Definitely, Diveristy in cell surface markers have been constantly exhibited between MSCs, isolated from humans. Ultimate clusters of differentiation of hAMSCs not only distinguish them from other cell populations in cell culture, but also facilitate clearance.

3.2.2. Flow cytometry for cell surface expression assay

Characterization of the cell surface antigen expression of hAMSCs were analyzed by flow cytometry during passage four to eight. Human clusters of differentiation that used in our study: [CD13, CD14, CD29, CD34, CD90 and CD105]. Flow cytometric analysis showed positive expression of CD13 (89%), CD29 (94%), CD 90 (92%) and CD 105 (92%) and negative expression of CD14 (4%) and CD34 (6%) (Fig. 3). This is in consistence with the findings of Dominici, M. *et. al.*, a working group within the International Society for Cytotherapy have recommended a number of surface markers as a minimal standards for determining the MSC.[28]

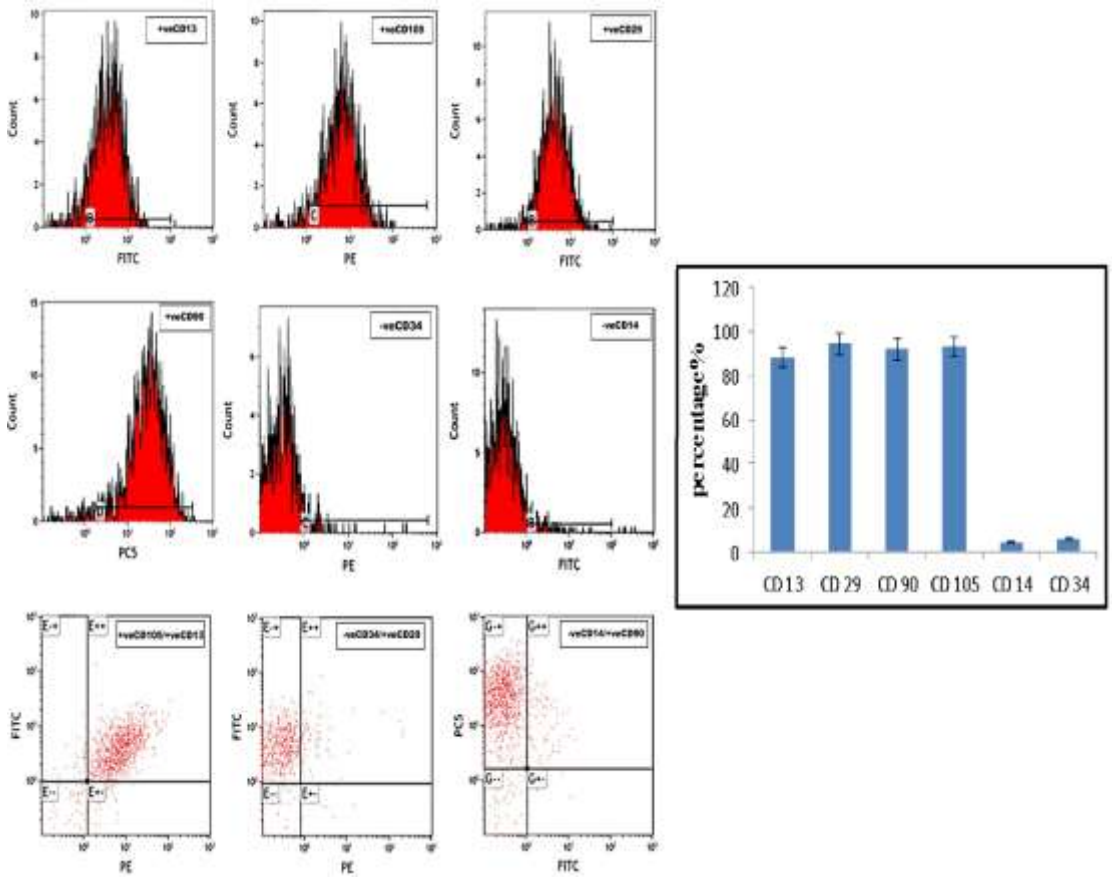

Fig. 3: Flow cytometric analysis of hAMSCs showed positive expression of CD13, CD29, CD 90 and CD 105 and negative expression of CD14 and CD34.

In order to assure the capacity of adipogenic multilineage differentiation of expanded MSC we used functional assays of *in vitro* cell differentiation.[29] These standard methods that help in testing the differentiation of MSC into the adipogenic lineages (see materials and methods). Testing this capacity require specific stimuli such as drugs, growth factors and hormones. These enhancers work in definite cell receptors which transport signals of differentiation. Our work supported many observations. Gregoire[30] demonstrated that preadipocytes differentiation into fats occurs in the existence of fetal bovine serum.

3.4. Adipogenic differentiation capacity

HAMSCs cultured with adipogenic medium. By using phase contrast microscopy we found that these cells have more fat vacuoles in comparison of the control ones. Adipogenic differentiation was slightly appeared at day 4 after incubation with adipogenic induction medium. Intensity of stained cells was gradually increased with days 7, 14 and 21 of culture periods (Fig.4). Detection the presence of neutral lipids in these vacuoles revealed by Oil Red-O staining for fat (Fig.5(A-D)). While control cells appeared negative for fat vacuoles (Fig.5E). Moreover, the stain showed appearance in nearly 80–90% of the cells treated with induced medium. This confirms that adipogenic differentiation of hAMSCs was highly efficient.

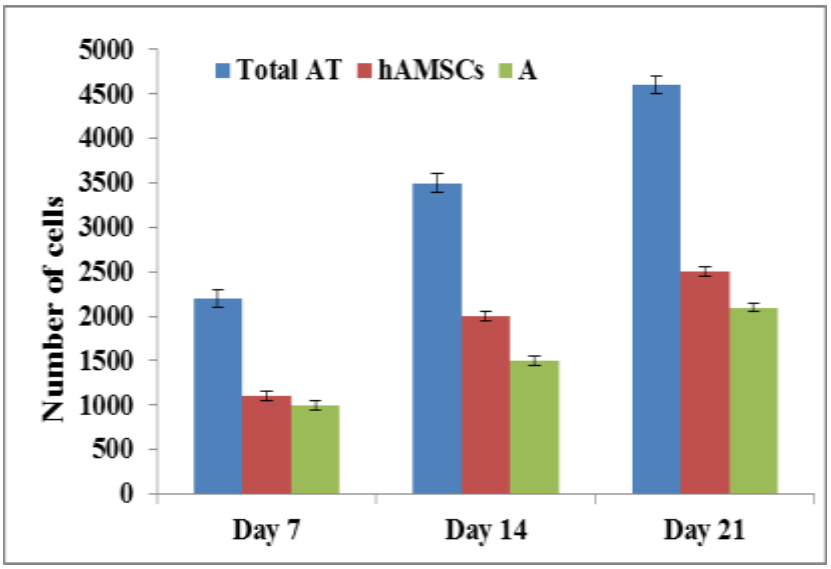

Fig. 4: Adipocyte (A) differentiation from human adipose tissue (AT). Total hAMSCs and AT significant at (P < 0.05).

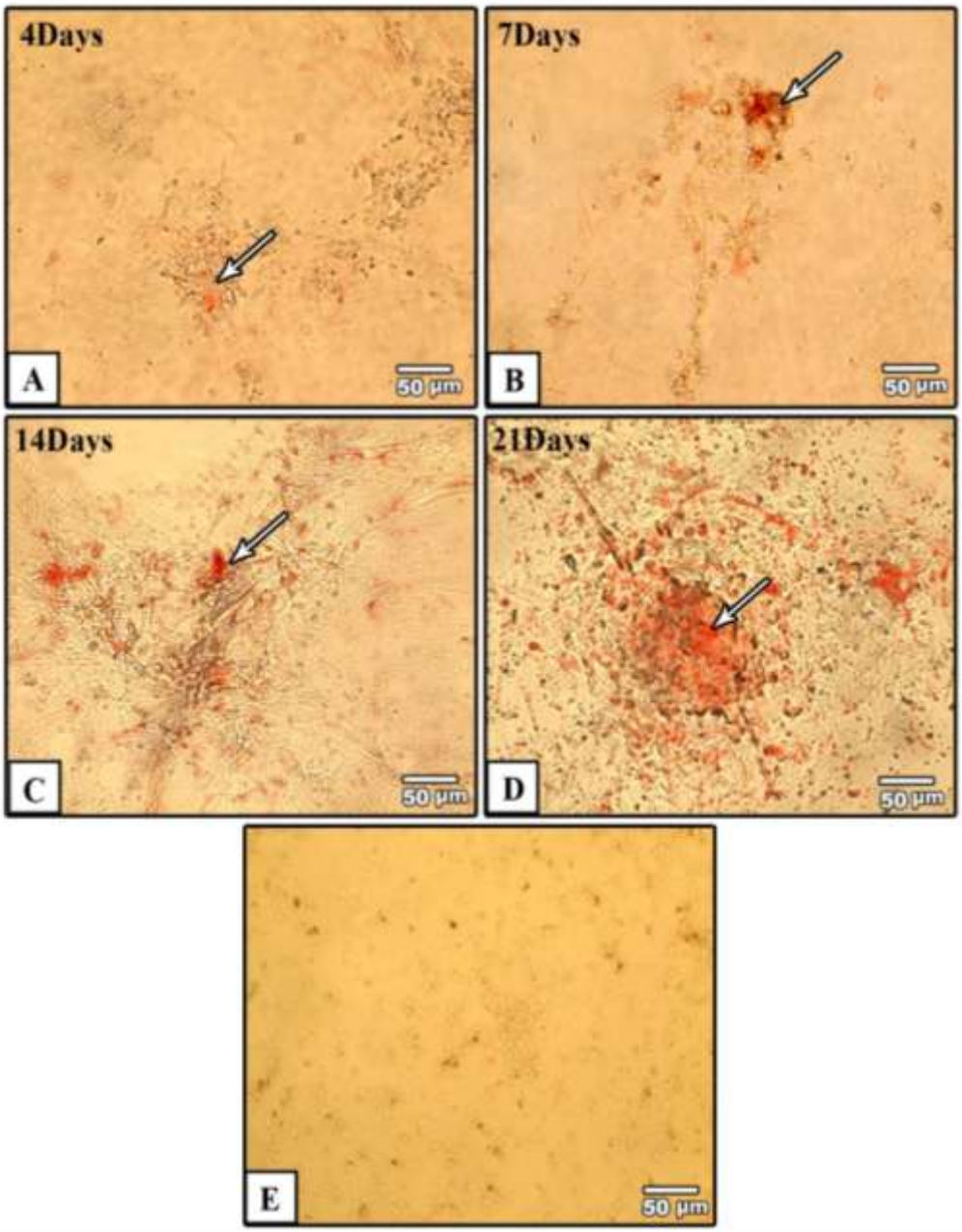

Fig. 5: Explanations of adipogenic differentiation capacity with Oil Red O staining using light microscopic. Observation of abundant quantities of fat vacuoles in the cells (A-D) in the cultures with induced medium. Detection the presence of neutral lipids in these vacuoles revealed by Oil Red-O staining for fat (arrows). While control cells appeared negative for fat vacuoles (E). Scale bar = 50 µm (A-E).

From the present findings the potentiation of proliferation of MSCs derived from human liposuction into adipocyte were confirmed by marked increase of Oil Red-O staining.

3.5- Gene expression during and after differentiation

Confirmation of differentiation occurred by RT-PCR analysis of adipocytic markers gene expression was observed at different time intervals after 4, 7, 14 and 21 days (Fig. 6). Gel electrophoresis for mRNA expression of PPAR-γ showed that: lane 4d, 7d, 14d and 21d made bands appeared at 130 bp and assured the existence of PPAR-γ adipocytic transcription factor. Gel electrophoresis for mRNA expression of LPL, Leptin and Adiponectin showed that: lanes 4d, 7d, 14d and 21d made bands appeared of adipocytic marker, at 150 bp, 120 bp and 100 bp, respectively proved the formation of LPL, Leptin and Adiponectin adipocytic marker during adipogensis which is consistant with the findings of Linehan, C., *et.al.*[31]

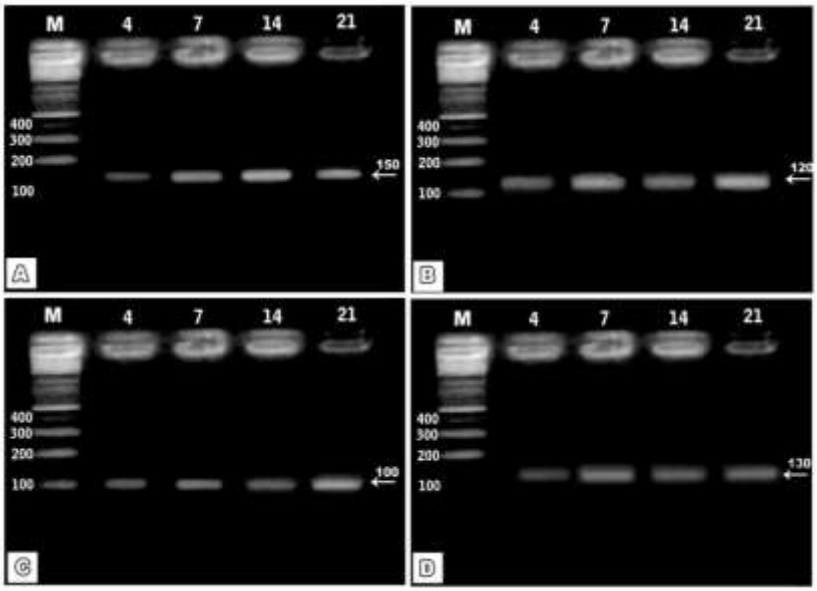

Fig. 6: Gel electrophoresis of adipose MSCs: lane M (DNA marker) and lane 4d, 7d, 14d and 21d showed bands present and assured the formation of adipocytic marker. (A) LPL at 150Bp, (B) Leptin at 120Bp, (C) adiponectin at 100Bp and (D) PPAR-γ at 130Bp.

Adiponectin is a protein secreted by adipose tissue that demonstrates both anti-atherogenic and insulin-sensitizing effects. Insulin resistance is closely caused reduced the production of adiponectin.[32] Adiponectin plasma concentration is inversely proportional with body mass so appeared at low concentration in obese subjects.[33] Reduced endothelium-dependent dilatation in both diabetic and nondiabetic patients is related with hypoadiponectinemia.[34] Adipose tissue produce anti-inflammatory vasoactive hormones like adiponectin that have an essential role in protecting vascular dysfunction in diabetes.

4. CONCLUSION

The presented data has confirmed that hAMSCs have the ability to multilineage potential and differentiate into specialized adipocytes when cultured with the adipogenic induction medium. Furthermore, during adipogensis they have the ability to express PPAR-γ, LPL, Leptin and Adiponectin. These differentiated cells have the same structural features as cells presented in the main tissues as demonstrated by phase microscopy and gene expression techniques. To enhance the role of hAMSCs in regenerative medicine in the future, a great effort into the differentiation potential of hAMSCs should be done.

5. REFERENCES

1. Sekiya, I., Larson, B. L., Smith, J. R., Pochampally, R., Cui, J. G., Prockop, D. J., Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. Stem Cells, 2002; 20(6): 530-41.
2. Pittenger, M.F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., Moorman, M. A., Simonetti, D. W., Craig, S., Marshak, D. R., Multilineage potential of adult human mesenchymal stem cells. Science, 1999; 284(5411): 143-7.
3. Owen, M., Marrow stromal stem cells. J Cell Sci Suppl, 1988; 10: 42-60.
4. Lee, M.W.Y., M. S. Park, J. S. Kim, H. C. Kim, Y. J. Choi, J., Isolation of mesenchymal stem cells from cryopreserved human umbilical cord blood. Int J Hematol, 2005; 81(2): 126-30.
5. Rebelatto, C.K.A., A. M. Moretao, M. P. Senegaglia, A. C. Hansen, P. Barchiki, F. Oliveira, J.Martins, J. Kuligovski, C. Mansur, F. Christofis, A. Amaral, V. F. Brofman, P. S. Goldenberg, S. Nakao, L. S. Correa, A., Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. Exp Biol Med (Maywood), 2008; 233(7): 901-13.
6. Sakaguchi, Y.S., I. Yagishita, K. Muneta, T., Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum, 2005; 52(8): 2521-9.
7. Bourin, P.G., M. Peyrafitte, J. A. Fleury-Cappellesso, S. Gomez, M. Rage, C. Sensebe, L., Mesenchymal Progenitor Cells: Tissue Origin, Isolation and Culture. Transfus Med Hemother, 2008; 35(3): 160-167.
8. Im, G.I.S., Y. W. Lee, K. B., Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? Osteoarthritis Cartilage, 2005; 13(10): 845-53.

9. Nambu, M., Ishihara, M., Nakamura, S., Mizuno, H., Yanagibayashi, S., Kanatani, Y., Hattori, H., Takase, B., Ishizuka, T., Kishimoto, S., Amano, Y., Yamamoto, N., Azuma, R., Kiyosawa, T., Enhanced healing of mitomycin C-treated wounds in rats using inbred adipose tissue-derived stromal cells within an atelocollagen matrix. Wound Repair Regen, 2007; 15(4): 505-10.
10. Owen, M., Marrow stromal stem cells. J Cell Sci Suppl, 1988; 10: 63-76.
11. Mandrup, S.L., M. D., Regulating adipogenesis. J Biol Chem, 1997; 272(9): 5367-70.
12. Barquissau, V., Ghandour, R. A., Ailhaud, G., Klingenspor, M., Langin, D., Amri, E. Z., Pisani, D. F., Control of adipogenesis by oxylipins, GPCRs and PPARs. Biochimie, 2017; 136: 3-11.
13. Suryawan, A., Swanson, L.V., Hu, C.Y., Insulin and hydrocortisone, but not triiodothyronine, are required for the differentiation of pig preadipocytes in primary culture. J Anim Sci, 1997; 75(1): 105-11.
14. Ross, M., Kaye, G. and Pawlina, W., Histology: A Text and Atlas. . Philadelphia, Pa: Lippincott Williams & Wilkins, 2003.
15. Rosen, E.D., Spiegelman, B.M., Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol, 2000; 16: 145-71.
16. Ntambi, J.M., Young-Cheul, K., Adipocyte differentiation and gene expression. J Nutr, 2000; 130(12): 3122S-3126S.
17. Aust, L., Devlin, B., Foster, S.J., Halvorsen, Y.D., Hicok, K., du Laney, T., Sen, A., Willingmyre, G.D., Gimble, J.M., Yield of human adipose-derived adult stem cells from liposuction aspirates. Cytotherapy, 2004; 6(1): 7-14.
18. Zuk, P.A.Z., M. Mizuno, H. Huang, J. Futrell, J. W. Katz, A. J. Benhaim, P. Lorenz, H. P. Hedrick, M. H., Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng, 2001; 7(2): 211-28.
19. Brustle, O.C., K. Karram, K. Huttner, A. Murray, K. Dubois-Dalcq, M. McKay, R. D., Chimeric brains generated by intraventricular transplantation of fetal human brain cells into embryonic rats. Nat Biotechnol, 1998; 16(11): 1040-4.
20. MacLimans, W.F., Davis, E.V., Glover, F.L., Rake, G.W., The submerged culture of mammalian cells; the spinner culture. J Immunol, 1957; 79(5): 428-33.
21. Kawazoe, N., et al., Adipogenic differentiation of mesenchymal stem cells on micropatterned polyelectrolyte surfaces. J Nanosci Nanotechnol, 2009; 9(1): 230-9.

22. Hauner, H.S., P. Pfeiffer, E. F., Glucocorticoids and insulin promote the differentiation of human adipocyte precursor cells into fat cells. J Clin Endocrinol Metab, 1987; 64(4): 832-5.
23. Pearse, A.A., Manual for histologic techniques. Boston, Little, Brown, 1972: p. 259-260.
24. Peng, H.H.W., T. H. Chao, A. S. Chang, S. D., Isolation and differentiation of human mesenchymal stem cells obtained from second trimester amniotic fluid; experiments at Chang Gung Memorial Hospital. Chang Gung Med J., 2007; 30(5): 402-7.
25. Mittal, R., Mesenchymal Stem Cells: The New Players in the Pathogenesis of Tuberculosis. J. Microbial Biochem. Technol., 2011; 3: ii-0.
26. Lennon, D.P.C., A. I., Isolation of rat marrow-derived mesenchymal stem cells. Exp Hematol, 2006; 34(11): 1606-7.
27. Skorobogatova, N.G., Volkova, N. A., Petrenko, AIu, Osteogenic and adipogenic capacity of fibroblast-like progenitor cells derived frow human fetal liver. Tsitologiia, 2008; 50(4): 317-22.
28. Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, Dj Horwitz, E., Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 2006; 8(4): 315-7.
29. Davies, O.G., Cooper, P. R., Shelton, R. M., Smith, A. J., Scheven, B. A., Isolation of adipose and bone marrow mesenchymal stem cells using CD29 and CD90 modifies their capacity for osteogenic and adipogenic differentiation. J Tissue Eng, 2015; 6: 2041731415592356.
30. Gregoire, F.M., Smas, C.M., Sul, H.S., Understanding adipocyte differentiation. Physiol Rev., 1998; 78(3): 783-809.
31. Linehan, C., Gupta, S., Samali, A., O'Connor, L., Bisphenol A-mediated suppression of LPL gene expression inhibits triglyceride accumulation during adipogenic differentiation of human adult stem cells. PLoS One, 2012; 7(5): e36109.
32. Zhu, W.C., K. K. Vanhoutte, P. M. Lam, K. S. Xu, A., Vascular effects of adiponectin: molecular mechanisms and potential therapeutic intervention. Clin Sci (Lond), 2008; 114(5): 361-74.
33. Arita, Y.K., S. Ouchi, N. Takahashi, M. Maeda, K. Miyagawa, J. Hotta, K. Shimomura, I. Nakamura, T. Miyaoka, K. Kuriyama, H. Nishida, M. Yamashita, S. Okubo, K. Matsubara, K. Muraguchi, M. Ohmoto, Y. Funahashi, T. Matsuzawa, Y., Paradoxical

decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun, 1999; 257(1): 79-83.

34. Torigoe, M.M., H. Ogawa, Y. Murakami, H. Murakami, R. Cheng, X. W. Numaguchi, Y. Murohara, T. Okumura, K., Impact of the high-molecular-weight form of adiponectin on endothelial function in healthy young men. Clin Endocrinol (Oxf), 2007; 67(2): 276-81.