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Differentiated Stem Cells Derived from Rabbit Adipose Tissue Exhibited *in Vitro* Adipogenesis and Osteogenesis

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ABSTRACT

The multipotent characteristic of rabbit adipose-derived stem cells makes them available and convenient sources for isolating mesenchymal stem cells. The aim of this study was to assess the differentiation in rabbit adipose-derived stem cells pre-committed to produce several mesenchymal lineages in response to inductive extracellular cues to multipotent stromal cells. Three grams of adipose tissue was taken from a subcutaneous region of the nape of the neck and was carefully isolated to obtain mesenchymal stem cells for expanded by fourth passage. In the 4th passage, active growth of mesenchymal stem cells was observed. Furthermore, the research demonstrated the inherent ability of rabbit MSCs to induce differentiation in osteogenic and adipogenic lineages. These mesenchymal stem cells were successfully isolated from adipose tissue which differentiated into either osteocytes or adipocyte-like cells after 21 and 14 days of culturing in specific osteogenic and adipogenic media, respectively. The remarkable differentiation potential of rabbit mesenchymal stem cells is indicated by mineralized deposition to the osteocytes and lipid droplets accumulated in the cytoplasm lipid vacuoles in the adipocytes.

Keywords: adipogenesis, osteogenesis, MSCs, ASCs, rabbit

INTRODUCTION

Adipose-derived stem cells (ASCs) are derived from the Adipose tissue that has different and distinct lineage potential. Adipose tissue may be easily isolated and exhibit a multipotent nature, including the ability to be greatly expanded in vitro and induced to differentiate into multiple MSCs types (1). In fact, ASCs are precursors for various mesoderm-type cells which include osteoblasts, chondroblasts and adipocytes (2). Induction of adipogenic and osteogenic differentiation pathways with lineage-specific induction agents like dexamethasone, IBMX, insulin, and ascorbate (3,4). In addition, ASCs have strong osteogenic differentiation capabilities, widely applied in cellular therapy, tissue repair, and regenerative medicine (5) due to their numerous advantages and several recent studies that have shown a preference for adipose-tissue-derived mesenchymal stem cells. Zuk et al. (6) described a multilineage stem cell population originating from the adipose tissue stroma in 2001. Thus, ASC can be easily and repeatedly extracted and obtained in large quantities with minimal discomfort (7). Adipose tissue (AT) has much more MSCs than bone marrow mesenchymal stem cells (BMSCs), about 1% vs. 0.01%, compared with other sources such as the dermis, umbilical cord, dental pulp, and placenta (8). The adipose-derived stem cells differentiate into several

cell types: bone, cartilage, fat, heart, skin, and neurons. This study aimed to highlight the differentiation of adipose-derived stem cells into various mesenchymal lineages in vitro.

MATERIALS AND METHODS

Ethical Approval

The experimental design and procedures carried out in this study were reviewed and approved in accordance with animal welfare ethical standards by the Research Ethics Committee at the University of Baghdad's College of Veterinary Medicine with ethics number 1305 P.G. dated on January 18, 2023.

Isolation and cultivation of ASCs

The rabbit was administered general anesthesia including xylazine (2%) 5 mg/kg (Alasan™, Holland) and ketamine (10%) 35 mg/kg (Alasan™, Holland) (9, 10) via intramuscular injection. Aseptic surgical preparation was carried out on the skin in the neck region, followed by a sharp incision of the skin. Three g of subcutaneous adipose tissue were harvested. The harvested tissue was immediately immersed in 50 mL conical tubes containing sterilized phosphate buffered saline (PBS). The surgical incision was closed as routine work, and the animals were administered systemic antibiotics ceftriaxone (LDP, Spain) 20 mg/kg intramuscularly for 5 days. All techniques for adipose-derived stem cell (ASC) processing were carried through a stem cell lab of College of Veterinary Medicine, University of Baghdad. The adipose tissue sample was transferred to a biosafety cabinet II and rinsed with dulbecco phosphate buffer saline (DPBS) (Gibco™, USA) in order to eliminate any residual blood. Then, the AT was minced using a sterile blade into very small pieces (Figure 1 A, B).

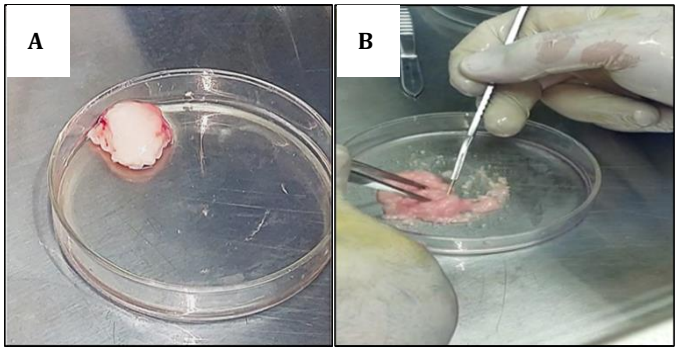

Figure 1. A photograph display the isolation process of MSCs obtained from adipose tissue. **(A)** Adipose tissue sample was cleansed using DPBS within a sterile laminar flow environment. **(B)** Adipose tissue segments were minced thoroughly with a blade until they turned into a mush

The minced tissue was collected in a fifty ml tube. After that, 10 mL of 2 mg/mL collagenase I (Gibco™, USA) was added in a ratio of 10 mL solution per 3 grams of tissue and incubated at 37 °C. Then, after the digestion, this sample was diluted using dulbecco's modified eagle medium (Gibco™, USA) (DMEM) 10% fetal bovine serum (Gibco™, USA) 1:1, and filter cell suspension by using a 40 µm cell strainers in with 50 mL conical tubes to eliminate the undigested fragment. Then, the filtrate material was centrifuged at 700 g (g-force) for seven min. Remove and discard the resulting lipid layer upon top as well as the supernatant that was collected using pipette. Add three ml of erythrocyte lysis buffer (155 mMNH 4 Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) to one mL of basal medium to re-suspend the pellet, allowing it to remain at the room temperature (RT) for 5 min. After that, 10 mL of DPBS was added; then mixture was centrifuged at 700 g for seven min. Remove supernatant and re-suspend the pellet with 10 mL medium and - seeded in tissue culture T-75 Flask (Thermo Scientific™). Finally, the cell culture flasks were incubated at 37 °C in a humidified environment consisting of 90% and 5% CO_2 incubator (Sanyo, Japan) for duration of 3-4 days. After that, cells that did not adhere were removed by changing media every 3 days through 12 days or until reached 80% cell confluency. The culture at 12 days was considered passage zero (P0). At this stage the monolayer cell cultures were subcultured to collect homogeneous stromal stem cells. As the following: After the old medium was discarded, the cell was harvested when the monolayer was washed 2-3 times with PBS pH 7.2. Then, 3 mL of trypsin with EDTA (0.25%) and phenol red (Gibco™, USA) solution was added and incubated for 2-3 min to detach the cell culture. The flask was gently shaken to make cell detachment faster. Then, trypsin with EDTA (0.25%) and phenol red solution action was stopped by adding 5-8 mL of DMEM (Gibco™, USA) containing 10% fetal bovine serum and an antibiotic-antimycotic (100×) 1% (Gibco™, USA). The cell solution T-75 culture flask was divided into two T-75 (Thermo Scientific™) flasks were incubated at 37 °C in 5% CO_2 and 90% humidity. The growth of cells was monitored daily until reached 80% cell confluency.

Adipose-Derived Stem Cells Adipogenesis

The differentiation of ASCs to adipocytes was performed according to the protocol described by Wall et al. (11). Mesenchymal stem cells were harvested after complete confluence during the fourth passage and seeded in a T-25 tissue culture flask (SPL®, Korea) with a density of 105 cells per cm^2 using 5 mL of MSCs expansion medium DMEM per flask. The flask was incubated at 37 °C in 5% CO_2. When the cells in each flask reached 80-90 % confluence, they were aspirated then 5 mL of adipogenesis induction media (DMEM-low glucose, 10% fetal bovine serum, 10 mM Dexamethasone, 0.5 M 3-isobutyl-1-methylxanthine (IBMX), recombination human insulin 10 mg/ml, 10 mM indomethacin, and penicillin-streptomycin) (Gibco™, USA) was added and changed every 2-3 days . An inverted microscope (Olympus, Japan) was used to observe droplets of lipids. Lipid droplets accumulated in the cytoplasm

served as an indication for adipogenic differentiation detected by Oil Red O staining.

Protocol for Oil Red O Staining (ORO)

The media of the culture was discarded from the tissue culture flask after 14 days of differentiation, and the remaining tissue culture flask was preserved in a 4% paraformaldehyde solution for forty min at RT. The fixative agent was then aspirated and the cells were washed with PBS three times for 10 min each. The cells were then covered with sufficient Oil Red O solution, and incubated at RT for 15 min. After removing the Oil Red O solution, five milliliters of distal water were used to wash the cells in the flasks three times. After washing, hematoxylin for 1-2 min to counterstain before observing the inverted microscope.

Oil Red O Solution Preparation

To prepare the Oil Red O solution, 0.35 gram of Oil Red O stained were dissolved in 100 milliliters of absolute isopropanol and placed on the magnetic stirrer for at least one hour. The Oil Red O solution should be filtered through a 0.4 µ pore size and stored at RT. Take 3 milliliters of Oil Red O solution Solution with 2 milliliters of double distilled water, allow stand at RT for 20 min, and filter solution through 0.22 µ pore size.

Adipose-Derived Stem Cells Osteogenesis

The differentiation of ASCs to osteocytes was performed according to the protocol described by Wall et al. (11) Mesenchymal stem cells were initially collected in 1 ml of DMEM, and then inoculated in a T-25 tissue culture flask at 105 cells per cm^2 density, then grown in DMEM at 37 °C with 5% CO_2. When the cells had attained complete confluency, after removing the medium, five milliliters of osteogenesis induction media (DMEM-low glucose with 10% FBS, 10 mM dexamethasone solution, ascorbic acid 2-Phosphate solution, L-glutamine and penicillin and streptomycin) (Gibco™, USA) were applied to flask. The osteogenic induction medium was changed every 2-3 days during the 21 days of culture. An inverted microscope (Olympus, Japan) was determined by mineralized calcium deposit in the extracellular matrix to indicate osteocyte differentiation detected by Alizarin Red S stain (Sigma-Aldrich).

Protocol for Alizarin Red S Staining (ARS)

The medium was carefully removed from the flask after 21 days of culturing and rinsed once with DPBS. Fix cells with 4% formaldehyde solution for 30 min at RT. After fixation, rinse the flask twice with distilled water for ten min each. After aspirating the tap water, the flask received sufficient 2% Alizarin Red S solution to cover the cells completely and was then incubated at the RT for 30 min. The Alizarin Red S was removed, and the cells were rinsed four times with 5 ml of distal water and aspirated after each wash. Finally, 5 milliliters of distal water were added to the flask to prevent the dryness of the cells in the culture. The Alizarin Red S stained cell culture was examined under an inverted microscope.

Alizarin Red S Solution Preparation

To prepare the Alizarin Red S solution, 2 g of Alizarin Red S were dissolved in 100 milliliters of double distilled water. The pH of the solution was then adjusted to 4 using HCL (1 N). The solution should be filtered through a filter 0.22 µ pore size and stored at 4 °C in a dark place.

RESULTS

Adipose-derived stem cells were obtained from adipose tissue in the subcutaneous portion of the nape of the neck in rabbits. They displayed a heterogeneous cell population upon seeding and growth in a cell culture flask. The adherent cells exhibited various morphologies that include small rounded, spindle and large flattened shapes. As the cells proliferated and approached the confluence, most of them displayed a fibroblast-like morphology. Notably, the small, rounded cells attached to the surface of the cell layers, but these cells gradually diminished with repeated passages. In contrast, the fibroblast-like cells became more abundant and homogeneous in morphology, reaching a purity of over 80-90% by passage 4 (Figure 2 A). Rabbit ASCs were adipogenic differentiation after 14 days to adipocytes. Lipid droplets accumulated in the cytoplasm lipid vacuoles (red-orange color) in the adipocytes that were stained with Oil red O (Figure 2 B). Rabbit ASCs were osteogenic differentiated after 21 days and were demonstrated the mineralized deposition to the osteocytes by staining with Alizarin Red S. (Figure 2 C).

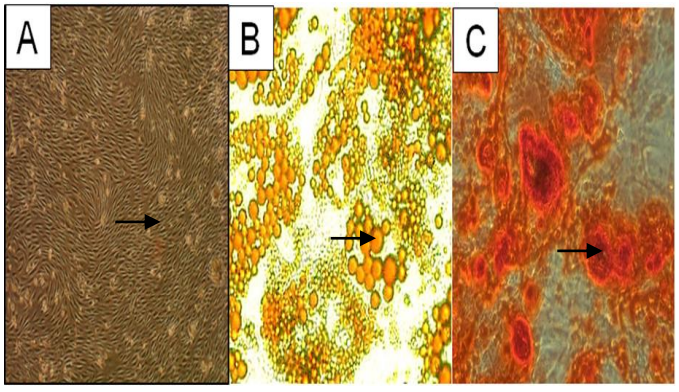

Figure 2. (A) Adipose-derived stem cells of fibroblast-like cells were observed attached to the tissue culture plastic at passage 4, cells exhibited characteristic spindle shape (arrow). **(B)** Adipose-derived stem cells adipogenic differentiation. Lipids droplets were detected by the orange color of Oil red O stain (arrows). **(C)** Adipose-derived stem cells osteogenic differentiation. Calcium deposition is demonstrated by the red color of Alizarin Red S stain (arrow (arrow)

DISCUSSION

This study found that ASCs adhered to tissue culture plastic surfaces within 3-4 days of incubation. The cells had fibroblast-like small, rounded, spindle, and large flattened shapes. Cells grew like fibroblasts. Previous observations were similar (12). ASCs are a diverse population of undifferentiated cells that can be obtained from different organs and differentiated into various cell lineages depending on specific stimuli and culture conditions (13, 14). To differentiate ASCs, in vitro osteogenic and adipogenic factors are directly added to the culture medium. In addition to their capability of adhering to plastic, stem cells have differentiating ability into osteoblasts, adipocytes, and chondroblasts both in vitro and in vivo (15). Mesenchymal stem cells with adipogenic induction medium contain specific factors, dexamethasone and 3-isobutyl-1-methylxanthine (IBMX), to induce differentiation of Adipose-derived stem cells to adipocytes (16). The available evidence indicates that the secretion of different factors that promote substances will likely play a role in the functioning of mesenchymal stem cells. This is performed to investigate a putative mechanism that explains the regeneration ability of ASCs (17). The findings of this study demonstrated the multipotentiality of adipose-derived stem cells up to the fourth passage. This characterizes the osteogenic induction of mesenchymal stem cells to osteocyte differentiation, a characteristic of osteogenesis and the initiation of mineralization (18). The deposition of minerals such as calcium is essential for the mineralization process during osteogenic differentiation. (19, 20), and dexamethasone, L-glutamine, and ascorbic acid can promote ASCs to undergo accelerated maturation during osteogenic differentiation (21, 22), these results agree with (23).Similarly, adipocyte differentiation has been achieved by studying lipid-filled vacuoles (24). The results agree with previous reports of osteogenic and adipogenic capability to transdifferentiation (25, 26). The results also align with previous studies, which show that a passage in mouse MSCs caused adipogenic and osteogenic to decline with aging (27, 28). An earlier investigation revealed that isolated bone marrow-derived mesenchymal cells were more adipogenic (29). According to the evidence, adipogenic MSCs can be encouraged to trans-differentiate along the osteogenic path. It has also been demonstrated that human ADSCs in the late passage have better osteogenic properties (30).In conclusion, rabbit ASCs were isolated from adipose tissue and differentiated into osteocytes by Alizarin Red S staining and adipocytes by Oil Red O staining. According to this study, Indomethacin, dexamethasone, and insulin promote ASC differentiation. These findings may help tissue engineering researchers develop sequential growth factor delivery systems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

EDITORIAL PROCESS TRANSPARENCY

Hameed A AL-Timmemi is the Editorial Board of The Iraqi Journal of Veterinary Medicine. Despite this role, the peer review process and the final publication decision were made independently and impartially, ensuring no influence from the author's editorial position.

REFERENCES

1. Ong WK, Sugii S. Adipose-derived stem cells: fatty potentials for therapy. Int J Biochem Cell Biol. 2013;45(6):1083-1086. 10.1016/j.biocel.2013.02.013
2. Baer PC, Geiger H. Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. Stem Cells Int. 2012;1-11. 10.1155/2012/812693
3. Liu H, Wei LK, Jian XF, Huang J, Zou H, Zhang SZ, et al. Isolation, culture and induced differentiation of rabbit mesenchymal stem cells into osteoblasts. Exp Ther Med. 2018;15(4):3715-3724. 10.3892/etm.2018.5894.
4. Langenbach F, Handschel J. Effects of dexamethasone, ascorbic acid and β-glycerophosphate on the osteogenic differentiation of stem cells in vitro. Stem Cell Res Ther. 2013;4(5):1-7. 10.1186/scrt328
5. Mimeault M, Hauke R, Batra SK. Stem cells: A revolution in therapeutics Recent advances in stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies. Clin Pharmacol Ther. 2007;82(3):252-264. 10.1038/sj.clpt.6100301
6. Zuk PA, Zhu MI, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7(2):211-228. 10.1089/107632701300062859
7. Hayder HA, Ahameed FB. Clinical and Histopathological Study of the Effect of Adipose-Derived Mesenchymal Stem Cells on Corneal Neovascularization following Alkali Burn in a Rabbit Model. Arch Razi Inst. 2022;77(5):1715-1721. 10.22092/ARI.2022.357998.2136
8. Costela-Ruiz VJ, Melguizo-Rodríguez L, Bellotti C, Illescas-Montes R, Stanco D, Arciola CR, et al. Different sources of mesenchymal stem cells for tissue regeneration: a guide to identifying the most favorable one in orthopedics and dentistry applications. Int J Mol Sci. 2022;23(11):6356. 10.3390/ijms23116356
9. Abed AF. Evaluation of midazolam and ketamine preceding by xylazine as general anesthesia in rabbits. Iraqi J. Vet. Med. 2013;37(2):144-148. 10.30539/iraqijvm.v37i2.274
10. Eesa MJ, Omar RA, Nazhat HH. Evaluation of general anaesthesia by using Propionylpromazine, Xylazine and Ketamine in rabbits. Iraqi J. Vet. Med. 2010;34(1):208-17. 10.30539/iraqijvm.v34i1.681
11. Wall ME, Bernacki SH, Loboa EG. Effects of serial passaging on the adipogenic and osteogenic differentiation potential of adipose-derived human mesenchymal stem cells. Tissue Eng. 2007;13(6):1291–1298. 10.1089/ten.2006.0275
12. Bakhtina A, Tohfafarosh M, Lichtler A, Arinzeh TL. Characterization and differentiation potential of rabbit mesenchymal stem cells for translational regenerative medicine. In Vitro Cell. Dev. Biol. Anim. 2014;50:251-260. 10.1007/s11626-013-9702-5
13. Al-Timmemi H, Dhurgham H, Zaid A. Efficacy of bone marrow stromal cells implantation on regeneration of peripheral nerve injury in dogs. Am J Res Commun. 2014;2(8):115–130.
14. Nombela-Arrieta C, Ritz J, Silberstein LE. The elusive nature and function of mesenchymal stem cells. Nat. Rev. Mol. Cell Biol. 2011;12(2):126-31. 10.1038/nrm3049

15. Dominici ML, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. Cytotherapy. 2006;8(4):315-317. 10.1080/14653240600855905
16. Thirumala S, Gimble JM, Devireddy RV. Evaluation of methylcellulose and dimethyl sulfoxide as the cryoprotectants in a serum-free freezing media for cryopreservation of adipose-derived adult stem cells. Stem Cells Dev. 2010;19(4):513-522. 10.1089/scd.2009.0173
17. Sowa Y, Imura T, Numajiri T, Takeda K, Mabuchi Y, Matsuzaki Y, et al. Adipose stromal cells contain phenotypically distinct adipogenic progenitors derived from neural crest. PLoS One. 2013;8(12): 1-11. 10.1371/journal.pone.0084206
18. Lian JB, Stein GS. Development of the osteoblast phenotype: molecular mechanisms mediating osteoblast growth and differentiation. Iowa Orthop J. 1995;15: 118-140. PMID: 7634023.
19. Essa HH, Jasim HS, Kadhim HA. Immunological and Hematological Response to Local Transplantation of Stem Cells in Injured Radial Nerve of Dogs. Iraqi J. Vet. Med. 2020;44(2):45-55. 10.30539/ijvm.v44i2.976
20. Murshed M, Harmey D, Millán JL, McKee MD, Karsenty G. Unique coexpression in osteoblasts of broadly expressed genes accounts for the spatial restriction of ECM mineralization to bone. Genes Dev. 2005;19(9):1093-1104. 10.1101/gad.1276205
21. Salasznyk RM, Williams WA, Boskey A, Batorsky A, Plopper GE. Adhesion to vitronectin and collagen I promotes osteogenic differentiation of human mesenchymal stem cells. J Biomed Biotechnol. 2004;24-34. 10.1155/S1110724304306017
22. Madhu V, Dighe AS, Cui Q, Deal DN. Dual inhibition of activin/nodal/TGF-β and BMP signaling pathways by SB431542 and dorsomorphin induces neuronal differentiation of human adipose derived stem cells. Stem cells international. 2016;1-16.10.1155/2016/1035374
23. Kwist K, Bridges WC, Burg KJ. The effect of cell passage number on osteogenic and adipogenic characteristics of D1 cells. Cytotechnology. 2016;68(4):1661–1667. 10.1007/s10616-015-9883-8
24. Barry FP, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. Int J Biochem Cell Biol. 2004;36(4):568-584. 10.1016/j.biocel.2003.11.001
25. Scott MA, Nguyen VT, Levi B, James AW. Current methods of adipogenic differentiation of mesenchymal stem cells. Stem Cells Dev. 2011;2(10):1793-1804. 10.1089/scd.2011.0040
26. Al-Mutheffer EA, Reinwald Y, El Haj AJ. Donor variability of ovine bone marrow derived mesenchymal stem cell-implications for cell therapy. Int J Vet Sci Med. 2023;11(1):23-37. 10.1080/23144599.2023.2197393
27. Kretlow JD, Jin Y-Q, Liu W, Zhang WJ, Hong T-H, Zhou G, et al. Donor age and cell passage affects differentiation potential of murine bone marrow-derived stem cells. BMC Cell Biol. 2008;9 (60):1-13. 10.1186/1471-2121-9-60
28. Thanoon MG, Eesa MJ, Alkenanny ER. Histopathological evaluation of the platelets rich fibrin and bone marrow on healing of experimental induced distal radial fracture in local dogs: Iraqi J.Vet.Med. 2019;43(1):11-20. 10.30539/iraqijvm.v43i1.465
29. Al-Timmemi H, Ibrahim R, Al-Jashamy K, Zuki A, Azmi T, Ramassamy R. Identification of adipogenesis and osteogenesis pathway of differentiated bone marrow stem cells in vitro in rabbit. Ann Microsc. 2011;11:24-29.
30. Yang Y-HK, Ogando CR, Wang See C, Chang T-Y, Barabino GA. Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro. Stem Cell Res Ther. 2018;9:1-14. 10.1186/s13287-018-0876-3

قابلية تمايز الخلايا الجذعية المأخوذة من النسيج الدهني للأرانب الى خلايا دهنية وعظمية مختبرياً

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الخلاصة

تمتلك الخلايا الجذعية اللحمية ذات المنشاء الدهني من الارنب خصائص متعددة منها سهولة الحصول عليها، وذلك لعزل الخلايا الجذعية. تهدف هذه الدراسة الى تقييم تمايز الخلايا الجذعية اللحمية ذات المنشاء الدهني غير المتخصصة الى خلايا متخصصة استجابةً للاشارات من خارج الخلايا الى الخلايا الجذعية المتعددة. حيث تم اخذ ثلاثة غرام من النسيج الدهني تحت الجلد لمنطقة مؤخرة العنق لغرض استزراع الخلايا الجذعية ولغاية اربع زرعات ثانوية. ولوحظ النمو النشط للخلايا في الزرعة الثانية الرابعة.بالاضافة الى ذلك أظهر البحث قدرة الخلايا الجذعية في الأرانب على إحداث تمايز الى خلايا عظمية ودهنية. وتمايزت الخلايا الجذعية بنجاح إلى خلايا عظمية و خلايا شحمية بعد ٢١ و ١٤ يومًا من الزراعة في وسط مخصص للعظم والدهون، وعلى التوالي. وتم التأكيد في هذه الدراسة على قدرة التمايز للخلايا الجذعية وذلك بترسبات المعدنية الملحوضة في الخلايا العظمية والفجوات الدهنية في سايتو بلازم الخلايا الشحمية.

الكلمات المفاحية: الخلايا الدهنية،الخلايا العظمية،الخلايا الجذعية المزنكايمية،الخلايا الجذعية المشتقة من الدهون،ارنب