Iran Red Crescent Med J. 2020 March; 22(3):e98809. doi: 10.5812/ircmj.98809.

Published online 2020 March 14. **Research Article**

Effect of Adipose-Derived Mesenchymal Stem Cell Repair in Nicotine Model Rat: A Femoral Bone Diaphyseal Defect Study

Mahyar Riahi [1], Kazem Parivar [1, *], Javad Baharara [2, 3] and Reza Zandi [4]

[1] Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Biology Department, Mashhad Branch, Islamic Azad University, Mashhad, Iran
[3] Research Center for Animal Develoment Applied Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran
[4] Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[*] *Corresponding author*: Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran. Tel: +98-9121304550, Fax :+98-2188921118, Email: parivarkaze@gmail.com

Received 2019 October 11; **Accepted** 2020 February 12.

Abstract

Background: The major function of the bone in the skeletal system is to provide structural support to the body and its vital organs. Many patients suffer from the disability to restore bone lesions following bone fractures during crashes or accidents. The use of mesenchymal stem cells such as adipose-derived mesenchymal stem cells (ADMSCs), along with collagen scaffolds, and its transfer to the lesion site can be valued as one of the available treatment options.
Objectives: In the current paper, a study was conducted on the level of mesenchymal stem cell repair from the rat adipocytes, where it was evaluated in bone defects.
Methods: In this study, mesenchymal stem cells were isolated from the rat adipocytes and their stem cell lines were determined with the standard cell tests. The isolated cells were differentiated in the next step and transferred to the two main groups: nicotine modeled and non-modeled (non-nicotine) along with collagens. The repair of the defect caused by a 2 mm drill in the diaphyseal region of the rat bone was evaluated after four weeks using radiographic examination and histopathologic staining.
Results: Radiographic data analysis indicated that bone density was much higher in the non-nicotine group than in the nicotine group. Histopathologic staining showed that bone formation was higher in the non-nicotine group than in the nicotine group. The new bone formation was about 80% and 60% in the non-nicotine and nicotine groups with differentiated osteocytes of ADMSCs, respectively.
Conclusions: Adipose-derived mesenchymal stem cell transplantation is effective in bone defect repair and nicotine plays an important role in the bone repair process as an inhibitory agent.

Keywords: Adipocyte Mesenchymal Stem Cell, Diaphyse, Nicotine, Rat

1. Background

The major function of the bone in the skeletal system is to provide structural support to the body and its vital organs. Bone is also considered to be the main source of minerals, as it provides a suitable leverage system for muscle contraction. Taking these effects into consideration, the consequence of bone loss in body homeostasis can be easily imagined (1, 2). Wars, diseases, accidents, and natural disasters among other types of events can cause damage to the bone tissue, including the skull. The most common cause of mortality in accidents is a hit on the head. Minimal treatment can spontaneously recover the majority of bone damages. However, sometimes spontaneous healing cannot be performed in some cases such as misplaced bone fusion, complete bone loss because of a tumor, and lesion site infection, necessitating further treatment. Orthopedists use a variety of methods to fully repair the damaged bone, such as bone grafting, herbal drugs (antibacterial compounds), or metal implants (2-5). Mesenchymal stem cells can differentiate into bone marrow cells. This seems promising since these cells could be used in the cell therapy of large bone lesions in the near future. Some researchers believe that if the cell therapy strategy is used in bone lesions, the cells should be well-differentiated (6, 7).

The adipose and connective tissues contain different adipocytes retained by collagen fibers. The cytoplasm of these cells is made up of approximately 90% fat and stromal-vascular cells including smooth muscle cells, endothelial cells, fibroblasts, and pre-adipocytes. Pre-adipocytes are located in the vascular-stromal region of the tissue. The appearance of these cells before differenti-

ation is pseudo-fibroblastic. Morphological and biochemical changes in these cells take place due to differentiation, in such a way that the cells become spherical with accumulated fat vacuoles. Pre-adipocytes, owing to their good ability to isolate, cultivate, expand, and differentiate into different cell types, are thought to be beneficial cellular sources (1, 8-10).

Some studies have shown that stem cells can be a significant hope for regenerative medicine and many researchers have studied these cells in many animal models such as planarians. The fatty tissues contain high levels of adult adipocytes, pre-adipocytes, fibroblasts, capillary smooth muscle cells, endothelial cells, established macrophages, and lymphocytes. Adipose-derived stem cells (ADSCs) have been isolated recently. Evidence suggests that these cells have the potential to differentiate into organ cells with mesodermic origins. Neurons, pancreatic endocrine cells, liver cells, endothelial cells, and heart muscle cells are all examples of increasing evidence based on the ability of ADSCs to differentiate into non-mesodermic cells (10-14). The surgical procedure is simple and access to subcutaneous fatty tissue and non-complex enzymatic separating process that provides ADSCs is repeatable. Such simplicity and repeatability are more attractive to researchers and clinicians. Also, the growth of ADSCs is easier and faster than that of BMSCs. It is estimated that 1% of the cells in the adipose tissue become mesenchymal stem cells in comparison with 0.001 - 0.002% of the cells from the bone marrow (1, 10, 12, 15). There are more numerous reasons why mesenchymal stem cells are suitable for bone tissue engineering as cell sources. These reasons include rapid and relatively easy separation for the patient, the presence of a high level of mesenchymal stem cells for isolation, immune system moderating properties, the secretion of effective factors in the treatment, the ability to identify and enter to the damaged site, the lack of ethical problems in their use, the low risk of mesenchymal stem cell tumorigenicity, and the power of proliferation and differentiation of mesenchymal stem cells, in addition to the availability of extensive studies on the therapeutic role of mesenchymal stem cells (16-23).

Studies have demonstrated that what can play a vital role in improving the functional activity of the organ is the number of live stem cells in the graft region. It has been shown that free radicals play a major role in reducing the viability of the transplanted cells. One of the most common abnormalities in bone defects is no rapid and complete repair. The reduction of bone density by age and non-welding after a fracture not only can disable the person but also may lead to heavy costs for the individual and society. Researchers have always sought to find a suitable treatment for these patients, especially because of the relative limitation of repair in these conditions. Several treatments have been proposed for the rapid repair of bone defects (10, 12, 15, 24).

2. Objectives

In this study, the repair effect of mesenchymal stem cells from rat adipocytes was investigated on bone defects in rats.

3. Methods

3.1. Isolation and Culture of Mesenchymal Stem Cells of Rat Adipocyte

First, male Wistar rats (Rattus Norvegicus) (200 to 250 g) were purchased to isolate and culture the mesenchymal stem cells of the bone marrow. The rats were purchased from the Pasteur Institute (Tehran, Iran). The animals were anesthetized by the inhalation of carbon dioxide. In the beginning, the adipose tissue was taken from the rat testicles, crashed, and washed with sterile salt buffer to isolate fatty stem cells. Then, the adipose tissue was transferred into a solution of salt-buffered saline and 0.3% collagenase (SERVA, Germany) for 45 - 60 minutes at 37°C, allowing the whole tissue to completely digest. After digestion, the enzyme was neutralized by adding cold buffer solution (PBS) (MiltenyiBiotec, Germany) and centrifugation was performed (25-27). The supernatant containing debris and fat droplets was discarded. The resulting pellet was collected and filtered using a diluted salt solution passed through a 70 μm filter. Following filtration, washing with buffer solution was performed twice (400 g/5 min). The cellular precipitate, containing adipocyte mesenchymal stem cells, was used for further experiments. The cells were transferred to a culture container at 30,000 cells/cm^2. After 48 hours, the floating cells were removed and the attached cells were cultured to reach 90% confluency. After three passages, the resulting cells were frozen and stored for further use.

3.2. Identification of Isolated Mesenchymal Cells

Based on the expression and lack of expression of surface markers, the cells were identified by flow cytometry according to the defined criteria. In this test, CD90 and CD106 were positive markers, while the negative markers were CD34 and CD45. In this study, the bone differentiation test was used to verify the mesenchymal nature of the cells and to evaluate their power of differentiation into osteocytes. After reaching the confluence of the third subculture cells, the cell culture medium was replaced with bone and fat differentiation medium and then evaluated

for the differentiation using Oil Red and Alizarin Red staining.

3.3. PCR Confirmation of Adipocyte Mesenchymal Cells Differentiated into Osteocytes

The primers were designed using NCBI and Primer 3 software and synthesized by Sinaclon Co. (17). During the PCR assay, primers were bonded on both sides of the target sequence. This allowed for the polymerase activity of DNA polymerase (28, 29). Therefore, the matching of the primer sequence with the template was of particular importance. The specifications of the primers are shown in Table 1.

3.4. Preparation, Surgery, and Grouping

Experiments were all carried out following the instructions of the Animal Ethics Committee of Islamic Azad University. This protocol observes the ethical principles and protection of animals used for laboratory and other scientific purposes. The collagen needed for preparing hydrogel was extracted from the rat's tail. The extracted collagen (0.04 mg/mL) was first dissolved in acetic acid to prepare the collagen solution. Then, this mixture was mixed with cell culture media (DMEM 1X, 350 μm), NaOH 0.4 N (26 μm) and FBS (10%). As we needed the pH to be close to the body pH, NaOH was added to neutralize the scaffold. In the next step and before injection to the damaged area, 100,000 extracted cells were mixed with it. The present study was conducted on 24 adult male rats weighing approximately 150 - 200 g. All the rats were kept under similar circumstances (12 h light and 12 h dark cycle at 20 - 25°C).

The rats were assigned to two groups: a control group (with no nicotine injection) and a nicotine group. To do experiments on the rats, 200 μL of the mixture of nicotine and saline (1/10 μL dilution) was injected into the control group (three times every 48 hours). Access to water and food was free for both groups. After shaving the thighs, a thorough cleaning was performed in the area using iodine and alcohol. To anesthetize each rat, 90 mg/kg of ketamine and 10 mg/kg of xylazine were used intraperitoneally. After shaving the thighs, the area was cleaned with iodine and alcohol. During the surgery, the rats' hands and feet were fixed on the specified points on the surgery bed and then, a cut was made onto the skin. For adequate access to the relevant area, the fascia and muscles were cut slowly. Trephine was used to create a defect (2 mm diameter). In this step, the cells of each group were cemented to a critical size for bone regeneration (differentiated osteocytes from the adipose-derived mesenchymal stem). Consecutively, the post-care was done (stitching up, disinfecting the wound with tetracycline spray, and injecting amoxicillin (0.1 mg/kg)). The rats were all kept in a similar condition in the animal house (clean cage, suitable temperature, and appropriate nutrition). After four weeks, the rats were sacrificed with a high dose of anesthesia and the specimens were removed.

3.5. Radiographic Examination

Four weeks after the cell transplantation, a radiographic examination of the right thigh femoral bone was performed with a Senographe 600T Senix H.F device at a dose of 22 kV and 9 mAs. The test was performed from the anterior-posterior (AP) and lateral (Lat).

3.6. Hematoxylin-Eosin Staining

To do the histopathology test, the samples were immersed in 10% formalin for fixation. Then, all samples were immersed in 10% nitric acid. After decalcification, the sample was sent to the histopathology laboratory. Tissue processing and sectioning (5 microns) were the next steps performed. The specimens were stained using Hematoxylin-Eosin (H&E) and examined and photographed with a light microscope (30-35).

4. Results

4.1. Isolation and Culture of Bone Mesenchymal Cells

As already stated, mesenchymal cells have two main characteristics, i.e., easy isolation and proliferation compared to other types of stem cells and the potential for conversing the different types of cells. Considering these characteristics, mesenchymal cells were selected for culture and evaluation of the damaged tissue repair. To do so, the isolated adipocyte cells were first centrifuged as mesenchymal cells and then were filtered and purified (being cleaned from other impurities using PBS). Since binding to the surface is one of the characteristics of mesenchymal cells, other cells were washed out by intermittent media changes. Having the cells being sub-cultured four times, they were relatively purified; a part of them was then frozen. Flow cytometry showed that the tested cells were cells with very low expression of CD34 and CD45 and high expression of CD90 and CD106 markers, indicating they were mesenchymal stem cell types. The differentiated mesenchymal stem cells to osteocytes and adipocytes were recognized by alizarin red and oil red, respectively. Their differentiation is shown in Figure 1.

4.2. PCR Analysis

The results of the PCR confirmed the expression of specific osteocyte genes differentiating ADMSCs into osteocytes including alkaline phosphatase, osteocalcin, and osteopontin (Figure 1).

Table 1. Primer Specifications

Primer	Sequence	Size (bp)	TM
SPP1	F: 5'CGCAGTTCTCCTGGCTGAAT-3'	150	59.35
	R: 5'-AGTGTTTGCTGTAATGCGCC-3'		57.30
ALP1	F: 5'-GGACCCTGCCTTACCAACTC-3'	245	61.40
	R: 5'-AACTTGTCCATCTCCAGCCG-3'		59.35
BGLAP	F: 5'-GAATAGACTCCGGCGCGCTACC-3'	229	61.40
	R: 5'-TCGAGTCCTGGAGAGTAGCC-3'		61.40

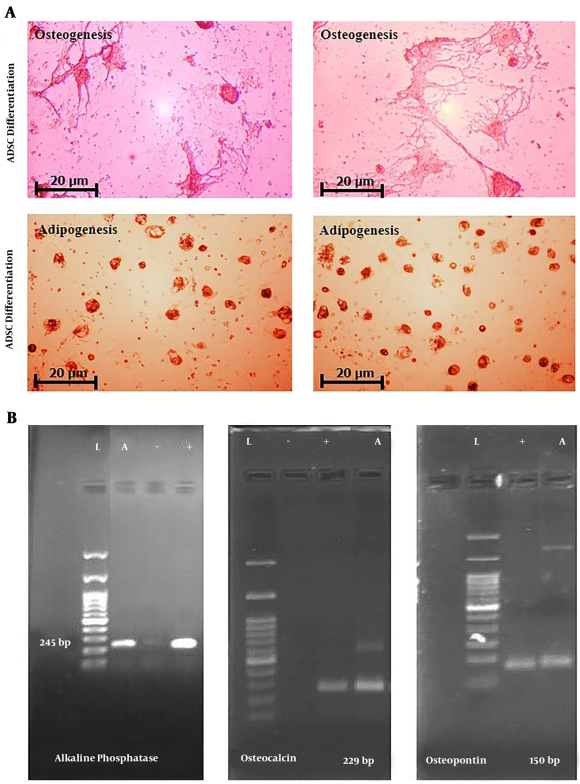

Figure 1. A: ADMSCs differentiation into osteocytes and adipocytes. Alizarin Red and Oil Red staining were used for confirming the osteogenesis and adipogenesis differentiation, respectively. B: osteopontin, alkaline phosphatase, and osteocalcin gene expression in the differentiation of ADMSCs. L, ladder; -, negative control; +, positive control; A, ADMSCs.

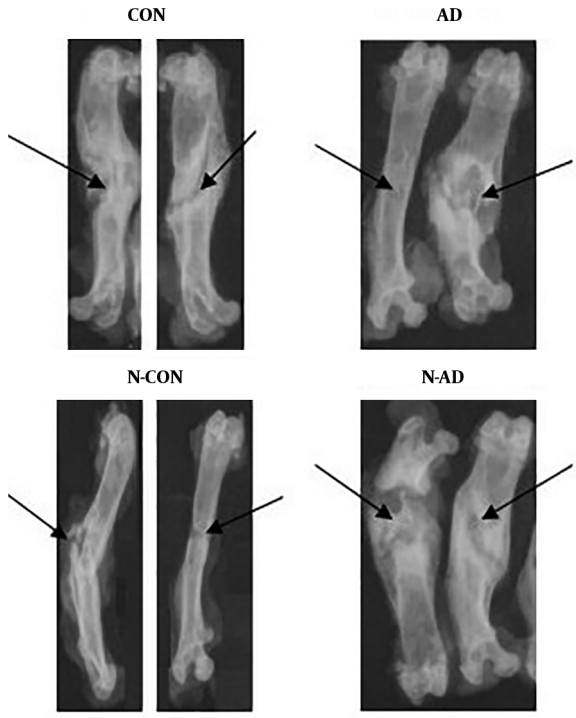

Figure 2. Radiographic images after four weeks. CON, control in the non-nicotine group; AD, differentiated osteocytes of ADMSCs in the non-nicotine group; N-CON, nicotine control; N-AD, differentiated osteocytes of ADMSCs in the nicotine group.

4.3. Radiographic Images

The results of radiography showed that the rate of osteogenesis in the non-nicotine group was much higher than that of the nicotine group. It also demonstrated that the level of this density was higher in the differentiated group. This issue was completely true for the nicotine group. The self-repair was evident in the control group without any cell transplantation compared to the nicotine group (Figure 2).

4.4. Histopathologic Images

According to the histopathologic staining, it was shown that the rate of osteogenesis and bone formation in the canal was more pronounced in the non-nicotine group than in the nicotine group. As shown in Figure 3, the level of regular bone formation was better and more in the group differentiated from adipocytes than in other groups. Comparing nicotine and non-nicotine groups, we observed the regular and continuous bone formation in the non-nicotine group differentiated from adipocytes, which was more distinct than that in the nicotine group with the same situation. The microscopic observations are shown in Figure 4 based on the repair percentage average in the defected area. Samples with severe fractures were

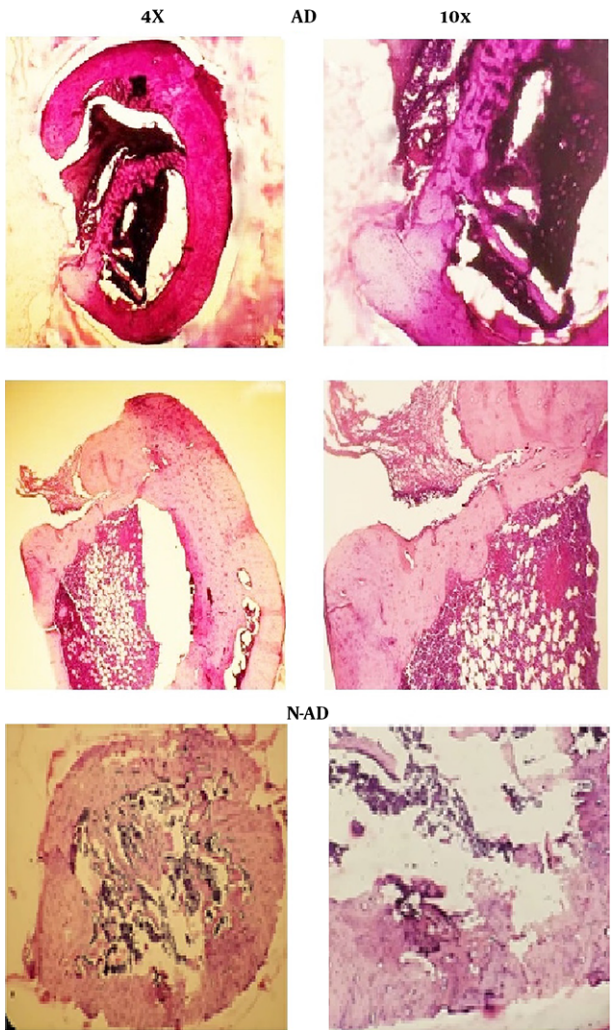

Figure 3. Hematoxylin-Eosin staining after four weeks. AD, differentiated osteocytes of ADMSCs in the non-nicotine group; N-AD, differentiated osteocytes of ADMSCs in the nicotine group (× 40 and × 100).

not reported and not entered in the percentage determination.

5. Discussion

The processes and mechanisms needed to be taken and acted to repair the damaged site are often difficult to handle in several cases. These cases include fractures in which wound healing and repair of the lesion are difficult or in diseases such as cancer, infection, osteoporosis, osteoarthritis, etc. Today, mesenchymal stem cells and biodegradable scaffolds are widely used to repair bone defects (2-4).

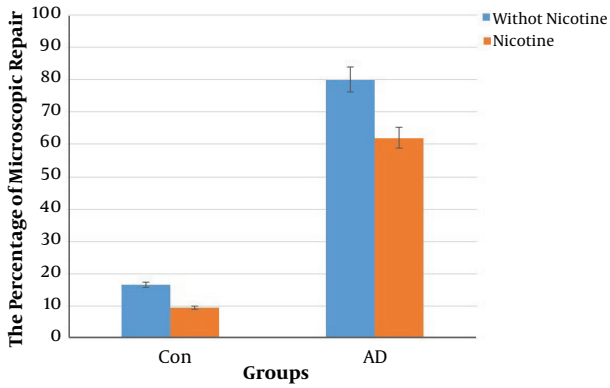

Figure 4. The percentage of microscopic repair based on new bone formation after four weeks. CON, control; AD, differentiated osteocytes of ADMSCs. Results based on the six sections of each sample ($P < 0.05$).

In this study, mesenchymal stem cells were isolated from the fat tissue of rats. Then, they were identified utilizing adhesion tests, flow cytometry, differentiation into adipocytes and osteocytes with specific staining, and expression of the desired genes by PCR. The tests confirmed the stem cell originality of the cells. Finally, the isolated cells from each group were differentiated into osteocytes (the third sub-culture cells that were placed in osteogenesis differentiation media for three weeks, undergoing morphological changes). They got denser in some places. The reddening of extracellular materials in the osteocyte differentiation culture was the result of staining. Then, they were assembled with the prepared collagen to transfer to modeled and non-modeled animals. Niedzwiedzki et al. treated bone defects in the right arms of rabbits using non-autologous BMSC transplants and considered the left arms as controls. On days 20 and 40 after transplantation, the results of the radiographic analysis indicated that in the experimental organ, the matrix filled the bone defect. Meanwhile, in the control organ, the bone defect was not filled well with the matrix. Accordingly, the results showed a significantly higher bone mineral density in the experimental group than in the control group (36).

For the treatment of pediatric patients with osteogenic defects, Horwitz et al. used non-autologous bone marrow cell transplantation. They found that bone density increased after three months of treatment and that the rate of fracture reduced (37). Shih et al. also indicated that biomechanical tests in the group under the autologous transplantation of stem cells in the demineralized femur distal of dogs showed significant increases in comparison with the allograft treatment group, non-cellular demineralized bone marrow matrix (PDMB) group, and no treatment group (38).

In this study, we evaluated two main groups of nicotine and non-nicotine models. For each major group, a control group was also considered. The differentiated osteocytes were obtained from adipocytes. The cells were transferred to the lesions according to the grouping. After four weeks, radiography results showed that the rate of osteogenesis was much higher in the non-nicotine group than in the nicotine group. The radiography results also showed that the rate of this density was more in the non-nicotine group receiving differentiated cells from adipocytes than in the nicotine group. In contrast, the results of histopathologic staining showed that osteogenesis and bone formation were more in the non-nicotine group than in the nicotine group. In addition, the rate of regular bone formation was higher in the group receiving osteocytes differentiated from adipocytes. The amount of osteogenesis in the control groups toward the center was observed in the margin of the defect, which was more than that in the nicotine control group.

5.1. Conclusions

Autologous graft/transplantation of adipocyte stromal cells in rats with bone defects can lead to the accelerated recovery of the bone. Meanwhile, what basically applying more positive effects on bone defect repair is autologous ADMSCs transplantation.

Acknowledgments

The authors thank all colleagues in the Science and Research Branch, Islamic Azad University, Tehran, Iran.

Footnotes

Authors' Contribution: All authors done equally.

Conflict of Interests: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

Ethical Approval: Experiments were all carried out following the instructions of the Animal Ethics Committee of Islamic Azad University. This protocol observes the ethical principles and protection of animals used for laboratory and other scientific purposes.

Funding/Support: There is no financial support.

References

1. Chen Y, Shao JZ, Xiang LX, Dong XJ, Zhang GR. Mesenchymal stem cells: A promising candidate in regenerative medicine. *Int J Biochem Cell Biol*. 2008;**40**(5):815–20. doi: 10.1016/j.biocel.2008.01.007. [PubMed: 18295530].
2. Salgado AJ, Coutinho OP, Reis RL. Bone tissue engineering: State of the art and future trends. *Macromol Biosci*. 2004;**4**(8):743–65. doi: 10.1002/mabi.200400026. [PubMed: 15468269].
3. Logeart-Avramoglou D, Anagnostou F, Bizios R, Petite H. Engineering bone: Challenges and obstacles. *J Cell Mol Med*. 2005;**9**(1):72–84. doi: 10.1111/j.1582-4934.2005.tb00338.x. [PubMed: 15784166]. [PubMed Central: PMC6741343].
4. Khomarlou N, Aberoomand-Azar P, Lashgari AP, Tebyanian H, Hakakian A, Ranjbar R, et al. Essential oil composition and in vitro antibacterial activity of Chenopodium album subsp. striatum. *Acta Biologica Hungarica*. 2018;**69**(2):144–55. doi: 10.1556/018.69.2018.2.4. [PubMed: 29888667].
5. Mosaddad SA, Tahmasebi E, Yazdanian A, Rezvani MB, Seifalian A, Yazdanian M, et al. Oral microbial biofilms: An update. *Eur J Clin Microbiol Infect Dis*. 2019;**38**(11):2005–19. doi: 10.1007/s10096-019-03641-9. [PubMed: 31372904].
6. Satija NK, Gurudutta GU, Sharma S, Afrin F, Gupta P, Verma YK, et al. Mesenchymal stem cells: Molecular targets for tissue engineering. *Stem Cells Dev*. 2007;**16**(1):7–23. doi: 10.1089/scd.2006.9998. [PubMed: 17348802].
7. Koh CJ, Atala A. Tissue engineering, stem cells, and cloning: opportunities for regenerative medicine. *J Am Soc Nephrol*. 2004;**15**(5):1113–25. doi: 10.1097/01.asn.0000119683.59068.f0. [PubMed: 15100351].
8. Kobayashi M, Spector M. In vitro response of the bone marrow-derived mesenchymal stem cells seeded in a type-I collagen-glycosaminoglycan scaffold for skin wound repair under the mechanical loading condition. *Mol Cell Biomech*. 2009;**6**(4):217–27. [PubMed: 19899445].
9. Tuan RS, Boland G, Tuli R. Adult mesenchymal stem cells and cell-based tissue engineering. *Arthritis Res Ther*. 2003;**5**(1):32–45. doi: 10.1186/ar614. [PubMed: 12716446]. [PubMed Central: PMC154434].
10. Xu W, Hu R, Fan E, Han D. Adipose-derived mesenchymal stem cells in collagen-hyaluronic acid gel composite scaffolds for vocal fold regeneration. *Ann Otol Rhinol Laryngol*. 2011;**120**(2):123–30. doi: 10.1177/000348941112000209. [PubMed: 21391425].
11. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol*. 2002;**30**(1):42–8. doi: 10.1016/s0301-472x(01)00769-x.
12. Caplan AI. Mesenchymal stem cells. *J Orthop Res*. 1991;**9**(5):641–50. doi: 10.1002/jor.1100090504. [PubMed: 1870029].
13. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve*. 1995;**18**(12):1417–26. doi: 10.1002/mus.880181212. [PubMed: 7477065].
14. Tebyanian H, Karami A, Nourani MR, Motavallian E, Barkhordari A, Yazdanian M, et al. Lung tissue engineering: An update. *J Cell Physiol*. 2019;**234**(11):19256–70. doi: 10.1002/jcp.28558. [PubMed: 30972749].
15. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: A phase II study. *Lancet*. 2008;**371**(9624):1579–86. doi: 10.1016/S0140-6736(08)60690-X. [PubMed: 18468541].
16. Sato K, Ozaki K, Mori M, Muroi K, Ozawa K. Mesenchymal stromal cells for graft-versus-host disease: Basic aspects and clinical outcomes. *J Clin Exp Hematop*. 2010;**50**(2):79–89. doi: 10.3960/jslrt.50.79. [PubMed: 21123965].
17. Sueblinvong V, Loi R, Eisenhauer PL, Bernstein IM, Suratt BT, Spees JL, et al. Derivation of lung epithelium from human cord blood-derived mesenchymal stem cells. *Am J Respir Crit Care Med*. 2008;**177**(7):701–11. doi: 10.1164/rccm.200706-859OC. [PubMed: 18063840]. [PubMed Central: PMC2277209].
18. Gupta N, Su X, Popov B, Lee JW, Serikov V, Matthay MA. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. *J Immunol*. 2007;**179**(3):1855–63. doi: 10.4049/jimmunol.179.3.1855. [PubMed: 17641052].

19. Loi R, Beckett T, Goncz KK, Suratt BT, Weiss DJ. Limited restoration of cystic fibrosis lung epithelium in vivo with adult bone marrow-derived cells. *Am J Respir Crit Care Med*. 2006;**173**(2):171–9. doi: 10.1164/rccm.200502-309OC. [PubMed: 16179642]. [PubMed Central: PMC2662986].
20. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*. 2001;**105**(3):369–77. doi: 10.1016/s0092-8674(01)00328-2. [PubMed: 11348593].
21. Wong AP, Dutly AE, Sacher A, Lee H, Hwang DM, Liu M, et al. Targeted cell replacement with bone marrow cells for airway epithelial regeneration. *Am J Physiol Lung Cell Mol Physiol*. 2007;**293**(3):L740–52. doi: 10.1152/ajplung.00050.2007. [PubMed: 17616650].
22. Serikov VB, Popov B, Mikhailov VM, Gupta N, Matthay MA. Evidence of temporary airway epithelial repopulation and rare clonal formation by BM-derived cells following naphthalene injury in mice. *Anat Rec (Hoboken)*. 2007;**290**(9):1033–45. doi: 10.1002/ar.20574. [PubMed: 17661377].
23. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation*. 2004;**109**(10):1292–8. doi: 10.1161/01.CIR.0000121425.42966.F1. [PubMed: 14993122].
24. Shang YC, Wang SH, Xiong F, Zhao CP, Peng FN, Feng SW, et al. Wnt3a signaling promotes proliferation, myogenic differentiation, and migration of rat bone marrow mesenchymal stem cells. *Acta Pharmacol Sin*. 2007;**28**(11):1761–74. doi: 10.1111/j.1745-7254.2007.00671.x. [PubMed: 17959027].
25. Heidari MF, Arab SS, Noroozi-Aghideh A, Tebyanian H, Latifi AM. Evaluation of the substitutions in 212, 342 and 215 amino acid positions in binding site of organophosphorus acid anhydrolase using the molecular docking and laboratory analysis. *Bratisl Lek Listy*. 2019;**120**(2):139–43. doi: 10.4149/BLL_2019_022. [PubMed: 30793618].
26. Taherian A, Fazilati M, Taghavi Moghadam A, Tebyanian H. Optimization of purification procedure for horse F(ab´)2 antivenom against Androctonus crassicauda (Scorpion) venom. *Trop J Pharmaceut Res*. 2018;**17**(3):409. doi: 10.4314/tjpr.v17i3.4.
27. Goli HR, Ebrahimzadeh MA, Tebyanian H, Ghazi Mirsaeed SR, Tahmasebi E, Ranjbar R, et al. The effect of Citrullus colocynthis extracts on Streptococcus mutans, Candida albicans, normal gingival fibroblast and breast cancer cells. *J Biol Res (Boll Soc Ital Biol Sper)*. 2019;**92**(1):8201. doi: 10.4081/jbr.2019.8201.
28. Babavalian H, Latifi AM, Shokrgozar MA, Bonakdar S, Tebyanian H, Shakeri F. Cloning and expression of recombinant human platelet-derived growth factor-BB in Pichia Pink. *Cell Mol Biol (Noisy-le-grand)*. 2016;**62**(8):45–51. [PubMed: 27545214].
29. Tebyanian H, Mirhosseiny SH, Kheirkhah B, Hassanshahian M, Farhadian H. Isolation and Identification of Mycoplasma synoviae From Suspected Ostriches by Polymerase Chain Reaction, in Kerman Province, Iran. *Jundishapur J Microbiol*. 2014;**7**(9). e19262. doi: 10.5812/jjm.19262. [PubMed: 25485069]. [PubMed Central: PMC4255384].
30. Karami A, Tebyanian H, Barkhordari A, Motavallian E, Sayyad Soufdoost R, Nourani MR. Healing effects of ointment drug on full-thickness wound. *C R Acad Bulg Sci*. 2019;**72**(1):123–9. doi: 10.7546/crabs.2019.01.16.
31. Shakeri F, Tebyanian H, Karami A, Babavalian H, Tahmasbi MH. Effect of topical phenytoin on wound healing. *Trauma Mon*. 2017;**22**(5). e35488. doi: 10.5812/traumamon.35488.
32. Tebyanian H, Karami A, Motavallian E, Aslani J, Samadikuchaksaraei A, Arjmand B, et al. Histologic analyses of different concentrations of TritonX-100 and Sodium dodecyl sulfate detergent in lung decellularization. *Cell Mol Biol (Noisy-le-grand)*. 2017;**63**(7):46–51. doi: 10.14715/cmb/2017.63.7.8. [PubMed: 28838339].
33. Tebyanian H, Karami A, Motavallian E, Aslani J, Samadikuchaksaraei A, Arjmand B, et al. A comparative study of rat lung decellularization by chemical detergents for lung tissue engineering. *Open Access Maced J Med Sci*. 2017;**5**(7):859–65. doi: 10.3889/oamjms.2017.179. [PubMed: 29362610]. [PubMed Central: PMC5771286].
34. Tebyanian H, Karami A, Motavallian E, Samadikuchaksaraei A, Arjmand B, Nourani MR. Rat lung decellularization using chemical detergents for lung tissue engineering. *Biotech Histochem*. 2019;**94**(3):214–22. doi: 10.1080/10520295.2018.1544376. [PubMed: 30516069].
35. Sayyad Soufdoost R, Yazdanian M, Tahmasebi E, Yazdanian A, Tebyanian H, Karami A, et al. In vitro and in vivo evaluation of novel Tadalafil/β-TCP/Collagen scaffold for bone regeneration: A rabbit critical-size calvarial defect study. *Biocybern Biomed Eng*. 2019;**39**(3):789–96. doi: 10.1016/j.bbe.2019.07.003.
36. Niedzwiedzki T, Dabrowski Z, Miszta H, Pawlikowski M. Bone healing after bone marrow stromal cell transplantation to the bone defect. *Biomaterials*. 1993;**14**(2):115–21. doi: 10.1016/0142-9612(93)90221-m.
37. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med*. 1999;**5**(3):309–13. doi: 10.1038/6529. [PubMed: 10086387].
38. Shih HN, Shih LY, Sung TH, Chang YC. Restoration of bone defect and enhancement of bone ingrowth using partially demineralized bone matrix and marrow stromal cells. *J Orthop Res*. 2005;**23**(6):1293–9. doi: 10.1016/j.orthres.2005.04.005.1100230609. [PubMed: 15946821].