

Received: 2020.11.06

Accepted: 2020.12.20

Available online: 2021.01.12

Published: 2021.03.10

Exosomes from Adipose-Derived Stem Cells Can Prevent Medication-Related Osteonecrosis of the Jaw

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ADEF 1 **Dong Xian**
CD 1 **Li-Hang Shen**
D 1 **Zheng Yi**
AEFG 2 **Lin-hai He**
AEF 1 **Zhang Yi**

1 Laboratory of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, Beijing, P.R. China
2 First Clinical Division, Peking University School Hospital of Stomatology, Beijing, P.R. China


Corresponding Authors: Lin-hai He, e-mail: helinhai07@bjmu.edu.cn, Zhang Yi, e-mail: zhangyi2000@263.net

Source of support: This work was supported by grants from the National Natural Science Foundation of China (No. 81371117 and No. 81801000) and the Beijing Natural Science Foundation (No. 7202234 and No. 7192230)

The treatment measures of medication-related osteonecrosis of the jaw (MRONJ) is a worldwide challenge in oral and maxillofacial surgery because of its unclear pathogenesis. Previous studies suggested that mesenchymal stem cells played important roles in promoting MRONJ lesion healing, but the detailed mechanisms were unknown. Increasing numbers of studies have demonstrated that exosomes derived from mesenchymal stem cells, especially adipose-derived stem cells, have key roles in stem cell-based therapies by accelerating bone remodeling, facilitating angiogenesis, and promoting wound healing. We hypothesized that exosomes derived from adipose-derived stem cells can prevent MRONJ by accelerating gingival healing and enhancing bone remodeling processes. Our results may provide a promising therapeutic option for MRONJ clinical therapy.

Keywords: **Bisphosphonate-Associated Osteonecrosis of the Jaw • Bone Remodeling • Mesenchymal Stromal Cells • Wound Healing**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/929684>

 1924

 —

 —

 67



Background

Medication-related osteonecrosis of the jaw (MRONJ) is a serious disease that only occurs in the jawbones. Its occurrence is related to anti-bone resorption drugs and anti-angiogenesis drugs, such as bisphosphonates and denosumab, among others [1]. Since MRONJ was first reported in 2003, the number of patients has dramatically increased because of the wide application of antiresorption drugs in cancer and osteoporosis treatments [2,3]. The quality of life for patients with MRONJ is markedly reduced due to persistent necrotic jawbone exposure and pain [4]. Moreover, MRONJ lesions are difficult to repair, and because viable jawbone margins are difficult to obtain, the recommended surgery might even expand the lesion area and worsen symptoms [5,6]. Thus, the treatment of MRONJ has drawn much attention among clinical surgeons and researchers. Although some conservative regimens have been explored to treat MRONJ, their success rate is controversial [1]. Therefore, more effective therapies are needed to prevent or treat MRONJ.

Therapeutic Potential of Adipose-derived Stem Cells on Bone And Soft Tissues

Adipose-derived stem cells (ASCs), a kind of self-renewing multipotent stem cell, seem to be the most advantageous adult stem cells for cell therapy. These cells are abundant and accessible, and their collection is associated with low donor morbidity [7]. Accumulated studies have shown that ASC-derived cellular therapy exerts important functions in the treatment of various human skeletal and soft tissue diseases, such as osteoporosis, bone nonunion, and skin wounds, by differentiating into the types of cells that are damaged and by secreting paracrine factors or regulatory mediators of immune function [8-10]. Previous research suggested that the local implantation of allogeneic ASCs directly participated in osteogenesis and enhanced rehabilitation within bone defects [11]. Wagner et al [12] found that local application of ASCs repaired bone defects by enhancing bone regeneration after sufficient debridement of infected bone tissue in posttraumatic osteomyelitis [12]. Furthermore, ASCs were reported to upregulate bone morphogenetic protein (BMP)-2 and vascular endothelial growth factor (VEGF) in perfracture tissues, differentiate into osteoblasts or endothelial cells, and promote M2 macrophage polarization, thus effectively accelerating fracture healing [13,14]. Previous research also suggested that the systemic transplantation of ASCs improved bone quality in ovariectomized-induced osteoporosis in vivo, and the conditioned medium derived from ASCs promoted proliferation and differentiation of osteoblasts and stimulated survival and differentiation of osteoclasts in vitro [8]. In rheumatoid arthritis, systemic administration of ASCs alleviated the progression of arthritis by reducing the total number of pathogenic GM-Th17 cells and

increasing the number of regulatory T cells [15]. Interestingly, Alonso-Rodriguez et al [16] and Barba-Recreo et al [17] reported ASCs effectively promoted vascularization, bone remodeling, and regeneration, and prevented the onset of MRONJ.

Aside from the bone repair process, ASCs were also shown to play important roles in cutaneous wound healing and regeneration [18-20]. Altman et al [21] confirmed that local administration of ASCs significantly accelerated full-thickness skin wound healing by endothelial and fibroblastic differentiation. Apart from direct differentiation, accumulated studies have shown that ASCs can secrete various cytokines and chemokines, such as VEGF, basic fibroblast growth factor (b-FGF), interleukin (IL)-6, IL-8, and hepatocyte growth factor (HGF) to regulate angiogenesis and immunity during wound healing [10,22]. Oryan et al [23] reported that ASCs suppressed the expression of IL-1 β on the 7th day after injury, increased the level of TGF- β 1 on the 14th day after injury, upregulated the level of TGF- β 1 and bFGFs on the 28th day after injury, and promoted burn wound healing [23]. Furthermore, ASCs were shown to accelerate diabetic wound healing by secreting VEGF, b-FGF, and HGF, which may have enhanced the epithelialization rate and granulation tissue formation [24]. With regard to MRONJ, our previous study suggested that local administration of ASCs accelerated gingival closure via TGF- β 1 in zoledronate-treated mice and could prevent MRONJ [25]. Hence, ASCs are a useful source of stem cell therapies for bone and soft tissue wound healing.

Exosomes Exert Important Roles in ASC-based Therapy Function

Exosomes are 30- to 100-nm extracellular vesicles that contain various proteins, nucleic acids, and lipids, and they have attracted much attention as acellular therapy [26]. Recently, studies have increasingly suggested that transplanted stem cells are more likely to secrete extracellular vesicles as cell-to-cell communicators to exert their influence on targeted tissues, rather than home in on the injured sites and subsequently differentiate into the target cells [27,28]. Many exosomal proteins from ASCs, some of which are associated with cell migration and regeneration, have been identified by proteomic analysis [29]. In addition, the use of ASC-derived exosomes (ASC-Exos) may avoid the biosafety and ethics problems associated with direct cell transplantation. Stem cell therapy is potentially controversial due to the risks of pulmonary embolism and tumorigenicity [30,31]. Furthermore, ASC-Exos have the advantage of being more easily manufactured, handled, characterized, and stored than stem cells [32]. In the past few years, accumulated studies have demonstrated that ASC-Exos play important roles in wound healing, bone repair, myocardial repair, hepatic repair, and other aspects of diseases [33-35]. ASC-Exos were found to deliver miR-21, miR-181b, MMPs, MFG-E8,

and ANGPTL1 to activate intercellular signaling pathways in endothelial cells and promote vascularization in soft tissue repair [36-38]; to transmit miR-375 to help induce osteoblast differentiation and bone regeneration in bone defects [39]; and to transfer miR-126 to inhibit inflammation and fibrosis and to promote neovascularization to alleviate myocardial damage in acute myocardial ischemic animal models [40]. Therefore, exosomes from ASCs show great potential as acellular factors for various disease therapies.

Hypothesis

MRONJ is a disease of obvious clinical manifestation with persistent gingival nonhealing and osteonecrotic bone exposure. Several theories have been reported regarding the main pathogenesis of MRONJ, including oversuppression of jawbone remodeling, soft tissue toxicity, and anti-angiogenesis [41-43]. Based on a brief review of recent studies of the effects of ASCs and exosomes on bone and soft tissue wound healing as well as angiogenesis, we hypothesize that exosomes from ASCs might contribute to preventing MRONJ by enhancing bone remodeling, accelerating gingival wound healing, and promoting angiogenesis.

Evaluation of the Hypothesis

Exosomes Derived from ASCs Enhanced Bone Remodeling

Bone remodeling is a lifelong process that serves to adjust the skeleton's architecture and repair microdamage to maintain functional integrity [44]. This process is characterized by coupling of bone remodeling-related cells, osteoclasts for bone resorption and osteoblasts for bone formation, which are organized in bone multicellular units [45]. Since exosomes are considered primary paracrine effectors of intercellular messengers, they have attracted considerable attention in the context of bone physiology and diseases [26]. Physiologically, exosomal miRNAs derived from MSCs have been found to be enriched in bone coupling-related signaling and proteins (Wnt/ β -catenin, BMP/Smad, TGF- β , mTOR) [46,47], which not only transmit signals to mediate bone resorption and formation and promote the proliferation, differentiation, and activity of most bone remodeling-related cells, but also play a pivotal part in extracellular matrix mineralization [34,46-49]. In the process of bone resorption, exosomal miR-31a-5p from bone marrow-derived MSCs promoted osteoclastic differentiation and bone resorption activity by directly binding to the 3'UTR of RhoA [50]. In the process of bone formation, exosomes from ASCs contained let-7i-5p, miR-22-3p, miR-17, miR-20a, miR-20b, and miR-106a, which are capable of promoting bone MSC migration and homing to bone defects, thereby remarkably enhancing bone

regeneration [51,52]. Taken together, the evidence suggests that exosomes from ASCs might exert important functions in promoting bone remodeling and might be a promising tool to rescue bone remodeling suppression in MRONJ.

Exosomes from ASCs Accelerating Wound Healing

An increasing number of studies have demonstrated that exosomes from ASCs can promote healing of wounds, such as diabetic foot ulcers and chronic cutaneous ulceration [53,54]. Wound healing includes 4 phases: hemostasis, inflammation, proliferation, and tissue remodeling [55]. Zhao et al [56] found that exosome-borne active STAT3 from ASCs could induce macrophages into M2 phenotypes and significantly suppress the secretion of proinflammatory factor tumor necrosis factor- α , while increasing the anti-inflammatory factor IL-10. In a separate study, Zhang et al [57] showed that ASC-derived exosomes can promote fibroblast proliferation and migration and optimize collagen deposition via the PI3K/Akt signaling pathway to further accelerate full-thickness skin wound healing. Furthermore, ASC-Exos were reported to promote re-epithelialization and inhibit apoptosis of HaCat cells via upregulating the Wnt/ β -catenin signaling pathway [58]. Thus, exosome-derived ASCs showed potent abilities to promote wound healing.

Exosomes from ASCs Promote Angiogenesis

Multiple studies have suggested that exosomes could promote cutaneous wound healing or bone healing by accelerating angiogenesis, which is a vital factor determining the outcome of healing [59,60]. Exosomes from MSCs have been found to contain pro-angiogenic factors (VEGF, TGF- β 1, IL-8, HGF, and multiple microRNAs) that induce vascularization by promoting endothelial cell proliferation and migration [34]. Huang et al [61] reported that systemic administration of MSC-Exos enhanced microvessel density, and reduced target cell apoptosis and tissue inflammatory infiltration in a rat spinal cord injury model. Another study showed that ASC-Exos enhanced endothelial cell tube formation both in vitro and in vivo through miR-125a inhibiting the expression of the angiogenesis inhibitor delta-like 4 (DLL4) [62]. Consistent with these findings, Xue et al [54] also showed that ASC-derived exosomes induced neovascularization and accelerated wound healing by overexpressing the transcription factor nuclear factor-E2-related factor 2 (Nrf2) in a diabetic foot ulcer rat model. In a study of experimental ischemia-reperfusion injury, ASC-Exos protected skin flap survival through enhanced neovascularization and reduced the inflammation and apoptosis in the skin flap [63]. Taken together, these studies suggested exosomes from ASCs might play key roles in stimulating vascularization owing to their enriched angiogenesis-related factors. Nevertheless, the definitive mechanisms of the interaction between exosomes and endothelial cells still need to be explored.

Discussion

Promoting gingival healing, bone remodeling, and angiogenesis are key interventions to prevent the development of MRONJ. Over the past few years, therapies based on stem cells have arisen as a very promising approach to prevent MRONJ development [16,17,25]. Our previous study suggested that the local administration of ASCs prevented the occurrence of MRONJ through secreting TGF- β 1, inducing primary gingival closure, and rescuing the bone regeneration process in the alveolar sockets [25]. However, MRONJ predominantly occurs in patients with malignant underlying diseases [64]. ASC-based treatment may increase the risks of systemic immunosuppression [30]. Furthermore, the efficiency of stem cell-based therapies is often influenced by many factors, such as high cell passages and cells being derived from aged or diseased patients [65,66]. Thus, it is necessary to find a more secure way to prevent the development of MRONJ.

Extensive evidence exists in the literature that shows that ASCs predominantly act in a paracrine manner, with secretory factors being the mediators of soft tissue repair and bone remodeling [34,55,67]. Exosomes from ASCs might exert a similar function in promoting wound healing in both bone tissues and soft tissues, as they are one of the key factors released by MSCs [26]. Furthermore, exosome-based therapy has

its advantages as an acellular therapy compared with direct treatment with ASCs. Therefore, exosomes may be a promising therapy in the treatment of MRONJ by enhancing bone remodeling, gingival healing, and angiogenesis. To achieve a higher concentration and more effective action in the targeted area and to eliminate the adverse effects on systemic immunomodulation, we generally apply the exosomes locally at the same time as dental surgery or dental extractions. Future studies are needed to focus on the therapeutic effects of exosomes on MRONJ and the therapeutic mechanisms.

Conclusions

The pathogenesis of MRONJ is now widely accepted as having a multifactorial etiology, including oversuppression of jawbone remodeling, soft tissue toxicity, and anti-angiogenesis. ASC-derived exosomes have potential for promoting bone remodeling and enhancing soft tissue wound healing and angiogenesis. As such, exosomes from ASCs might contribute to preventing MRONJ.

Conflict of Interest

None.

References:

- Ruggiero SL, Dodson TB, Fantasia J et al, American Association of Oral and Maxillofacial Surgeons. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw – 2014 update. *J Oral Maxillofac Surg*, 2014;72(10):1938-56
- Di Fede O, Fusco V, Matranga D, et al. Osteonecrosis of the jaws in patients assuming oral bisphosphonates for osteoporosis: A retrospective multi-hospital-based study of 87 Italian cases. *Eur J Intern Med*, 2013;24(8):784-90
- Lazarovici TS, Yahalom R, Taicher S, et al. Bisphosphonate-related osteonecrosis of the jaws: A single-center study of 101 patients. *J Oral Maxillofac Surg*, 2009;67(4):850-55
- Miksad RA, Lai KC, Dodson TB, et al. Quality of life implications of bisphosphonate-associated osteonecrosis of the jaw. *Oncologist*, 2011;16(1):121-32
- Ruggiero SL, Dodson TB, Assael LA, et al. American Association of Oral and Maxillofacial Surgeons. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws – 2009 update. *J Oral Maxillofac Surg*, 2009;67(5 Suppl.):2-12
- Marx RE, Sawatari Y, Fortin M, Broumand V. Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: Risk factors, recognition, prevention, and treatment. *J Oral Maxillofac Surg*, 2005;63(11):1567-75
- Wallner C, Abraham S, Wagner JM, et al. Local application of isogenic adipose-derived stem cells restores bone healing capacity in a type 2 diabetes model. *Stem Cells Transl Med*, 2016;5(6):836-44
- Lee K, Kim H, Kim JM, et al. Systemic transplantation of human adipose-derived stem cells stimulates bone repair by promoting osteoblast and osteoclast function. *J Cell Mol Med*, 2011;15(10):2082-94
- Tawonsawatruk T, West CC, Murray IR, et al. Adipose derived pericytes rescue fractures from a failure of healing – non-union. *Sci Rep*, 2016;6:22779
- Li P, Guo X. A review: Therapeutic potential of adipose-derived stem cells in cutaneous wound healing and regeneration. *Stem Cell Res Ther*, 2018;9(1):302
- Liu G, Zhang Y, Liu B, et al. Bone regeneration in a canine cranial model using allogeneic adipose derived stem cells and coral scaffold. *Biomaterials*, 2013;34(11):2655-64
- Wagner JM, Reinkemeier F, Wallner C, et al. Adipose-derived stromal cells are capable of restoring bone regeneration after post-traumatic osteomyelitis and modulate B-cell response. *Stem Cells Transl Med*, 2019;8(10):1084-91
- Shoji T, Ii M, Mifune Y, et al. Local transplantation of human multipotent adipose-derived stem cells accelerates fracture healing via enhanced osteogenesis and angiogenesis. *Lab Invest*, 2010;90(4):637-49
- Li Y, Kong N, Li Z, et al. Bone marrow macrophage M2 polarization and adipose-derived stem cells osteogenic differentiation synergistically promote rehabilitation of bone damage. *J Cell Biochem*, 2019;120(12):19891-901
- Lopez-Santalla M, Mancheño-Corvo P, Menta R, et al. Human adipose-derived mesenchymal stem cells modulate experimental autoimmune arthritis by modifying early adaptive T cell responses. *Stem Cells*, 2015;33(12):3493-503
- Alonso-Rodriguez E, González-Martín-Moro J, Cebrián-Carretero JL, et al. Bisphosphonate-related osteonecrosis. Application of adipose-derived stem cells in an experimental murine model. *Med Oral Patol Oral Cir Bucal*, 2019;24(4):e529-36
- Barba-Recreo P, Del Castillo Pardo de Vera JL, Georgiev-Hristov T, et al. Adipose-derived stem cells and platelet-rich plasma for preventive treatment of bisphosphonate-related osteonecrosis of the jaw in a murine model. *J Craniomaxillofac Surg*, 2015;43(7):1161-68
- Sivan U, Jayakumar K, Krishnan LK. Constitution of fibrin-based niche for in vitro differentiation of adipose-derived mesenchymal stem cells to keratinocytes. *Biores Open Access*, 2014;3(6):339-47
- Hu R, Ling W, Xu W, Han D. Fibroblast-like cells differentiated from adipose-derived mesenchymal stem cells for vocal fold wound healing. *PLoS One*, 2014;9(3):e92676
- Nie C, Yang D, Xu J, et al. Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis. *Cell Transplant*, 2011;20(2):205-16
- Altman AM, Matthias N, Yan Y, et al. Dermal matrix as a carrier for in vivo delivery of human adipose-derived stem cells. *Biomaterials*, 2008;29(10):1431-42

22. Park BS, Jang KA, Sung JH, et al. Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging. *Dermatol Surg*, 2008;34(10):1323-26
23. Oryan A, Alemzadeh E, Mohammadi AA, Moshiri A. Healing potential of injectable Aloe vera hydrogel loaded by adipose-derived stem cell in skin tissue engineering in a rat burn wound model. *Cell Tissue Res*, 2019;377(2):215-27
24. Nambu M, Kishimoto S, Nakamura S, et al. Accelerated wound healing in healing-impaired db/db mice by autologous adipose tissue-derived stromal cells combined with atelocollagen matrix. *Ann Plast Surg*, 2009;62(3):317-21
25. Zang X, He L, Zhao L, et al. Adipose-derived stem cells prevent the onset of bisphosphonate-related osteonecrosis of the jaw through transforming growth factor β -1-mediated gingival wound healing. *Stem Cell Res Ther*, 2019;10(1):169
26. Hao ZC, Lu J, Wang SZ, et al. Stem cell-derived exosomes: A promising strategy for fracture healing. *Cell Prolif*, 2017;50(5):e12359
27. Fontaine MJ, Shih H, Schäfer R, Pittenger MF. Unraveling the mesenchymal stromal cells' paracrine immunomodulatory effects. *Transfus Med Rev*, 2016;30(1):37-43
28. Ophelders DR, Wolfs TG, Jellema RK, et al. Mesenchymal stromal cell-derived extracellular vesicles protect the fetal brain after hypoxia-ischemia. *Stem Cells Transl Med*, 2016;5(6):754-63
29. Ni J, Li H, Zhou Y, et al. Therapeutic potential of human adipose-derived stem cell exosomes in stress urinary incontinence – an in vitro and in vivo study. *Cell Physiol Biochem*, 2018;48(4):1710-22
30. Djouad F, Plence P, Bony C, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood*, 2003;102(10):3837-44
31. Jung JW, Kwon M, Choi JC, et al. Familial occurrence of pulmonary embolism after intravenous, adipose tissue-derived stem cell therapy. *Yonsei Med J*, 2013;54(5):1293-96
32. Liu X, Yang Y, Li Y, et al. Integration of stem cell-derived exosomes with *in situ* hydrogel glue as a promising tissue patch for articular cartilage regeneration. *Nanoscale*, 2017;9(13):4430-38
33. Fang Y, Zhang Y, Zhou J, Cao K. Adipose-derived mesenchymal stem cell exosomes: A novel pathway for tissues repair. *Cell Tissue Bank*, 2019;20(2):153-61
34. Masaoutis C, Theocharis S. The role of exosomes in bone remodeling. Implications for bone physiology and disease. *Dis Markers*, 2019;2019. 9417914
35. Lu Z, Chen Y, Dunstan C, et al. Priming adipose stem cells with tumor necrosis factor- α preconditioning potentiates their exosome efficacy for bone regeneration. *Tissue Eng Part A*, 2017;23(21-22):1212-20
36. Yang Y, Cai Y, Zhang Y, et al. Exosomes secreted by adipose-derived stem cells contribute to angiogenesis of brain microvascular endothelial cells following oxygen-glucose deprivation in vitro through microRNA-181b/TRPM7 axis. *J Mol Neurosci*, 2018;65(1):74-83
37. An Y, Zhao J, Nie F, et al. Exosomes from adipose-derived stem cells (ADSCs) overexpressing miR-21 promote vascularization of endothelial cells. *Sci Rep*, 2019;9(1):12861
38. Lopatina T, Bruno S, Tetta C, et al. Platelet-derived growth factor regulates the secretion of extracellular vesicles by adipose mesenchymal stem cells and enhances their angiogenic potential. *Cell Commun Signal*, 2014;12: 26
39. Chen S, Tang Y, Liu Y, et al. Exosomes derived from miR-375-overexpressing human adipose mesenchymal stem cells promote bone regeneration. *Cell Prolif*, 2019;52(5):e12669
40. Luo Q, Guo D, Liu G, et al. Exosomes from miR-126-overexpressing ADSCs are therapeutic in relieving acute myocardial ischaemic injury. *Cell Physiol Biochem*, 2017;44(6). 2105-16
41. Reid IR, Bolland MJ, Grey AB. Is bisphosphonate-associated osteonecrosis of the jaw caused by soft tissue toxicity? *Bone*, 2007;41(3):318-20
42. Otto S, Hafner S, Mast G, et al. Bisphosphonate-related osteonecrosis of the jaw: Is pH the missing part in the pathogenesis puzzle? *J Oral Maxillofac Surg*, 2010;68(5):1158-61
43. Boff RC, Salum FG, Figueiredo MA, Cherubini K. Important aspects regarding the role of microorganisms in bisphosphonate-related osteonecrosis of the jaws. *Arch Oral Biol*, 2014;59(8):790-99
44. Hadjidakis DJ, Androulakis II. Bone remodeling. *Ann NY Acad Sci*, 2006;1092: 385-96
45. Arias CF, Herrero MA, Echeverri LF, et al. Bone remodeling. A tissue-level process emerging from cell-level molecular algorithms. *PLoS One*, 2018;13(9):e0204171
46. Peng S, Gao D, Gao C, et al. MicroRNAs regulate signaling pathways in osteogenic differentiation of mesenchymal stem cells (review). *Mol Med Rep*, 2016;14(1):623-29
47. Bhushan R, Grünhagen J, Becker J, et al. miR-181a promotes osteoblastic differentiation through repression of TGF- β signaling molecules. *Int J Biochem Cell Biol*, 2013;45(3):696-705
48. Narayanan R, Huang CC, Ravindran S. Hijacking the cellular mail: Exosome mediated differentiation of mesenchymal stem cells. *Stem Cells Int*, 2016;2016: 3808674
49. Crane JL, Cao X. Bone marrow mesenchymal stem cells and TGF- β signaling in bone remodeling. *J Clin Invest*, 2014;124(2):466-72
50. Xu R, Shen X, Si Y, et al. MicroRNA-31a-5p from aging BMSCs links bone formation and resorption in the aged bone marrow microenvironment. *Aging Cell*, 2018;17(4):e12794
51. Jia B, Zhang Z, Qiu X, et al. Analysis of the miRNA and mRNA involved in osteogenesis of adipose-derived mesenchymal stem cells. *Exp Ther Med*, 2018;16(2):1111-20
52. Zhang ZJ, Zhang H, Kang Y, et al. miRNA expression profile during osteogenic differentiation of human adipose-derived stem cells. *J Cell Biochem*, 2012;113(3):888-98
53. Hu L, Wang J, Zhou X, et al. Exosomes derived from human adipose mesenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci Rep*, 2016;6: 32993
54. Li X, Xie X, Lian W, et al. Exosomes from adipose-derived stem cells over-expressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. *Exp Mol Med*, 2018;50(4):29
55. Rani S, Ritter T. The exosome – a naturally secreted nanoparticle and its application to wound healing. *Adv Mater*, 2016;28(27):5542-52
56. Zhao H, Shang Q, Pan Z, et al. Exosomes from adipose-derived stem cells attenuate adipose inflammation and obesity through polarizing M2 macrophages and being in white adipose tissue. *Diabetes*, 2018;67(2):235-47
57. Zhang W, Bai X, Zhao B, et al. Cell-free therapy based on adipose tissue stem cell-derived exosomes promotes wound healing via the PI3K/Akt signaling pathway. *Exp Cell Res*, 2018;370(2):333-42
58. Ma T, Fu B, Yang X, et al. Adipose mesenchymal stem cell-derived exosomes promote cell proliferation, migration, and inhibit cell apoptosis via Wnt/ β -catenin signaling in cutaneous wound healing. *J Cell Biochem*, 2019;120(6):10847-54
59. Zhang B, Wang M, Gong A, et al. HucMSC-exosome mediated-Wnt4 signaling is required for cutaneous wound healing. *Stem Cells*, 2015;33(7):2158-68
60. Zhang J, Guan J, Niu X, et al. Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis. *J Transl Med*, 2015;13: 49
61. Huang JH, Yin XM, Xu Y, et al. Systemic administration of exosomes released from mesenchymal stromal cells attenuates apoptosis, inflammation, and promotes angiogenesis after spinal cord injury in rats. *J Neurotrauma*, 2017;34(24):3388-96
62. Liang X, Zhang L, Wang S, et al. Exosomes secreted by mesenchymal stem cells promote endothelial cell angiogenesis by transferring miR-125a. *J Cell Sci*, 2016;129(11):2182-89
63. Bai Y, Han YD, Yan XL, et al. Adipose mesenchymal stem cell-derived exosomes stimulated by hydrogen peroxide enhanced skin flap recovery in ischemia-reperfusion injury. *Biochem Biophys Res Commun*, 2018;500(2):310-17
64. Otto S, Schreyer C, Hafner S, et al. Bisphosphonate-related osteonecrosis of the jaws – characteristics, risk factors, clinical features, localization and impact on oncological treatment. *J Craniomaxillofac Surg*, 2012;40(4):303-9
65. Scruggs BA, Semon JA, Zhang X, et al. Age of the donor reduces the ability of human adipose-derived stem cells to alleviate symptoms in the experimental autoimmune encephalomyelitis mouse model. *Stem Cells Transl Med*, 2013;2(10):797-807
66. Siddappa R, Licht R, van Blitterswijk C, de Boer J. Donor variation and loss of multipotency during in vitro expansion of human mesenchymal stem cells for bone tissue engineering. *J Orthop Res*, 2007;25(8):1029-41
67. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: Toward cell-free therapeutic applications. *Mol Ther*, 2015;23(5):812-23