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REVIEW ARTICLE

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Stem cell-derived exosomes: A promising strategy for fracture healing

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Abstract

Objectives: To describe the biological characteristics of exosomes and to summarize the current status of stem cell-derived exosomes on fracture healing. Meanwhile, future challenges, limitations and perspectives are also discussed.

Methods: Search and analyze the related articles in pubmed database through the multi-combination of keywords like “stem cells”,”exosomes”,”bone regeneration” and “fracture healing”.

Conclusion: Stem cell-derived exosome therapy for fracture healing has been enjoying popularity and is drawing increasing attention. This strategy helps to promote proliferation and migration of cells, as well as osteogenesis and angiogenesis, in the process of bone formation. Although the exact mechanisms remain elusive, exosomal miRNAs seem to play vital roles. Future studies are required to solve multiple problems before clinical application, including comprehensive and thorough understanding of exosomes, the exact roles of exosomes in regulating bone formation, and the optimal source, dose and frequency of treatment, as well as technical and safety issues. Moreover, studies based on fracture models of large animals are could offer guidance and are in demand.

1 | INTRODUCTION

Fractures are common traumatic injuries with an estimated prevalence of 16 million in the United States each year. Although bones possess excellent regenerative properties and most fractures heal normally, approximately 5%-10% of fractures are complicated by delayed healing or non-union.[1–3] These complications lead to prolonged treatment time, impaired quality of life and even additional remedial surgeries, which exert a heavy burden on the patients and impact heavily on society.[3,4] In established non-union fractures, surgical intervention with autologous bone graft is the gold standard at present due to the superior clinical safety and graft biocompatibility compared with allogenic or artificial grafts.[5–8] Nevertheless, it requires a second surgery with associated pain and additional damage to harvest sites. It is also limited by the quantity of harvested bone.[9,10] Therefore, obtaining in-depth knowledge of the fracture healing process and associated mechanisms and providing appropriate interventions to accelerate bone regeneration is critical to avoid adverse outcomes.

Fracture repair is a complex process, regulated by thousands of genes and impacted significantly by cytokines, chemokines, growth factors and other molecules.[11,12] Initially, a haematoma develops following fracture to create a microenvironment that is rich in hormones, growth factors and cytokines. Subsequently, progenitor cells and bone marrow stromal cells are induced to recruit, proliferate, migrate and differentiate into osteoblasts and chondrocytes for intramembranous ossification and endochondral ossification.[13–15] Collagen matrices are secreted by chondrocytes and osteoblasts to calcify and bridge the fracture site. During endochondral ossification, under the regulation of angiogenesis-related genes, angiogenesis is stimulated and the circulatory system is restored.[16–18] The surrounding matrix is digested by chondroclasts under the effect of vascular endothelial growth factor (VEGF) from endothelial cells and then infiltrated by blood vessels and osteoblasts.[19–22] Finally, bone tissues achieve regeneration and return to their original architecture and function.

A variety of currently available approaches have been developed to enhance fracture healing, typically including biophysical and biological

means.[1] Biophysical treatments primarily consist of electromagnetic stimulation and low-intensity pulsed ultrasonography, which show uncertain and controversial effects on fracture healing.[23–26] Biological strategies primarily indicate the use of osteogenic materials, such as bone marrow grafting,[27] injection of active substances,[28,29] gene modification,[30–32] stem cell implantation[33,34] and tissue engineering.[35] Among these, stem cell therapy has been shown to be promising for bone regeneration as well as for regeneration of various tissues.[33,34,36–39] Nevertheless, the molecular mechanisms are still undefined. Moreover, the clinical application of stem cell therapy is still hampered by the limited number of donors, invasive harvesting procedures and safety hurdles.[40,41]

With the in-depth understanding of stem cells in tissue regeneration, mounting evidence has suggested that the positive effects primarily depend on exosomes released from stem cells.[42–45] These 40-100 nm extracellular vesicles from cells are considered important players in intercellular communication due to their ability to transfer proteins, genetic information and diverse molecules to target cells.[43,46–48] Exosomes provide researchers with a novel way to promote regeneration of various tissues, including bone. The aim of this review was to describe the biological characteristics of exosomes and to summarize the current status of research on the use of stem cell-derived exosomes to promote fracture healing.

2 | BIOLOGICAL CHARACTERISTICS OF EXOSOMES

To achieve certain physiological functions, cells can release different types of extracellular vesicles, including microvesicles, exosomes, membrane fragments and apoptotic bodies. Commonly, these vesicles are distinguished from one another with regard to subcellular origin, size, content and the formation mechanism (Table 1).[46,47,49–52] Exosomes are 40-100 nm cup-shaped vesicles that are derived from the inward budding of the endosome membrane and are released into the extracellular environment through fusion of multivesicular bodies with the plasma membrane. Released exosomes may either fuse directly with the plasma membrane of target cells or be endocytosed by target cells.[53–57]

Exosomes derived from sheep red blood cells were initially described by biochemist Rose Johnstone in the 1970s.[58] Since then, related studies to explore the mysteries of exosomes have been ongoing. Numerous studies have demonstrated that exosomes can be generated by various cell types, including dendritic cells,[59] epithelial cells,[60] tumour cells,[61] immune cells[62] and stem cells,[42–45] and they can also be detected in biological fluids such as blood plasma,[63] saliva,[64] urine[65] and breast milk.[66] Exosomes contain various molecular constituents from the originating cells, including lipids, proteins, mRNA, miRNA and other components.[58,67–69] Although exosomal components differ substantially on the basis of the specific donor cell type, exosomes are generally enriched with proteins, including Alix, TSG101, annexin, glycosylphosphatidylinositol-anchored proteins, flotillin and tetraspanins (CD9, CD63 and CD81), which can be considered identifying signatures of an exosome.[67–71]

As to the roles of exosomes, to remove the unnecessary substances from parent cells was regarded as the primary function.[58,69] With further studies, exosomes appear to reflect the behaviour of the originating cells and are active in diverse aspects of physiology and pathophysiology, including immunological regulation, tumour progression, virus spreading, epithelial activities, neuronal survival and so forth. Their functional characteristics are not completely clear to this day. It is commonly accepted that exosomes play important roles in intercellular communication between cells locally or at a distance through receptor-mediated interactions or by delivering their protein, lipid and genetic contents.[58,59,72–74]

3 | REGENERATIVE CAPACITIES OF STEM CELL-DERIVED EXOSOMES IN VARIOUS TISSUES

In the last few years, stem cell-derived exosomes have gained prominence in regenerative medicine research. Ruenn Chai Lai found that stem cell-derived exosomes reduced infarct size in a mouse model of myocardial ischaemia/reperfusion injury, highlighting the role of exosomes as mediators of tissue repair.[75] Later, this novel treatment was further applied to repair injured cardiac tissue, exhibiting a positive

TABLE 1 Basic characteristics of different extracellular vesicles groups

	Exosomes	Microvesicles	Apoptotic bodies
Size	40-100 nm	100-1000 nm	500-4000 nm
Density	1.13-1.19 g/mL	Undescribed	1.16-1.28 g/mL
Morphology	Cup-shaped	Heterogeneous	Heterogeneous
Biogenesis	Produced in multivesicular bodies and released into the extracellular environment through fusion of multivesicular bodies with the plasma membrane	Outward budding from the plasma membrane	Released when cells become apoptotic and formed by blebbing of the plasma membrane
Markers	Alix, TSG101 GTPases, annexins, flotillins, CD9, CD63 and CD81	CD40 ligand, adenosine diphosphate ribosylation factor 6, several integrins and selectins	Thrombospondin, complement component C3b, organelles, DNA fragments and histones
Composition	Lipids, proteins, mRNA and miRNA	Lipids, proteins, mRNA and miRNA	Lipids, proteins, mRNA, miRNA and DNA

effect.[75–77] Additionally, stem cell-derived exosomes have also been shown to protect against renal injury by stimulating cell proliferation and inhibiting apoptosis in a mouse remnant kidney model.[78–81] As studies progressed, regeneration of a multitude of tissues, including cutaneous wound healing,[82,83] skeletal muscle regeneration[84] and limb ischaemia repair,[44] has been shown to be accelerated by stem cell-derived exosomes.

Accumulating in vivo and in vitro studies have suggested that the potential regenerative capacities of stem cell-derived exosomes are achieved through activating the native cells, enhancing angiogenesis and other indeterminate actions. Thus, based on the promising performance in tissue repair, this novel therapy might be a potential method for bone regeneration as well.

4 | REGENERATIVE CAPACITY OF STEM CELL-DERIVED EXOSOMES IN BONE TISSUES

Stem cell implantation has been widely studied for bone regeneration in pre-clinical investigations, showing promising prospects.[33,34] There is increasing support that transplanted stem cells play important roles in bone regeneration, mainly through paracrine signalling effects.[43–45] Therefore, exosomes, as primary paracrine effectors, have attracted more and more attention in the area of promoting fracture healing (Table 2).

Through in vitro investigations, Ji-Feng Xu et al.[85] demonstrated that exosomes could be generated by undifferentiated BMSCs as well as by osteogenic differentiated BMSCs. Furthermore, they analysed miRNA profiles and mRNA transcripts of exosomes from osteogenic differentiated BMSCs at different time points and consequently explored the biological pathways involved in related dysregulated exosomal miRNA signatures. As a whole, this report indicated that exosomes might exert a vital regulatory function in osteogenic differentiation of BMSCs.

Precise and reliable evidence for the osteogenic potential of exosomes in regenerative medicine was given in another study from Raghuvaran Narayanan et al.[86] To clarify the osteogenic potential of stem cell-generated exosomes in regenerative medicine, they incubated human marrow-derived stromal cells (HMSCs) with exosomes isolated from HMSCs or from osteogenic differentiated HMSCs. Next, RNA from the incubated HMSCs was extracted to analyse the expression levels of genes representative of osteogenic differentiation. It turned out that both types of exosomes internalized by HMSCs triggered a very robust and statistically significant upregulation in several genes spanning growth factors such as bone morphogenetic protein 9 (BMP9) and transforming growth factor β1 (TGFβ1), transcription factors and ECM molecules. In addition, the researchers performed an in vivo investigation by implanting clinical grade collagen membranes with HMSCs and exosomes in the back of athymic nude mice for 4 weeks. The results indicated that the scaffolds containing exosomes showed more robust vascularization and calcium phosphate nucleation than the control scaffolds. The expression levels of specific proteins involved in matrix mineralization, vascularization and osteogenic differentiation were enhanced as well. Together, the in vitro and in vivo experiments showed that exosomes have the potential to induce osteogenic differentiation of HMSCs.

Comparable in vitro tests were conducted with human BMSC-derived exosomes by Yunhao Qin et al.,[87] demonstrating the promising capacity of osteogenic differentiation. In addition, they examined whether exosomes had a marginal effect on osteoblast proliferation through cell cycle analysis using FACS and cell proliferation analysis using MTT assays. Furthermore, *in vivo* functional tests in SD rats with calvarial defects suggested that BMSC-derived exosomes substantially enhanced bone regeneration.

Furuta et al.[90] evaluated the role of exosomes in a particular way, comparing the healing condition in femur fracture models of wild-type mice and another CD92/2 mice that is known to produce reduced levels of exosomes. As expected, there was a significant retardation of fracture healing in CD92/2 mice and the retardation was rescued with accelerated formation of hypertrophic chondrocytes, woven bone and vascularization by the subsequent injection of MSC-derived exosomes. And not only that, the timing of bone union was also significantly shorter in wild-type mice treated with MSC-derived exosomes compared with the control groups.

Recent studies in bone tissue engineering combined hiPS-MSC-Exos with β-TCP scaffolds. Similarly, these studies confirmed the active role of exosomes in proliferation, migration and osteogenic differentiation of hBMSCs.[88,89] Among these studies, the study of Xin Qi et al. showed that the application of hiPSC-MSC-Exos promoted bone regeneration through enhanced angiogenesis as well as osteogenesis in an ovariectomized rat model.[88]

These investigations revealed that exosome treatment seems to enhance the proliferation, migration and differentiation of native cells, especially MSCs. In addition, angiogenesis was accelerated. As the resident MSCs are primary cells that differentiate to repair the injury, the proliferation and migration abilities of MSCs are critical for bone regeneration. During bone formation, osteoblasts produce calcium- and phosphate-based minerals to form mineralized bone. Thus, the regulation of osteoblast proliferation and the promotion osteogenic differentiation play key roles in skeletal development and bone formation.[91,92] During bone formation, blood vessels not only serve as a source of oxygen and nutrients but also supply calcium and phosphate, which are the building blocks for mineralization. The timely appearance of blood vessels in the fracture callus is a critical step in bone healing.[93,94] Therefore, it can be concluded that MSC-derived exosomes are a potential therapy for fracture healing.

5 | POTENTIAL MECHANISMS OF STEM CELL-DERIVED EXOSOMES FOR FRACTURE HEALING

Application of stem cell-derived exosomes in bone repair and regeneration has been reported by a number of studies. According to the experimental data, it is a promising measure for enhancing fracture healing. Nevertheless, the underlying mechanisms are still uncertain.

TABLE 2 Recent studies on stem cell-derived exosome therapy for promoting bone regeneration

Origin of exosomes	Experimental objective	Content analysis	Animal model	In vitro evaluation	In vivo evaluation	Inhibition test	Involved pathway
Osteogenic human BMSCs[85]	To characterize differences in exosomal miRNA during osteogenic differentiation of human BMSCs and to explore their biological functions	Nine upregulated miRNAs like let-7a and miR-199b five down-regulated miRNAs such as miR-221 and miR-155	None	None	None	None	Ten prominent pathways such as the mRNA surveillance pathway and Wnt signalling pathway
Osteogenic human BMSCs[86]	To explore the use of human BMSC-derived exosomes as agents to induce osteogenic differentiation of undifferentiated human marrow-derived stromal cells	Not mentioned	None	Significant upregulation of pro-osteogenic genes	1. More robust vascularization and calcium phosphate nucleation 2. Upregulated expression levels of specific proteins involved in matrix mineralization, vascularization and osteogenic differentiation	None	Not mentioned
Human BMSCs[87]	To explore the role of BMSC-derived exosomes in the regulation of osteoblast activity and bone regeneration	Highly enriched in miR-196a, miR-27a and miR-206	SD rats with calvarial defects	1. Substantial increases in calcium deposits 2. Upregulated expression of osteoblast markers and osteogenic genes 3. A marginal effect on osteoblast proliferation	Micro-CT and histological examinations indicated that the amount and area density of neo-formed bones were both significantly increased in the Exo-group compared to the Gel-group	A miR-196a inhibitor effectively attenuated the effects and grossly reduced the expression of osteogenic genes	Not mentioned
Human-induced pluripotent stem cell-derived MSCs[88]	To investigate the effects of hiPSC-MSC-exosomes in promoting bone regeneration through enhanced proliferation, angiogenesis and osteogenesis.	Not mentioned	Ovariectomized rats with critical-sized calvarial defects	1. CCK-8 assay indicated promoted proliferation 2. Enhanced ALP staining and alizarin red S staining 3. Up regulated expression of osteogenesis-related genes and bone-related proteins	1. Higher local BMD and BV/TV values 2. Three-dimensional micro-CT images and quantification showed increased angiogenesis 3. Positive immunohistochemical staining of the osteogenic markers OCN and OPN and the angiogenic marker CD31	None	Not mentioned

continues

Table 2 (continued)

Origin of exosomes	Experimental objective	Content analysis	Animal model	In vitro evaluation	In vivo evaluation	Inhibition test	Involved pathway
Human-induced pluripotent stem cell-derived MSCs[89]	To use exosomes to functionalize biomaterials and obtain an exosome/biomaterial combination in order to improve the osteogenesis ability of biomaterials	Not mentioned	SD rats with critical-sized calvarial defects	1. CCK-8 and scratch wound healing assays showed promoted proliferation and migration ability of MSCs 2. Enhanced ALP staining 3. Confirmed the PI3K/Akt pathway with microarray analyses, qRT-PCR detection and the protein levels of Akt and p-Akt	1. A larger amount of de novo bone formation, higher local BMDs and BV/TV levels 2. Histological evidence demonstrated increased newly formed bone tissues 3. OCN immunostaining suggested enhanced osteogenic responses	A PI3K inhibitor (LY294002) markedly 1. suppressed the levels of osteogenesis-related proteins, and 2. Reduced ALP generation, matrix mineralization and calcium nodule formation	PI3K/Akt signalling pathway
Human BMSC[90]	To address the function of MSC-derived exosomes as novel paracrine factors in fracture healing	1. Highly enriched in miR-21, miR-125b and miR-4454 2. SDF-1, MCP-1 and MCP-3 levels were significantly lower than the levels in conditioned medium and exosomes-free conditioned medium	Femur fracture models of wild-type mice and CD92/2 mice	1. A significant retardation of natural fracture healing in CD92/2 mice compared with wild-type mice. 2. Accelerated formation of hypertrophic chondrocytes, woven bone and vascularization in CD92/2 mice with MSC-derived exosomes. 3. The timing of bone union was significantly shorter in wild-type mice with MSC-derived exosomes.	None	None	Not mentioned

In the study from Ji-Feng Xu et al.,[85] the variations in miRNA and mRNA in exosomes during BMSC osteogenic differentiation were unveiled for the first time. They discovered that let-7a, miR-199b, miR-218, miR-148a, miR-135b, miR-203, miR-219, miR-299-5p and miR-302b were significantly increased, while miR-221, miR-155, miR-885-5p, miR-181a and miR-320c were significantly under-expressed. Furthermore, with DIANA-mirPath, they suggested that RNA degradation, the mRNA surveillance pathway, Wnt signalling pathway and RNA transport were the most prominent pathways enriched in quantiles with differential exosomal miRNA patterns related to osteogenic differentiation. After illuminating the correlation between molecules and signal alterations in bone formation through existing investigations, they inferred that exosomes regulated osteogenic differentiation through the modulatory effect of miRNAs on target genes and pathways.

Reports from Yunhao Qin et al.[87] and Furuta et al.[90] supported the role of exosomal miRNA as well. Yunhao Qin et al. analysed the miRNAs in BMSC-derived exosomes using RNA sequencing and suggested that three osteogenic-related miRNAs, miR-196a, miR-27a and miR-206, were highly upregulated. Among, miR-196a was considered the most important regulator of exosome-dependent osteogenic effects on the basis of Alizarin Red staining, qRT-PCR and miRNA-specific inhibitor testing. Meanwhile, they also confirmed that there were other undetermined mechanisms in addition to miR-196a. Furuta et al. analysed the cytokines and microRNAs in MSC-derived exosomes and speculated that the accelerated fracture healing process by MSC-derived exosomes was bound up with exosomal miRNA besides certain cytokines like MCP-1, -3, SDF-1 and angiogenic factors. The differentially expressed miRNA such as miR-21, miR-4532, miR-125b-5p and miR-338-3p in MSC-derived exosomes compared with HOS-derived exosomes or exosomes-free conditioned medium might contribute to the enhanced osteogenesis and angiogenesis.

Jieyuan Zhang et al.[89] proposed another possible mechanism. With microarray analyses and bioinformatics analyses, they revealed genetic alterations and the involved signalling pathways. Among the signalling pathways, the phosphatidylinositol 3-kinase (PI3K)-Akt signalling pathway was considered to play a key role in the exosome-mediated pro-osteogenesis effects on hBMSCs due to the reported involvement in MSC proliferation, migration and osteogenic differentiation. To confirm the inference, they further performed an inhibition test of the PI3K/Akt pathway and discovered significantly decreased levels of early osteogenesis-related marker proteins as well as decreased ALP production and calcium mineral deposition in hBMSCs. Thus, these results indicated that the enhanced osteogenic differentiation of hBMSCs could mainly be ascribed to activation of the PI3K/Akt signalling pathway.

It has been demonstrated that exosomes can act as mediators by transferring genetic information (mRNA and miRNAs), proteins and other molecules to recipient cells, thereby regulating the bioactivity of target cells. Based on the studies mentioned above, Ji-Feng Xu analysed alterations in related miRNAs and pathways, hypothesizing that the enhanced bone regeneration induced by exosome treatment depended on the regulation of multiple miRNAs. However, further investigation, such as a miRNA suppression test, was not conducted.[85] Yunhao Qin demonstrated that exosomal miR-196a is a critical mediator of the expression of osteogenic genes. However, equally important was the finding that there were also other indeterminate mechanisms that affected this regulation process.[87] Furuta et al.[90] discovered that exosomes from MSCs and human osteosarcoma cells exhibited efficient and invalid abilities respectively in fracture healing process despite they showed many similar highly expressed miRNAs. Thus, Furuta et al. considered that the therapeutic effect was extremely likely attributed to certain stem cell-specific miRNA. Jieyuan Zhang suggested that activation of the PI3K/Akt pathway might be the crux of the enhanced osteogenesis. Nevertheless, the molecular content of hiPS-MSC-exosomes that underlies the activation of the PI3K/Akt signalling pathway still requires clarification.[89]

The potential mechanisms proposed in the investigations mentioned above aimed to explain the enhanced osteogenesis. However, the authors did not account for the promoted proliferation, migration and angiogenesis. It is suggested that multiple miRNAs in stem cell-derived exosomes could regulate cell cycle progression and proliferation (miR-191, miR-222, miR-21 and let-7a) [95,96] and migration (miR-10b) [96,97] and modulate angiogenesis (miR-129 and miR-136).[98–101] Thus, the enhanced proliferation, migration and angiogenesis might also be attributed to the undetermined exosomal miRNA content. Moreover, another obvious possible scenario is that other molecules such as proteins, mounted inside the exosome, or on its surface, are either delivered to the interior of the recipient cells or directly activate the cells by direct exosome-to-cell contact.[87,102]

6 | ADVANTAGES/FEASIBILITIES OF STEM CELL-DERIVED EXOSOME STRATEGIES

The biological characteristics and particular structure of exosomes make their use a favourable strategy for tissue regeneration. Encapsulation by the lipid bilayer of the exosomal membrane protects proteins and miRNAs from degradation in body fluid, contributing to their ability to deliver the content across the cell membrane into the cytosol of recipient cells.[79,103] Compared with biomaterial treatment, this novel strategy resolves the problems of immunogenicity and toxicity. Exosomes can either intensify or suppress the immune response. It has been suggested that stem cell-derived exosomes maintain the immune privileged properties of their origins.[58,69,103–105] Additionally, exosomes are considerably stable and can be preserved for approximately 6 months in vitro at −20°C without loss of potency.[106]

7 | LIMITATIONS OF STEM CELL-DERIVED EXOSOME THERAPY AND DIRECTIONS OF FUTURE STUDIES

Mounting evidence suggests that stem cell-derived exosomes are capable of promoting fracture healing, providing a potential strategy to solve this clinical challenge in the future. However, there are still quite a few limitations to be addressed before stem cell-released exosomes can be developed into a practical and effective therapeutic.

In the last two decades, exosomes, as key mediators for intercellular communication, have been extensively investigated. Nevertheless, specific details still require clarification. Early exosomes are initiated by budding into multivesicular endosomes (MVEs), which then selectively recruit the cytoplasmic elements, including proteins, RNA and lipids, to form intact exosomes. Next, the MVEs move to the cell periphery, fuse with the cell surface, release from the plasma membrane and act on the recipient cells. It is a fairly complex process from formation to effect, and it is regulated by multiple factors, such as tetraspanins, cholesterol, endosomal sorting complex responsible for transport, sphingomyelinase, adhesion molecules and even the released microenvironment. The related mechanisms are still at an early stage of comprehension and require further investigations.[69,96]

Recent investigations have revealed that specific integrins expressed on tumour-derived exosomes, distinct from tumour cells, could dictate exosome adhesion to specific cell types in particular organs, such as the lungs, liver and brain.[107,108] For bone regeneration, it is also suggested that DiO-labelled exosomes can be found in the perinuclear region of hBMSCs, indicating that hBMSCs are recipient cells of hiPS-MSC-Exos.[88,89] However, a correlation between specific integrins of stem cell-derived exosomes and particular targeting cells has not been illustrated. Furthermore, in vivo tests only detect the local internalization of exosomes, lacking surveillance of the systematic distribution and clearance. Thus, the biosafety of this novel therapy is still to be confirmed.

As to clinical applications, one major challenge is to develop strategies to obtain sufficient amounts of exosomes. Current exosome isolation methods such as ultracentrifugation or ultrafiltration provide only a low exosome yield.[109] In addition, the exact mechanisms of the enhanced bone formation after exosome treatment remain elusive. miRNAs are considered one of the major functional components of exosomes. The exosome content varies according to the different parent cells, culture conditions and even separation methods.[87,90,110] Thus, exosomes from different sources may exhibit diverse effects on fracture healing. To intensively analyse the content, including genetic information, proteins and other molecules, within exosomes from various origins is conducive to defining the exact mechanisms. Meanwhile, it also would contribute to confirming the most effective exosomes. Moreover, there is no consensus on dose and frequency of exosome treatment to achieve an optimal effect.

8 | CONCLUSION

Stem cell-derived exosome therapy for fracture healing has been enjoying popularity and is drawing increasing attention. This strategy helps to promote proliferation and migration of cells, as well as osteogenesis and angiogenesis, in the process of bone formation. Although the exact mechanisms remain elusive, exosomal miRNAs seem to play vital roles. Future studies are required to solve multiple problems before clinical application, including comprehensive and thorough understanding of exosomes, the exact roles of exosomes in regulating bone formation, and the optimal source, dose and frequency of treatment, as well as technical and safety issues. Moreover, studies based on fracture models of large animals are could offer guidance and are in demand.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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