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Review

Exosomes from preconditioned mesenchymal stem cells: Tissue repair and regeneration

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ABSTRACT

As a prominent research area in tissue repair and regeneration, mesenchymal stem cells (MSCs) have garnered substantial attention for their potential in the treatment of various diseases. It is now widely recognized that the therapeutic effects of MSCs primarily occur through paracrine mechanisms. Among these mechanisms, exosomes play a crucial role by exerting a series of regulatory effects on surrounding cells and tissues. While exosomes have shown promise in treating various diseases, they do have some limitations, such as limited secretion, poor targeting, and single functionality. However, MSC preconditioning can enhance the production of exosomes, lead to more stable functionality and improve therapeutic effects. Moreover, exosomes could also serve as carriers for specific drugs or genes, enabling more precise treatments of diseases. This review summarizes the most recent literatures on how preconditioning of MSCs influences the regenerative potential of their exosomes in tissue repair and provides new insights into the therapeutic application of exosomes derived from MSCs.

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1. Introduction

The regenerative potential of human tissues is limited, making tissue/organ defects and necrosis caused by congenital or acquired factors significant threats to human health. To address this challenge, regenerative medicine has focused on stem cell research and the application of stem cells. Mesenchymal stem cells (MSCs) have garnered the attention of researchers due to their ability to differentiate towards multiple lineages, their immunomodulatory properties, low immunogenicity [1], and a wide range of sources. These characteristics make MSCs an ideal seed cell source [2] and have led to their extensive use in clinical trials [3]. The therapeutic effect of MSCs is not limited to their direct differentiation into effector cells needed for tissue repair; they also act as 'nutrient factories' for surrounding cells by secreting biologically active molecules. These molecules regulate tissue repair and regeneration processes through cellular autocrine or paracrine mechanisms, which are key to therapeutic effects [4]. Exosomes, which are vesicles formed outside the cell when intracellular multivesicular bodies (MVBs) are released upon fusion with the cell membrane surface, play important roles in cellular paracrine mechanisms [5,6]. They carry therapeutic payloads such as proteins, nucleic acids, enzymes, transcription factors, surface receptors, and metabolites. Exosomes also participate in signal transduction, immune response, antigen presentation, and tissue repair [7] Compared to MSCs, exosomes are considered less immunogenic, more biologically stable, and easier to preserve and control in terms of dosage [8,9]. As a result, exosomes have been effectively employed in the treatment of diseases associated with tissue repair and regeneration More importantly, as a novel strategy for regenerative therapy, stem cell preconditioning can enhance the proliferation, migration, directional differentiation, and anti-apoptotic ability of MSCs, as well as improving the secretion and function of exosomes [10,11], which have been investigated by numerous in vitro and in vivo studies showing enhanced biological effects of preconditioned MSCs exosomes and improved therapeutic effects in various diseases [12–15] Herein, we review the recent advancements in the field of exosomes derived from MSCs preconditioned with different approaches in regenerative medicine and address the application of exosomes in tissue repair and regeneration.

2. Exosomes from mesenchymal stem cells with different types of preconditioning

2.1. Hypoxia

Room air oxygen concentration is still typically used for cell culture. However, the partial pressure of oxygen in tissues, such as cartilage and bone marrow (1%) and peripheral blood (12%), is much lower than that [16]. Consequently, conventional cell culture methods fail to accurately replicate the environment in vivo. To simulate a hypoxic environment, researchers utilize either 1–5% oxygen (physical hypoxia) [17] or hypoxia-inducible factor 1-alpha (HIF-1α, chemical hypoxia) [18] during cell culture. Culturing MSCs in a hypoxic environment has been demonstrated to better preserve their proliferation [19] and multidirectional differentiation abilities [20]. It also enhances the survival rate [21] and expression level of angiogenesis-related growth factors [22] in stem cells, leading to a more desirable therapeutic effect. In a study involving bleomycin-induced pulmonary fibrosis mice, hypoxia-pretreated bone marrow mesenchymal stem cells (BMSCs) demonstrated superior therapeutic effects. Hypoxic conditions improved the survival rate of transplanted BMSCs and increased the expression of antiapoptotic, antioxidant, and growth factors in BMSCs [21]. Olfactory mucosa mesenchymal stem cells (OM-MSCs), a novel type of resident stem cell in the olfactory lamina propria, have a high proliferation rate, self-renewal capacity, and ability to differentiate into multiple lineages. Ge et al. investigated the effects of culturing OM-MSCs in normoxic and hypoxic environments. They discovered that exosomes from hypoxia-pretreated OM-MSCs were more effective in promoting the proliferation, migration, and angiogenesis of HBMECs than were exosomes from normoxic OM-MSCs [23]. This could be attributed to the upregulation of miR-612 levels in exosomes from hypoxia-pretreated OM-MSCs, which in turn stimulated HIF-1α-VEGF signaling in HBMECs. In a study conducted by Li et al. on BMSCs from crab-eating monkeys, it was observed that hypoxic preconditioning led to the upregulation of miR-486-5p in exosomes. This upregulation promoted angiogenesis and enhanced vascular density by inhibiting MMP19 and promoting VEGFA expression in cardiac fibroblasts [24]. Additionally, when exosomes derived from human embryonic stem cell-derived hCVPCs were injected into mice with acute-phase infarction, significant benefits were observed, such as reduced cardiomyocyte apoptosis, increased angiogenesis, and reduced scar formation. The effects were further enhanced by the hypoxic preconditioning of hCVPCs, resulting in the improved repair of infarcted myocardium [25].

2.2. Genetic modification

In recent years, extensive research has been conducted on MSCs genetic engineering and discovered that selective modifications of MSC DNA sequences can enhance their survival, proliferation, and immunomodulation and inflammatory suppression abilities [26]. Akt, known for promoting cell proliferation and inhibiting cell apoptosis, was transfected into hucMSCs using an adenoviral transfection system in a study conducted by Ma et al. They found that exosomes derived from Akt-transfected hucMSCs (referred to as Akt-Exo) had a significant impact on various cellular processes. Akt-Exos enhanced the proliferation and migration of endothelial cells, promoted the formation of tubular structures in vitro, and facilitated vascularization in vivo. In the treatment of a rat model of acute myocardial infarction (AMI), Akt-Exos were found to be more effective by upregulating the expression of platelet-derived growth factor D [27]. Shang et al. performed a high-throughput sequencing analysis of lung tissues from an asthma mouse model and controls. They discovered that the expression of mmu_circ_001359 was significantly reduced in asthma model mice. Conversely, exosomes derived from adipose-derived stem cells (ADSCs) overexpressing mmu_circ_001359 promoted macrophage M2 polarization mediated by FoxO1 and inhibited the macrophage-mediated expression of inflammatory cytokines, which resulted in reduced fibrosis and airway remodeling in mouse lung tissues and promoted angiogenesis [28].

2.3. Proinflammatory cytokines

The preconditioning of MSCs with proinflammatory cytokines has recently been recognized as a novel approach for cell-free therapies and not only stimulates cell proliferation and enhances cell survival but also improves the bioactivity of exosomes [29,30]. IFN-γ, which is produced by activated T cells and natural killer (NK) cells, is a cytokine that can promote immunomodulation and has been widely studied for its antiapoptotic activity [31]. Zhang et al. evaluated the therapeutic effect of IFN-γ-treated MSC-derived exosomes (IFN-γ-Exos) using both an in vitro model of glyco-oxidative deprivation and a rat infarction model. The results demonstrated that IFN-γ-Exos effectively suppressed apoptosis and promoted the neovascularization of H9c2 cells, surpassing the effects of MSC-derived exosomes. The underlying mechanism was partially attributed to the upregulation of miR-21 in IFN-γ-Exos, which played a crucial role in improving cardiac function after myocardial infarction in rats through the STAT1/miR-21/BTG2 signaling axis [32]. Furthermore, exosomes derived from interleukin-1β (IL-1β)-pretreated murine MSCs (βMSCs) effectively induced the M2 polarization of macrophages both in vitro and in vivo. This polarization significantly alleviated symptoms in septic mice and improved the survival rate. The results were attributed to increased miR-21 levels in βMSC exosomes, which were then transferred to macrophages, where they targeted and inhibited the function of PDCD4, thereby exerting therapeutic effects [29]. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine known for its involvement in various pathophysiological responses, including cell proliferation and migration. Zhu et al. used MIF to pretreat human umbilical cord mesenchymal stem cells (hucMSCs) to obtain MIF-engineered exosomes (MIF-Exos). The researchers also utilized MIF-Exo, MSC-Exos (exosomes from hucMSCs) and siMIF-Exos (exosomes from hucMSCs down-regulated by MIF) to treat an AMI rat model. The results demonstrated that MIF-Exos significantly enhanced the proliferation, migration, and angiogenesis of human umbilical vein endothelial cells (HUVECs), inhibited cardiomyocyte apoptosis, reduced cardiac fibrosis, and improved cardiac function in infarcted rats through the upregulation of miRNA-133a-3p in MSC-Exos and enhancement of AKT signal pathway in recipient cells, but the mechanisms of miR-133a-3p upregulation have not been clarified in the study [33].

2.4. Drugs and chemical agents

Atorvastatin (ATV) is an HMG-CoA reductase inhibitor that regulates cell growth-related proteins and cytokines and also promotes tissue regeneration in acutely diseased tissues [34]. In a study involving STZ-induced diabetic rats, it was found that exosomes derived from ATV-pretreated BMSCs (referred to as ATV Exos) had remarkable abilities to facilitate wound regeneration by promoting the formation of blood vessels compared with exosomes derived from BMSCs (Exos) without influencing liver and kidney function. The underlying mechanism behind this phenomenon was partially attributed to the upregulation of miR-221-3p in ATV Exos. This upregulation played a crucial role in enhancing endothelial cell proliferation, migration, tubular structure formation, and VEGF levels in rats with diabetic skin defects through the AKT/eNOS pathway [35]. Kartogenin (KGN), a small heterocyclic compound, can effectively induce MSC differentiation into chondrocytes [36] A study demonstrated that exosomes derived from infrapatellar fat pad MSCs pretreated with KGN (KGN-EXOs) have a potent ability to induce the chondrogenic differentiation of stem cells, effectively promoting the proliferation and expression of chondrogenic proteins and genes of chondrocytes [37]. In conclusion, MSCs primed with drugs can enhance the therapeutic efficacy of their derived exosomes, holding significant potential in the field of tissue regeneration.

In addition, certain chemical agents have been found to enhance the therapeutic efficacy of MSCs. For example, nitric oxide (NO) molecules increase the expression of proangiogenic cytokines in MSCs, thereby promoting angiogenesis [38]. Exosomes derived from ADSCs cultured with low concentrations of H_2O_2 have been found to enhance the migration rate of HUVECs, generate more cord-like structures, promote neovascularization and alleviate inflammatory reactions and apoptosis in the skin flap after ischaemia/reperfusion (I/R) injury; they have also been shown to promote the neovascularization and survival of the flap [39]. Wang et al. discovered that MSC-derived exosomes pretreated with dimethyloxalylglycine (DMOG) had a significant positive impact on cardiac function and survival in a mouse model of myocardial infarction. Furthermore, the results of in vitro experiments demonstrated that these exosomes exhibited enhanced proangiogenic and cardiomyocyte-protective properties [40].

2.5. Other

With extensive research on cell culture models, researchers have realized the limitations of conventional two-dimensional (2D) cell culture models, i.e., a low cell survival rate and an inability to maintain natural cellular morphology [41]. Moreover, there are noticeable discrepancies in cell differentiation and function when cells are cultured in a 2D environment compared with cells cultured in a physiological environment. To overcome these limitations, researchers have employed three-dimensional (3D) cell culture as a novel pretreatment method for MSCs. The approach closely mimics the physiological conditions of cells and consistently generates results that align with in vivo experiments. Notably, 3D cell culture models, such as spheroid culture, have been found to stimulate cells to produce a higher quantity of exosomes [42]and trophic factors [43], leading to improved cell survival, proliferation, and angiogenesis [44,45]. Among these models, 3D dynamic culture stands out because it involves the dynamic cultivation of single cells or small cell clusters in a liquid medium that is constantly stirred or rotated. It provides cells with a microenvironment that closely resembles living conditions in vivo. Yuan et al. conducted a study in which they placed BMSCs in a small dynamic fluctuating bioreactor. They observed that hMSCs grew in 3D aggregates, expressing more stemness genes (Oct4, Sox2 and Nanog) and secreting more exosomes. These exosomes contained therapeutically relevant miRNAs and anti-inflammatory cytokines, indicating their potential for applications in immunomodulation, pro-wound healing, and anti-ageing [46]. Additionally, Kronstadt and other scholars developed a 3D-printed scaffold-perfusion bioreactor system that not only enhanced the yield and purity of exosomes from MSCs but also preserved the proangiogenic bioactivities of exosomes and improved their therapeutic efficacy [47]. Although studies on obtaining exosomes through the 3D culture of MSCs are limited, we anticipate that the increasing biological relevance and improvements in the throughput of 3D cell culture techniques will lead to the widespread application of exosomes from 3D culture in the future.

Other researchers have developed novel methods for the surface editing of MSC-EXOs without affecting their inherent biological functions and yields. You et al. implemented a technique in which azide groups were introduced onto the surface of ADSCs through the metabolic glycoengineering-mediated sialic acid pathway. Subsequently, dibenzocyclooctyne-conjugated dextran sulfate (DBCO-DS) was ligated onto azide-containing ADSCs through click chemistry. The resulting DS-decorated MSC-EXOs (DS-EXOs) effectively regulated macrophage heterogeneity, which promoted

polarization towards the M2 type and modulated the synovial microenvironment of inflamed mouse joints. This innovative surface engineering strategy can be widely employed to introduce a broad range of targeting moieties onto MSC-EXOs and holds great potential for the treatment of challenging diseases using various types of therapeutic cell-derived exosomes [48]. A summary of different types of MSCs preconditioning is given in Table 1.

3. Exosomes derived from preconditioned MSCs in tissue repair and regeneration

3.1. Bone tissue

Bone tissue defects caused by trauma, diseases, and congenital factors not only have a negative impact on patients' physical health but also affect their psychological well-being. Bone tissue regeneration and repair has become a common yet challenging problem in clinical practice. In recent years, MSCs and their exosomes have emerged as prominent areas of research in tissue regeneration and repair, with extensive studies conducted in both basic and clinical research on bone tissue. Although the exact mechanism by which exosomes promote osteogenesis is not fully understood, it is evident that exosomes contain various signalling molecules that interact with different osteogenesis-related cells [49,50]. For instance, exosomes derived from human umbilical cord MSCs (hucMSCs) have shown promising results in ameliorating bone loss in a mouse model of osteoporosis. Exosomes transport a pro-osteogenic protein called CLEC11A (C-type lectin domain family 11, member A), which facilitates the transition of bone marrow MSCs from lipogenic to osteogenic differentiation, whereas the formation of osteoclasts, bone marrow fat accumulation and bone resorption are reduced [51]. Wang et al. discovered that in normal and diabetic rats with bone defects, diabetic model (DM) rat BMSC-derived exosomes (DM-Exos) had lower osteogenic capacity than did normal rat BMSC-derived exosomes (N-Exos). Their RNA deep-sequencing analysis revealed that miR-1440-3p expression was higher in N-BMSCs than in DM-BMSCs. Moreover, when miR-140-3p mimics were transfected into DM-BMSCs, their exosomes could carry miR-140-3p and translocate them into BMSCs and osteoblasts to promote bone regeneration by inhibiting plexin B1 expression and targeting the plexin B1/RohA/ROCK signalling pathway. Consequently, miR-140-3p-overexpressing Exos were able to accelerate bone injury healing in diabetic rats [52]. Lu et al. observed that Wnt-3a content was elevated in adipose tissue-derived MSC (ASC)-derived exosomes (ASC-EXO) when ASCs were preconditioned with tumour necrosis factor-α (TNF-α) and that inhibiting Wnt signaling decreased the osteogenic gene expression levels in HOBs cultured in TNF-α-preconditioned ASC-conditioned medium. Furthermore, the results of the study demonstrated that ASC-EXOs promoted the proliferation and osteogenic differentiation of HOBs, presenting a promising alternative to stem cell transplantation for bone defect repair [53].

Angiogenesis is believed to have a significant impact on the process of new bone formation. In the centre part of a bone defect, where the vascular network is sparse or even absent, cells cannot survive, and the formation of new bone is hindered. Therefore, it is crucial to enhance angiogenic activity in the region of a bone defect during the bone healing process. HIF-1α, which is involved in bone development, readily degrades in an environment with normal oxygen levels. Li et al. discovered that after modifying BMSCs with mutant HIF-1α, exosomes derived from these cells (BMSC-Exos^{MU}) exhibited a greater ability to promote the osteogenic differentiation of BMSCs than did wild-type exosomes (BMSC-Exos^{WT}). Additionally, BMSC-Exos^{MU} promoted the proliferation, migration, and tubule-forming ability of vascular endothelial cells. When BMSC-Exos^{MU} were injected into an animal model of hormone-induced ischaemic necrosis of the femoral head (SANFH), it resulted in trabecular reconstruction and increased microvessel density in the osteonecrotic area, thereby accelerating angiogenesis and osteogenesis [54]. Liang et al. primed BMSCs with low-dose DMOG and discovered that exosomes derived from these cells (DMOG-MSC-Exos) stimulated angiogenesis in human umbilical vein endothelial cells (HUVECs) through the activation of the AKT/mTOR pathway, which led to an increase in blood perfusion and in the number of microvessels, ultimately facilitating bone healing in a critical cranial defect model in rats [55]. In recent studies, magnetic nanoparticles such as Fe_3O_4 and γ-Fe_2O_3, along with a static magnetic field (SMF), have been widely used to facilitate wound healing and bone regeneration. Wu et al. conducted experiments using Fe_3O_4 nanoparticles and SMFs to prepare two types of exosomes from BMSCs; the exosomes derived from these sources were named BMSC-Fe_3O_4-Exos and BMSC-Fe_3O_4-SMF-Exos, respectively. Compared with BMSC-Exos, both types of exosomes showed enhanced osteogenesis and angiogenesis in both in vitro and in vivo experiments. Notably, BMSC-Fe_3O_4-SMF-Exos, which were derived from BMSCs primed with low doses of Fe_3O_4 nanoparticles and SMF, exhibited the highest osteogenic and angiogenic capacities. The effects were attributed to the abundant presence of miR-1260a in BMSC-Fe_3O_4-SMF-Exos, which promoted osteogenesis and angiogenesis by targeting and inhibiting HDAC7 and COL4A2 [56].

Scleraxis (Scx) is a transcription factor that contains a basic helix-loop-helix and drives the tendonogenic differentiation of MSCs. Wang et al. investigated the role of BMMSCscx-exos derived from Scx-overexpressing PDGFRα(+) BMMSCs in a tendon-bone healing mouse model. They found that BMMSCscx-exos were enriched with miR-6924-5p to inhibit bone resorption during the tendon-bone healing process by targeting the osteoclast regulatory factors CXCL12 and OCSTAMP [57]. Additionally, Kim et al. found that IL-1β-pretreated BMSC-derived MSC-IL-Exos exhibited stronger anti-inflammatory activity in osteoarthritic human synovial sarcoma cell lines than did MSC-Exos, which was attributed to the enhanced expression of anti-inflammatory factors (SOCS3 and SOCS6) in MSC-IL-Exos. The key mechanism underlying this effect was the enrichment of miR-147b in MSC exosomes, which targeted and inhibited the NF-κB pathway [31]. In conclusion, stem cell exosomes have been recognized as a promising therapeutic approach in the treatment of bone-related disorders [58].

3.2. Cartilage

Articular cartilage plays crucial roles in protecting the joints and spine by cushioning shock, reducing friction, and providing support; however, it only possesses limited regenerative capacity and is susceptible to degenerative changes such as osteoarthritis (OA). Shen et al. discovered that miR-205-5p expression was elevated in exosomes derived from BMSCs after hypoxic preconditioning. These exosomes activated the PTEN/AKT pathway, leading to the deposition of cartilage-specific matrix and the formation of hyaline cartilage-like tissue [59]. Exosomes from synovial mesenchymal stem cells (SMSCs) carry Wnt5a and Wnt5b to activate Yes-associated protein (YAP) via the alternative Wnt signaling pathway and promote chondrocyte proliferation and migration, nevertheless significantly decreasing the secretion of ECM components, such as aggregated proteoglycans and type II collagen. To overcome this, SMSCs were transfected with miR-140-5p, and their exosomes (SMSC-140-Exos) significantly enhanced chondrocyte proliferation and migration without damaging ECM secretion in vitro. Moreover, it was also found that MSC-140-Exos successfully prevented OA in a rat model [60]. Zheng et al. found that compared with SMSC-Exos, exosomes derived from miR-212-5p-

Table 1
Preconditioning of MSCs and therapeutic effects of MSCs-Exos.

MSCs	Priming Treatments	Exos Recipient	Functional Factors Detected	Biological Effects	Reference
OM-MSCs	Hypoxia	HBMSCs	miR-612	Promoted the proliferation, migration, and angiogenesis of HBMSCs	Ge et al. (2021)
hCVPC	Hypoxia	MI mice model	lncRNA MALAT1	Reduced cardiomyocyte apoptosis, scar formation, and increased angiogenesis	Wu et al. (2020)
SHEDs	Hypoxia	Endothelial cells	Let-7f-5p, miR-210-3p	Enhanced proangiogenic properties and improved the growth, migration, and tube formation of endothelial cells	Liu et al. (2022)
hucMSCs	Akt- modified	AMI rat model	platelet-derived growth factor D	Facilitated vascularization in vivo	Ma et al. (2017)
DM-BMSCs	miR-140-3p overexpression	Bone defects of DM rat model	miR-140-3p	Accelerated bone formation by increasing the numbers of osteoblasts	Wang et al. (2022)
BMSCs	oxygenase-1-modified	LT rat model	miR-124-3p	Inhibited hepatocyte ferroptosis and reduced ischaemia-reperfusion injury	Wu et al. (2022)
BMSCs	IL-1β	Sepsis mouse model	miR-21	Induced the M2 polarization of macrophages and alleviated symptoms in septic mice	Yao et al. (2021)
hucMSCs	MIF	AMI rat model	miR-133a-3p	Inhibited cardiomyocyte apoptosis, reduced cardiac fibrosis, and preserved heart function	Zhu et al. (2021)
GMSCs	TNF-α	Periodontitis model mice	miR-1260b	Ameliorated the inflammatory response and bone loss in periodontitis model mice	Nakao et al. (2021)
hNSCs	IFN-γ	Ischemic stroke rat model	SOD2	Improved neuroprotective and anti-inflammatory effects	Zhang et al. (2020)
BMSCs	ATV	Diabetic rat model	miR-221-3p	Facilitated wound repair by promoting the formation of blood vessels	Yu et al. (2020)
MSCs from infrapatellar fat pad	KGN	Chondrocyte	–	Induced the chondrogenic differentiation of stem cells and promoted expression of chondrogenic proteins and genes	Shao et al. (2021)
BMSCs	DMOG	Critical cranial defect rat model	PTEN	Facilitated bone healing via increasing blood perfusion and the number of micro-vessels	Liang et al. (2019)
hucMSCs	TSA	MI/RI rat model	miR-223-5p	Inhibited the activation of CCR2, reduce monocyte infiltration, and promoted angiogenesis to attenuate MI/RI in rats.	Li et al. (2023)
ADSCs	H_2O_2	Skin flap rat model	–	Promoted neovascularization , alleviated inflammatory reactions and apoptosis in the skin flap	Bai et al. (2018)
BMSCs	H_2S	HI brain injury mice model	miR-7b-5p	Decreased levels of proinflammatory mediators and improved HI-induced cognitive impairments in neonatal mice	Chu et al. (2020)
ADSCs	Surface editing	Macrophage, CIA mice model	let-7b-5p, miR-24-3p	Promoted macrophage M2 polarization, and modulated the synovial micro-environment of inflamed mouse joints	You et al. (2021)
BMSCs	Fe3O4, SMFs	Calvarial defect rat model	miR-1260a	Accelerated bone defect healing process via promoting bone regeneration and angiogenesis	Wu et al. (2021)
Dental follicle stem cells	LPS	PDLSCs, macrophages	Ros	Reduced the RANKL/OPG ratio in PDLSCs and promoted macrophage M2 polarization	Huang et al. (2022)
Embryonic stem cells	3D culture	Livers fibrosis mouse model	miR-6766-3p	Reduced hepatocyte necrosis and increased level of liver functioning proteins	Wang et al. (2021)

overexpressing SMSCs (SMSC-212-5p-Exos) successfully reversed the decrease in viability of degraded chondrocytes. The key mechanism underlying this effect was that the SMSC-212-5p-Exos inhibited the expression of the ELF3 oncogene and cellular inflammatory molecules (IL-6, MCP-1, TNF-α, COX-2, and iNOS) in chondrocytes induced by IL-1β, thus attenuating chondrocyte degeneration and matrix degradation as well as mitigating chondroarthritis [61]. Mao et al. observed significant exosomal miR-92a-3p upregulation during MSC-induced chondrogenesis and further investigated the potential of exosomes derived from miR-92a-3p-overexpressing hMSCs (MSC-miR-92a-3p-Exos) for the treatment of OA. MSC-miR-92a-3p-Exos promoted cartilage proliferation and matrix gene expression in chondrogenic hMSCs and OA primary human chondrocytes (PHCs) by downregulating WNT5A, inhibiting the progression of early OA and preventing severe articular cartilage damage in an OA mouse model [62]. The results of the aforementioned studies suggest that pretreatment, particularly genetic modification, can enhance the potential of MSC-derived exosomes to promote cartilage tissue repair and regeneration and even to treat OA.

3.3. Oral and maxillofacial region

Periodontitis is a prevalent chronic infectious disease worldwide, and maintaining periodontal health is crucial for both oral and general health. Dental follicle stem cell (DFC) exosomes have been found to contain essential molecules that play vital roles in maintaining periodontal tissue homeostasis and regulating the repair and regeneration processes of the periodontium and alveolar bone. Lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, can penetrate periodontal tissue and cause inflammation. However, LPS pretreatment can enhance the protective and regenerative properties of MSCs [63,64]. Weidong Tian's team investigated the potential of exosomes from LPS-pretreated DFC (L-D-sEV) in treating periodontitis. L-D-sEVs reduced the RANKL/OPG ratio in PDLSCs by inhibiting ROS/JNK signalling and promoted macrophage polarization towards the M2 phenotype via ROS/ERK signalling. Furthermore, a 0.2% hyaluronic acid (HA) gel injectable system loaded with L-D-sEV demonstrated the sustained release of sEVs and improved therapeutic efficacy for periodontitis in canines [65]. Nakao et al. used TNF-α to pretreat gingival tissue mesenchymal stem cells (GMSCs) and found that the secretion of their exosomes and the exosomal expression of CD73 were increased, thereby inducing anti-inflammatory M2 macrophage polarization. Moreover, the level of miR-1260b was elevated in exosomes, targeting the Wnt5a-mediated RANKL pathway to inhibit osteoclastogenic activity, which ameliorated the inflammatory response and bone loss in periodontitis model mice [66] The results of that study suggest that TNF-α pretreatment of GMSC-derived exosomes may hold promise in the treatment of periodontitis and other inflammatory bone loss diseases.

MSCs in a hypoxic microenvironment promote peripheral angiogenesis by secreting exosomes to compensate for the reduced blood supply caused by root resorption [67]. Wu et al. isolated exosomes from hypoxic-preconditioned stem cells of human deciduous exfoliated teeth (SHEDs) (Hypo-exos) and from normoxic cultured SHED cells (Norm-exos). They found that compared with Norm-exos, Hypo-exos significantly enhanced proangiogenic properties and improved the growth, migration, and tube formation of endothelial cells in vitro. The mechanism was attributed to the upregulation of Let-7f-5p and miR-210-3p in Hypo-exos [68]. Another study performed by Zhao et al. involved culturing periodontal ligament fibroblasts (PDLFs) under compressive force and found that the exosome secretion of PDLFs was decreased but that the YAP content and M1 polarization of THP-1 macrophages were increased. The study concluded that exosomes from compressive force-stimulated PDLFs may play a regulatory role in promoting alveolar bone remodeling and orthodontic tooth movement [69]. Furthermore, Liu et al. stimulated ADSCs with low concentrations of the cytokines TNF-a and IFN-γ to maintain stem cells in an inflammatory state. Compared with normal exosomes (referred to as AEs) of ADSCs, inflammatory exosomes (referred to as IAEs) significantly increased the proliferation and migration of BMSCs and promoted the M2 polarization of THP-1 macrophages. Compared with AEs, the combination of IAEs and scaffolding material was found to be more effective in repairing bone regeneration in the temporomandibular joint (TMJ) condylar osteochondral defect model of rabbits [70].

3.4. Skin

With the development of cell-based therapy, MSC-based biological products have been utilized in the treatment of chronic wounds. However, the limited survival of transplanted cells and the high cost of live cell transportation restrict their clinical application. Exosomes, on the other hand, not only possess the biological functions of their parent cells but also overcome the limitations associated with direct cell transplantation and have been widely used to promote wound re-epithelialization. For instance, exosomes derived from ADSCs can be absorbed and internalized by human keratinocytes, thereby improving the diminished biological function of keratinocytes and enhancing their ability to proliferate, migrate, and facilitate neovascularization. Furthermore, pretreatments of MSCs can greatly enhance the therapeutic effect of their exosomes [71]. TAO et al. discovered that exosomes derived from miR-126-3p-overexpressing SMSCs (SMSC-126-Exos) exhibited an enhanced pro-proliferative effect on human dermal fibroblasts and human dermal microvascular endothelial cells. SMSC-126-Exos not only stimulated neovascularization but also promoted collagen deposition, thereby accelerating the epithelial regeneration of wounds in diabetic model rats [72]. Ti et al. investigated the use of exosomes from LPS-pretreated hucMSCs (LPS pre-Exo) in the treatment of skin wounds in diabetic model rats. They discovered that LPS pre-Exos promoted wound healing by enriching let-7b, which inhibited TLR4/NF-κB signalling and activated the STAT3/AKT pathway, followed by the upregulation of M2 polarization and anti-inflammatory cytokines in macrophages [73]. Pioglitazone (PGZE), a peroxisome proliferator-activated receptor activator, is a common drug used to treat diabetes and was also used for MSC preconditioning. Hu et al. found that in a rat model of diabetic wounds, pioglitazone pretreatment enhanced the therapeutic efficacy of MSC-derived exosomes and accelerated diabetic wound healing via enhanced angiogenesis. In addition, PGZ-Exos promoted collagen deposition, ECM remodeling and VEGF and CD31 expression through the activation of the PI3K/AKT/eNOS pathway [74]. These findings highlight the potential of exosomes derived from pretreated MSCs as promising drug delivery systems for the future treatment of skin tissue injuries.

3.5. Neural tissue

Nerve injury repair remains a significant challenge in the clinic due to the complex pathologic process and slow rate of nerve regeneration. Cell-free therapies utilizing exosomes from preconditioned stem cells have shown some promise in repairing nerve injuries. Chang et al. used exosomes from BMSCs cultured in normoxic and hypoxic conditions to treat a rat model of TBI (traumatic brain injury) and observed that in the latter group, the formation of new neurons increased, brain damage decreased, and motor and cognitive function improved [75]. Zhang et al. found that

exosomes derived from human neural stem cells (hNSCs) preconditioned with IFN-γ (IFN-γ-hNSC-Exo) were more effective in promoting hNSC proliferation and survival and exhibited better neuroprotective and anti-inflammatory effects in an ischemic stroke rat mode [76]. Hydrogen sulfide (H_2S) is an endogenous gaseous transmitter that regulates various physiological and pathological processes in the brain [77]. Chu et al. focused on the neuroprotective role of modifying BMSC-derived extracellular vesicles (EVs) with H_2S preconditioning to treat hypoxia-ischemic (HI) brain injury in neonatal mice. They found that H_2S-EVs could be transferred into the ipsilateral hemisphere of damaged areas, which further induced miR-7b-5p expression and inhibited FOS. Mice that received H_2S-EVs exhibited substantially lower amounts of brain tissue loss and decreased levels of proinflammatory mediators towards a more anti-inflammatory condition than those that received only EVs [78]. Additionally, Zhang et al. examined the use of cell-free exosomes produced by hMSCs cultured under 2D and 3D conditions for treating a rat model of TBI. The results of the study revealed that the use of exosomes from hMSCs cultured in a 3D environment significantly increased the number of newborn endothelial cells and newborn mature neurons in the lesion boundary zone and dentate gyrus and that neuroinflammation decreased. Moreover, rats treated with exosomes from 3D culture exhibited enhanced brain angiogenesis and resulted in better spatial learning outcomes than did rats treated with exosomes from 2D culture [42].

Spinal cord injury (SCI) can result in severe motor and sensory dysfunction, leading to high rates of disability and mortality, yet lack of effective therapies. Exosomes derived from MSCs have shown promise in enhancing functional recovery after SCI by reducing neuroinflammation and promoting axonal regeneration [79]. However, the low targeting ability of unmodified exosomes has hindered their use as therapeutic agents. Lee et al. addressed this issue by injecting exosome-like nanovesicles (MF-NVs) derived from macrophage membrane-fused umbilical cord blood-derived MSCs (MF-MSCs) into mice with SCI. These nanovesicles, which contain macrophage membrane integrins α4 and β1, exhibited improved targeting of ischemic regions and inflammatory tissues, such as the injured spinal cord. As a result, MF-NVs provided better neuroprotection and anti-inflammatory and pro-angiogenesis effects and reduced glial scar formation, ultimately improving the function of the damaged spinal cord [26]. The development of nanovesicle-targeted enhanced exosomes holds great potential for the clinical treatment of SCI. Xu et al. also supposed that MSCs-EVs should be utilized more for the management of SCI due to higher safety and more efficient delivery of therapeutic substances to recipient cells relative to MSCs. Their research data suggested that HucMSCs-EVs improved neurological recovery post SCI by carrying miR-26b-5p to target the intracellular KDM6A/NOX4 axis, and the effect was enhanced after overexpression of miR-26b-5p in HucMSCs-Evs [80]. However, systemically injected exosomes undergo rapid clearance, which harms microenvironment regulation and limits the regenerative outcomes [81]. Mu et al. constructed a hydrogel composed of hyaluronic acid (HA) for spinal cavity replenishment and further modified with the adhesive peptide PPFLMLLKGSTR as the vector to encapsulate exosomes derived from hypoxia-inducible factor HIF-1α-pretreated MSCs (hypo-Exo), then transplanted them into the damaged spinal cord. The hypo-Exo significantly upregulated VEGF expression in peripheral endothelial cells at the site of injury and promoted angiogenesis and functional recovery [82]. Additionally, to address targeting issues during systemic administration of therapeutic exosomes and minimize additional damage from repeated injections at the injury site, Han et al. developed a controllable 3D-Exo microneedle array patch for the in situ repair of SCI, which not only increased the 3D-Exo yield but also obtained exosomes containing more neuroprotective substances, therefore effectively mitigating the inflammatory response and glial scarring caused by SCI [83].

3.6. Heart

In recent years, the focus on stem cells in regenerative medicine has brought new hope for treating cardiovascular diseases. Numerous studies have shown that stem cells participate in repairing damaged myocardial tissue through paracrine mechanisms. The therapeutic effects of MSCs were further enhanced by subjecting them to a hypoxic environment, genetic modification, drugs, or physical factors before usage, potentially amplifying their paracrine effects to the surroundings [84]. Compared with MSCs under normal conditions, exosomes derived from hypoxia-treated MSCs have been found to have higher levels of angiopoietin, VEGF, and insulin-like growth factor I (IGF-1), which effectively promoted angiogenesis [85]. Pan et al. used miR-146a to modify ADSC-derived exosomes to treat an AMI rat model and found that the exosomes reduced the apoptosis, inflammatory response, and fibrosis of cardiomyocytes by downregulating early growth response factor 1 (EGR1) [86]. issue inhibitor of matrix metalloproteinases 2 (TIMP2) plays a crucial role in maintaining the dynamic homeostasis of the extracellular matrix (ECM) by inhibiting the activity of matrix metalloproteinases (MMPs), which are essential for normal cardiac function [87]. Ni et al. used exosomes derived from hucMSCs overexpressing TIMP2 (huc-exo^{TIMP2}) to treat a rat model of myocardial infarction and found that huc-exo^{TIMP2} reduced oxidative stress induced by myocardial infarction through the Akt/Sfrp2 pathway, suppressed extracellular matrix (ECM) remodeling and facilitated angiogenesis in myocardial infarction injury, which significantly improved cardiac function [88].

Tanshinone IIA (TSA), a fat-soluble constituent of the traditional Chinese medicine Danshen, has been shown to attenuate the adverse consequences of myocardial ischaemia–reperfusion injury [89]. Li et al. investigated the therapeutic effect of exosomes derived from hucMSCs primed with TSA (TSA-MSCexo) on myocardial ischaemia–reperfusion injury. They found that the level of miR-223-5p was upregulated in TSA-MSCexos, which were then transferred by TSA-MSCexos to regulate monocyte infiltration and angiogenesis by targeting CCR2, leading to myocardial repair and improvements in MI/RI [90]. The findings provide experimental support for the use of exosomes from MSCs primed with tanshinone IIA as a cell-free therapy for cardiovascular diseases. Atorvastatin (ATV), one of the widely used lipid-lowering drugs for patients with coronary heart disease, could enhance the efficacy of MSC treatment for AMI [91]. Huang et al. discovered that exosomes from MSCs pretreated with ATV (MSCATV-Exo) promoted endothelial cell function and cardiac angiogenesis, increased the release of VEGF and ICAM-1, and decreased the expression of cellular inflammatory factors (lL-6 and TNF-a) and fibrotic genes (Col1a1 and Col3a1) in fibroblasts, which protected cardiomyocytes and improved post-infarction cardiac functions [92]. One possible explanation was that ATV pretreatment upregulated the level of lncRNA H19, which is known to be an important regulator of endothelial senescence and promotes endothelial cell proliferation [93], thus contributing to improved angiogenesis in the heart. Oridonin is a diterpenoid isolated from Rabdosia rubescens that can target cell cycle arrest, apoptosis and autophagy [94]. Fu et al. investigated the treatment of ischaemia/reperfusion (IR)-induced myocardial injury using exosomes derived from BMSCs preconditioned with oridonin and found that the exosomes inhibited apoptosis and promoted autophagy, which effectively protected cardiomyocytes from IR injury [95]. Similarly, Du et al. discovered that exosomes derived from human placental MSCs (hP-MSCs)

preconditioned with NO exhibited enhanced angiogenic effects on HUVECs. This finding was further supported by a murine model of hind limb ischaemia in which exosomes demonstrated superior angiogenic effects and improved limb functions. The underlying mechanism was attributed to the upregulation of VEGF and miR-126 levels in exosomes due to the NO pretreatment of hP-MSCs [96]. In conclusion, MSC-derived exosomes exhibited prominent protective effects against myocardial injury [97], and MSC preconditioning enhanced the therapeutic efficacy of exosomes in the treatment of cardiovascular diseases.

3.7. Liver

Studies have demonstrated the potential of embryonic stem cell-derived exosomes in repairing liver tissue. Wang et al. applied exosomes from human embryonic stem cells cultured in both 3D and 2D monolayer cultures to treat injured livers of a fibrosis mouse model. The results showed higher 3D-Exos accumulation in the liver, leading to a reduction in hepatocyte necrosis and an increased level of liver functioning proteins. Ultimately, the liver function of fibrotic mice was restored. The mechanism behind the effect could be attributed to the inhibition of SMAD signaling by upregulating miR-6766-3p in 3D-Exos, which suppresses TGFβRII expression, LX2 cell activation, and hepatic fibrosis [98]. Wu et al. explored treatment in a model of rat liver transplantation (LT) using heme oxygenase-1-modified BMSC-derived exosomes (HM-exos). HM-exos inhibit hepatocyte ferroptosis and reduce ischaemia–reperfusion injury in liver transplantation by upregulating miR-124-3p expression, which in turn reduces prostate transmembrane epithelial 3 antigen antibody (Steap3) levels, thereby attenuating graft IRI [99].

MiR-199a-3p has an impact on the proliferation, migration, and invasiveness of hepatocellular carcinoma cell lines and increases the sensitivity of these cell lines to adriamycin. Using miR-199a lentivirus infection, Lou et al. constructed exosomes (AMSC-Exo-199a) that drive miR-199a-modified AMSCs (AMSC-199a). Compared with the control group, AMSC-Exo-199a contained more than 10-fold higher levels of miR-199a-3p. Furthermore, overexpressed miR-199a-3p in exosomes targeted and inhibited the mTOR signaling pathway, leading to enhanced sensitivity of hepatocellular carcinoma to chemotherapeutic drugs [100]. Another study by Zhang et al. involved TNF-α pretreatment of umbilical cord mesenchymal stem cell-derived exosomes (T-Exos) for the treatment of an acute liver failure (ALF) mouse model. Researchers found that the level of miR-2993p, which has anti-inflammatory effects and inhibits the activation of the NLRP3 inflammation-related pathway, was increased in T-Exos, with decreased levels of serum alanine aminotransferase (ALT), glutamate aminotransferase (AST), and proinflammatory cytokines. As a result, ALF-induced inflammatory injury was attenuated, and hepatic tissue repair was promoted [101].

3.8. Others

Due to the low tolerance towards ischaemia in adipose tissues, early revascularization of transplanted tissues is crucial for the survival of grafts [102]. Therefore, Han et al. used exosomes to improve survival from graft rejection. Exosomes derived from hypoxia-treated human ADSCs (hypoxic ADSC-Exos) have been found to possess a high capacity to enhance angiogenesis in a nude mouse model of subcutaneous fat grafting; this effect was achieved by significantly increasing the protein expression of EGF, FGF, VEGF/

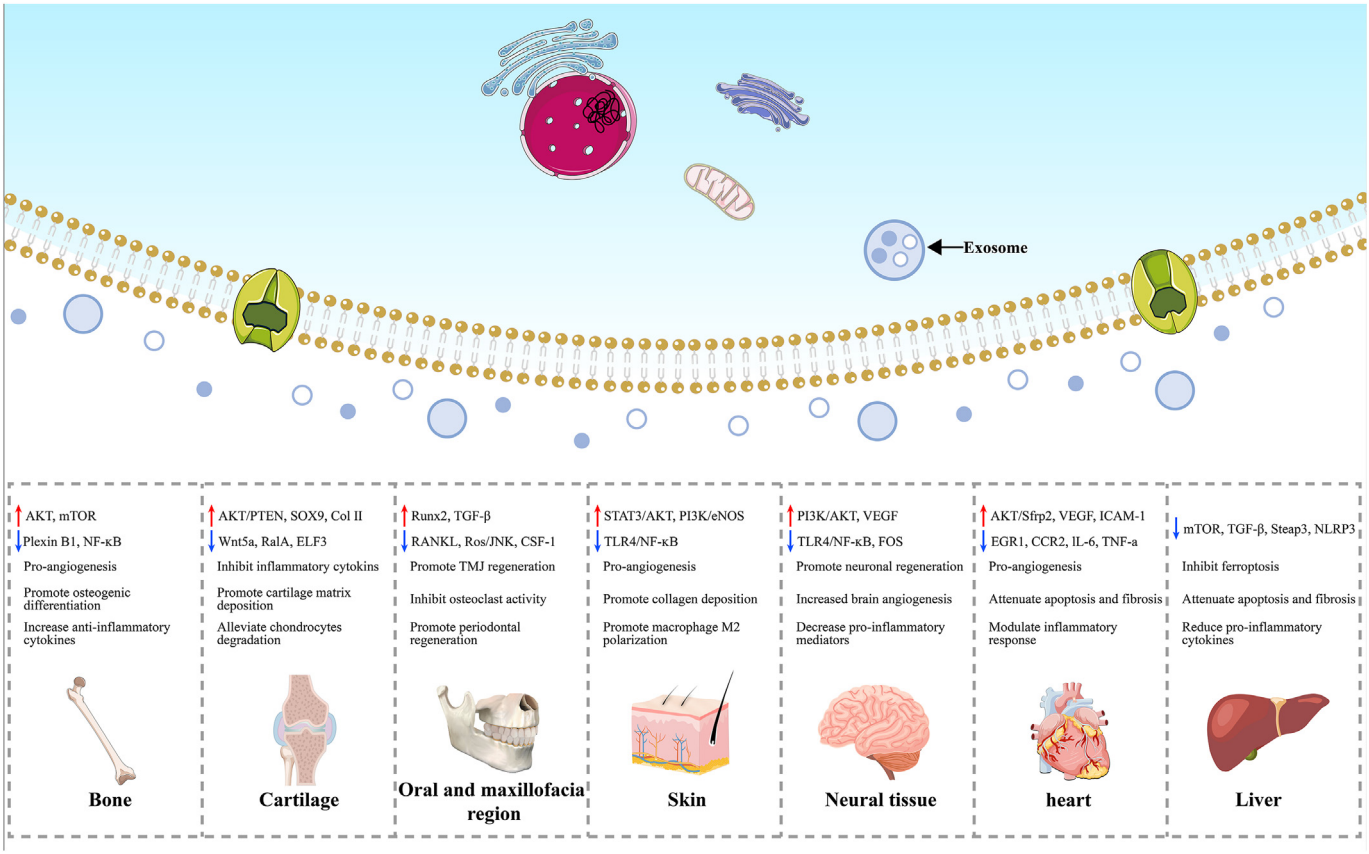

Fig. 1. Therapeutic effects of exosomes derived from preconditioned MSCs in tissue repair and regeneration.

VEGF-R, and angiopoietin-1 (Ang-1) to regulate VEGF/VEGF-R signaling. Hypoxic ADSC-Exo treatment significantly promoted the proliferation, migration and tube-formation capability of HUVECs [103]. To further investigate those findings, they injected hypoxic ADSC-Exos and ADSC-Exos around subcutaneous fat grafted tissues in a nude mouse model and found that ADSC-Exos effectively promoted graft survival, facilitated neovascularization, and attenuated inflammation in adipose grafts and that hypoxic preconditioning enhanced the therapeutic effect of ADSC-Exos [104]. Similarly, Guo et al. discovered that IL-35-modified ADSCs (IL-35-ASCs) exerted stronger immunosuppressive effects than did unmodified ADSCs, both in vivo and in vitro [105]. They found that IL-35-ASC-Exos, as the main effector of IL-35-ASCs, also possessed potent immunosuppressive properties, which effectively upregulated the regulatory T cell (Treg) ratio and prolonged the survival of grafts in a mouse heart transplantation model [106]. Additionally, exosomes derived from MSCs modified with PD-L1 and PD-L1-ITGB1 showed inhibitory effects on the proliferation of activated T cells and increased the proportion of Tregs, consequently, acute graft-versus-host-disease (GVHD) was ameliorated [107]. The results of those studies demonstrate that the immunomodulatory capacity and biological effects of exosomes can be enhanced through specific pretreatments and offer potential for the treatment of graft rejection.

All in all, the therapeutic effects of exosomes derived from preconditioned MSCs in tissue repair and regeneration have been widely investigated and shown promising results for clinical application (Fig. 1).

4. Conclusions and future prospects

Exosomes, especially exosomes derived from MSCs, have emerged as promising applications in various tissue repair treatments, serving as an important modality for cell-free therapies. Preconditioning, as an adjunct method of stem cell therapy, can be employed to improve proliferation, migration, and directional differentiation of MSCs, and has been demonstrated to enhance exosome secretion and functions through the activation of various signaling pathways [108,109]. However, it is generally accepted that MSCs are a heterogeneous cell population and their heterogeneity depends on their origin (biological niche) or the conditions of potential donors (age, diseases or unknown factors) [110], which naturally pass the heterogeneity to MSC-exos. Coupling with the different culture conditions mentioned in this review, it is next to impossible to fully understand the precise mechanisms by which exosomes of preconditioned MSCs exert their therapeutic effects and regulate cells involved in repair processes. The only way scientists could choose is to embrace the challenges and strive to fill research gaps which might provide valuable directions for the future study of exosome-based cell-free therapies.

Nevertheless, we believe it would be of great value in exploring the potential benefits of combining multiple pretreatment methods for the application of exosomes in specific diseases. Before conducting the studies, we should realize that there are several unresolved issues in the current strategies for tissue regeneration and disease treatment using preconditioned stem cell exosomes: a. MSC heterogeneity should be considered in the development of MSC and their exosomes, possibly using specific functional analysis to ensure homogeneity of action; b. Improved methods to mimic the in vivo microenvironment of MSCs need to be developed to obtain exosomes that closely resemble the in vivo environment; c. The precise and stable control of the exosome producing process needs to be achieved to increase the amount and improve the quality/purity of exosomes; d. More suitable delivery systems need to be designed to ensure the slow and continuous release of exosomes, as well as guiding exosomes to the targeting tissues. According to the literature reviewed, dynamic 3D culture was able to better mimic the natural growth environment of MSCs than was 2D culture, and the exosomes from 3D culture showed superior therapeutic efficacy. Additionally, the coupling of microfluidics and 3D bioprinting could allow the localized, sustained, and controlled release of therapeutic exosomes [111]. Surface engineering strategies can be widely employed to introduce a broad range of targeting moieties onto MSC-EXOs, which will be an important field of research for therapeutic success. Furthermore, exosomes can be used as a novel type of biomaterial or gene/drug carrier, showing promising applications in tissue damage repair. Despite the challenges, there is great potential for the development of new strategies to further boost the application of stem cell exosome-based cell-free therapies in regenerative medicine.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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