

REVIEW

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A paradigm shift in cell-free approach: the emerging role of MSCs-derived exosomes in regenerative medicine

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Abstract

Recently, mesenchymal stem/stromal cells (MSCs) due to their pro-angiogenic, anti-apoptotic, and immunoregulatory competencies along with fewer ethical issues are presented as a rational strategy for regenerative medicine. Current reports have signified that the pleiotropic effects of MSCs are not related to their differentiation potentials, but rather are exerted through the release of soluble paracrine molecules. Being nano-sized, non-toxic, biocompatible, barely immunogenic, and owning targeting capability and organotropism, exosomes are considered nanocarriers for their possible use in diagnosis and therapy. Exosomes convey functional molecules such as long non-coding RNAs (lncRNAs) and micro-RNAs (miRNAs), proteins (e.g., chemokine and cytokine), and lipids from MSCs to the target cells. They participate in intercellular interaction procedures and enable the repair of damaged or diseased tissues and organs. Findings have evidenced that exosomes alone are liable for the beneficial influences of MSCs in a myriad of experimental models, suggesting that MSC- exosomes can be utilized to establish a novel cell-free therapeutic strategy for the treatment of varied human disorders, encompassing myocardial infarction (MI), CNS-related disorders, musculoskeletal disorders (e.g. arthritis), kidney diseases, liver diseases, lung diseases, as well as cutaneous wounds. Importantly, compared with MSCs, MSC- exosomes serve more steady entities and reduced safety risks concerning the injection of live cells, such as microvasculature occlusion risk. In the current review, we will discuss the therapeutic potential of MSC- exosomes as an innovative approach in the context of regenerative medicine and highlight the recent knowledge on MSC- exosomes in translational medicine, focusing on in vivo researches.

Keywords: Mesenchymal stem/stromal cells (MSCs), Exosomes, Regenerative medicine, Micro-RNAs (miRNAs)

Introduction

Mesenchymal stem/stromal cells (MSCs) can be obtained from a variety of human tissues, ranging from bone marrow (BM) to umbilical cord (UC), typically showing multipotent differentiation competencies

[1–4]. Because of the pro-angiogenic, anti-apoptotic, and immunomodulatory attributes concomitant with fewer ethical concerns, MSCs are recently introduced as respectable candidates for regenerative medicine [5]. A large body of investigations has evaluated the potent applications of MSCs in a spectrum of disorders encompassing cardiomyopathy, neurodegenerative disorders, spinal cord injuries (SCI), kidney injuries, liver injuries, lung injuries, and also cancers [6–8]. Currently, studies have revealed that MSCs-derived

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extracellular vesicles (EVs) are responsible for MSCs-exerted therapeutic merits [9, 10].

Concerning the definition of the International Society for Extracellular Vesicles (ISEV), EVs are lipid bilayer particles which are naturally secreted from the cells, and do not show replication capacity. The ISEVs have also cited that the two classes (classes 1 and 2) of EV markers and one class of non-EV markers (class 3) are essential to be inspected for demonstration of the existence of EVs [11]. Class 1 involves transmembrane or glycosylphosphatidylinositol (GPI)-anchored proteins, representing the lipid bilayer construction of EVs; on the other hand, class 2 includes cytosolic proteins containing enzymes, cytoskeletal proteins, and proteins actively merged into EVs through membrane binding. Also, class 3 includes proteins of non-EV constructions co-isolated with EVs which enable determining of the EVs purity during preparation [11].

Generally, EVs are categorized into 3 subclasses regarding sizes and biogenesis procedures, surrounding exosomes (50–150 nm), microvesicles (MVs) (100–1000 nm), and apoptotic bodies (ApoBDs) (500–5000 nm) [12]. Exosomes are produced through multiple steps; creation of endosomes from the plasma membrane, construction of intraluminal vesicles inside of multivesicular bodies (MVB) by inward budding, merging of MVB with the plasma membrane, and finally releases of internal vesicles. Besides, both MVs and ApoBDs are formed via local distortion and straight outward budding from the plasma membrane [13, 14]. MSC- exosomes elicited their activities by the transmission of their molecules, including proteins, messenger RNA (mRNAs), and microRNAs (miRNAs), to target cells that result in phenotypic change and then modification of regenerative programs of target organs [15, 16]. These phenotypic alterations are triggered by some mechanisms, such as prevention of apoptosis in target cells, cell proliferation, immunomodulatory reactions, attenuation of oxidative stress in recipient cells, as well as improvement of oxygen supply [17]. For instance, MSC- exosomes could support mitochondrial transfers, and consequently, suppress the inflammatory cytokine generation and also induce phenotype 2 of alveolar macrophages (M2) leading to acute lung injury (ALI) rescue in vivo [18]. In this regard, the transmission of miRNAs from MSC- exosomes to recipient cells has been proven as the causal mechanism in the restoration of the kidney [19, 20], heart [21], liver [22, 23], and brain damages [24, 25]. Herein, we will emphasize the therapeutic potential of MSC- exosomes as a cell-free-based therapeutic option in human organ disorders and highlight their superiority over parental MSCs, as evident from in vivo reports.

Overview of MSCs

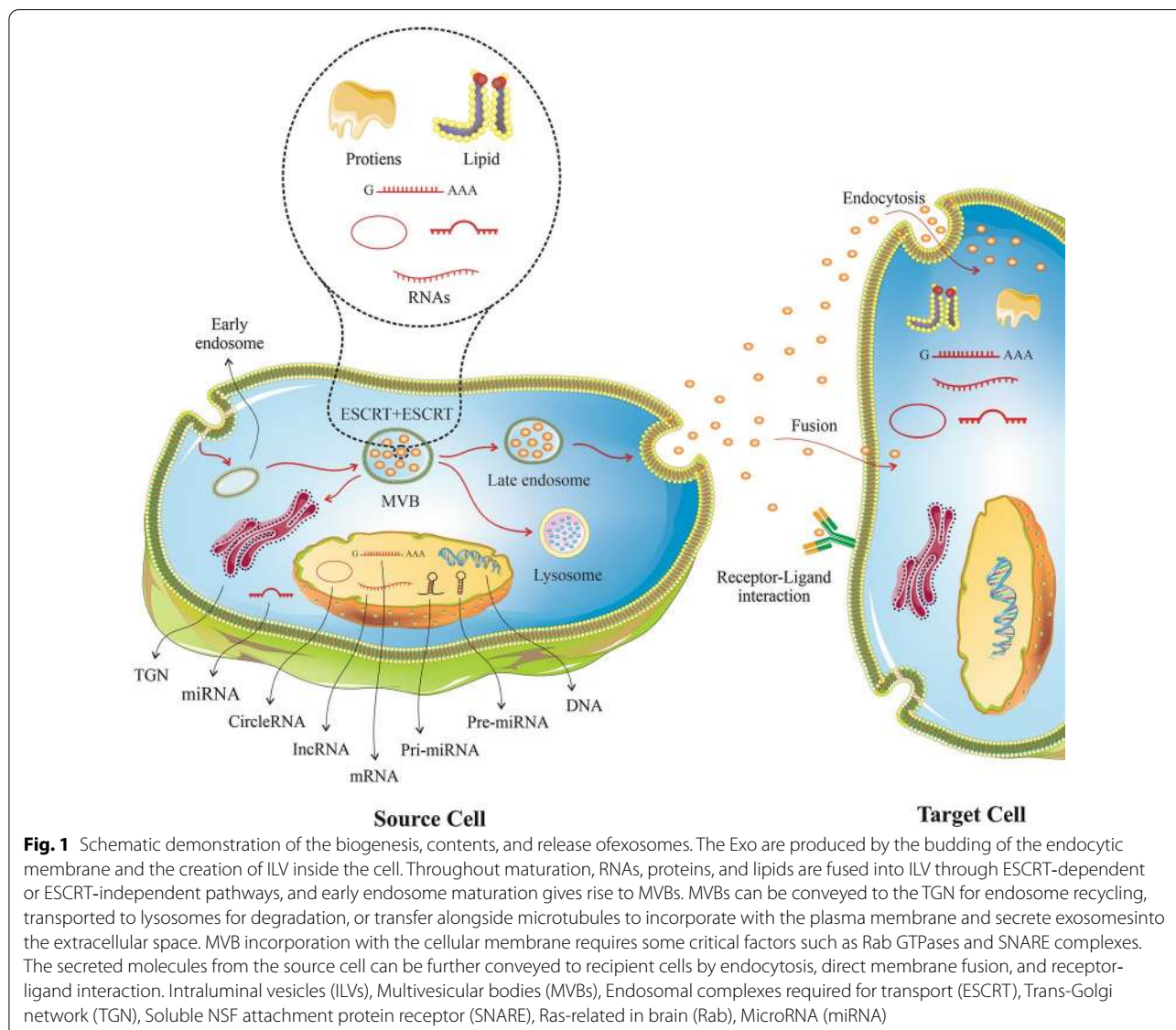
In the 1960s, Friedenstein and his colleagues for the first time found that the osteogenic capability, as demonstrated through heterotopic transplantation of BM cells, was dependent on a BM cell's minor subpopulation [26]. They found that these cells were different from the common hematopoietic cells because of their speedy adherence to tissue culture vessels and also their fibroblast-like morphology in culture. Friedenstein et al. delivered a chief advance by presenting that seeding of BM cell suspensions at clonal density lead to the formation of separate colonies introduced by single cells, known as the colony-forming unit fibroblastic (CFU-Fs) [27]. As described, MSC's unique attributes, including self-renewability, multipotency, and accessibility concurrently with lower ethical concern and immunoregulatory competencies represent their prominent position in regenerative medicine [28, 29]. In addition to the BM, MSCs can be procured from a broad range of tissues, including the perivascular area. Though there is no discrete clarification and quantitative assay, providing confident detection of MSCs in miscellaneous cells population, the International Society for Cellular Therapy (ISCT) has delivered minimum values for MSCs definition. These principles consist of donating plastic adherence possessions, expressing CD73, D90, CD105 in the absence of CD14, CD34, CD45, and human leucocyte antigen-DR (HLA-DR), and finally potent in vitro differentiation into adipocyte, chondrocyte, and osteoblast [30]. Given these principles, cells that demonstrate MSC's minimal properties can be obtained from adipose tissue (A-MSCs), dental pulps (DP-MSCs), endometrium (En-MSCs), peripheral blood (PB-MSCs), skin (S-MSCs), placenta (PL-MSCs), umbilical cord (UC-MSCs), tendon (T-MSCs) synovial fluid (SF-MSCs), muscles (M-MSCs), Wharton's jelly (WJ-MSCs), etc. [31].

Despite of the expression of similar levels of the surface antigen by MSCs derived from diverse sources, some reports have evidenced differences between them. In this regard, UC-MSCs demonstrated higher proliferation rate, and lower expression of p53, p21, and p16 against BM-MSCs and A-MSCs [32]. Besides, BM-MSCs and WJ-MSC showed higher proliferation and clonality compared with A-MSCs, and also WJ-MSCs exposed higher immunoregulatory ability in comparison to other cells possibly mediated by deterring the function of Th1 and Th17 but not Th2 and Treg [33]. On the other hand, surveying of the cells obtained from BM, UC, adipose tissue, and tendons have exposed that MSC's numbers attained from adipose tissue, tendon, and UC were prominently higher than those attained from BM. Moreover, A-MSCs and T-MSCs experienced more rapid proliferation, and

also BM-MSCs showed more powerful osteogenic differentiation than those derived from other origins [34]. As well, Urrutia and her coworkers found that BM-MSCs, S-MSCs, and UC-MSCs can differentiate into neuron-like cells, as evidenced by the demonstration of either progenitors or mature neural markers, A-MSCs can express significantly higher levels of neural markers and also show quicker proliferation rate [35]. Correspondingly, it seems that choosing the distinct type of MSCs from specific tissue origin considering the patient's conditions and disorder's characteristics and stages may affect the therapeutic outcome.

MSCs- exosomes biogenesis

In 1983, exosomes were firstly identified by Johnstone et al. as vesicles that contribute to mammalian reticulo-cyte differentiation and maturing [36]. Exosomes as one of the three subclasses of EVs are shaped through budding as intraluminal vesicles (ILVs) within the luminal space of late endosomes or multivesicular bodies (MVBs) [37] through endosomal complexes required for transport (ESCRT)-dependent or ESCRT-independent pathways (Fig. 1). In the ESCRT-dependent pathway, ESCRT as cytoplasmic proteins participating in membrane budding play a central role to generate a coated subdomain on endosomes to eventually shape the ILVs. Following MVB's incorporation into the cellular membrane, these ILVs are released as exosomes [38]. MVBs include



endocytic markers encompassing Rab proteins and lysosome-associated membrane glycoproteins (LAMPs), which make them distinguishable from autophagic bodies or multilamellar lysosomes [39]. Using the described mechanism, exosomes are continually formed and secreted via diverse cells, ranging from B and T lymphocytes, platelets, mast cells, intestinal epithelial cells, dendritic cells, neoplastic cell lines, microglia, and neurons to MSCs [40]. Collecting information has shown that exosomes are responsible for a range of cell-to-cell communication axis allied with several physiological and pathological activities. About their molecular components and also morphology, there exist various types of MVBs, creating several sorts of exosomes within a cell. Meanwhile, only MVBs comprising a higher ratio of cholesterol could merge with the cellular membrane of B cells and release exosomes [41]. Remarkably, reports have indicated that Exo released from the apical and basolateral sides of polarized cells possess dissimilar molecular components [42]; however, it seems that exosomes components relatively reveal their parent cell's compositions.

Despite their inherent biological activities, exosomes are recently introduced as encouraging drug carriers that rely on their small size, supreme biocompatibility, and aptitude to load particular and different therapeutic ingredients such as proteins, nucleic acids, and small molecules [43]. However, restricted secretion of exosomes from parent cells obstructs their large-scale production. Following a few passages, MSCs experience senescence, and their exosomes commonly show impaired regenerative capability compared with young cells. Though MSCs transfection with vectors carrying MYC results in the establishment of immortalized cells [44], MYC is a well-known proto-oncogene and may stimulate unwanted effects, making its applications challenging. Moreover, varied approaches have been suggested to augment the Exo production, including inducing hypoxia, overexpressing tetraspanin CD9 along with overexpressing hypoxia-inducible factor-1 alpha (HIF-1 α). On the other hand, the use of hollow fibers bioreactors is other strategies that can boost the biogenesis and secretion of MSCs- exosomes [45]. For instance, it has been offered that the three-dimensional culture of MSCs in a hollow-fiber bioreactor leads to the promoted Exo yield and could support therapeutic efficacy for cisplatin-induced acute kidney injury (AKI) in murine models [46]. Moreover, other investigations have suggested that widely recognized biomaterial 45S5 Bioglass[®] (BG) can considerably promote MSCs-derived exosomes quantity by stimulating the expression of neutral sphingomyelinase-2 (nSMase2) and Rab27a, which promote nSMases and Rab GTPases axis and eventually improve Exo generation as well as secretion. Moreover, alginate

hydrogel led to the the highest rates of cytokines and growth factors, such as fibroblast growth factor 2 (FGF-2), insulin-like growth factor (IGF), hepatocyte growth factor (HGF) and leukemia inhibitory factor (LIF) generated by BM-MSCs compared to control BM-MSCs [47], and thereby could affect EVs biogenesis. Further, Avitene Ultrafoam collagen hemostat induced the BM-MSCs to secrete higher exosomes with more prominent therapeutic influences on the rat model of traumatic brain injury due to the supporting continued delivery to the wound area [48]. As well, EVs derived from bioreactor-inoculated hBM-MSCs in addition to the demonstration of consistency in size and concentration revealed a high frequency of immuno-regulatory and angiogenic factors VEGF-A and IL-8 compared to control cells. Accordingly, EVs from hBM-MSCs with immuno-regulatory competences could be established using bioreactors, circumventing the challenge for clinical use [49]. Besides, metformin, which largely used to treat people with type 2 diabetes, could improve EVs secretion and modify the protein profile of EVs. Metformin promotes EVs generation by an autophagy-related axis concurrently with the phosphorylation of synaptosome-associated protein 29 (SNAP29). Interestingly, this ingredient affect both the quantity and the quality of MSCs-derived EVs, as documented by an elevation in the content of EVs participated in cell growth through proteomic analysis [50]. Another study showed that preconditioning of MSCs with lithium could ameliorate the neuroregenerative capacities of MSCs mediated by elevated secretion of EVs. Meanwhile, EVs derived from preconditioned MSCs demonstrated improved levels of miR-1906, acting as a possible regulator of toll-like receptor 4 (TLR4) signaling, and finally decreased the rates of poststroke cerebral inflammation in a stroke murine model [51]. On the other hand, it has been found that ultrasonication of ultracentrifuged MSC-EVs tracked by regular centrifugation and filtration enables enhancing the EV yield by 20-fold [52].

MSCs-Exo contents

The exosome involves numerous cytoplasmic and membrane proteins, including receptors, enzymes, transcription factors, extracellular matrix (ECM) proteins, nucleic acids (mtDNA, ssDNA, dsDNA, mRNA, and miRNA), and also lipids [53]. Examinations of the exosomal protein compositions have shown that some of them are limited to special cell/tissue types, while others are mutual among all exosomes. Although cell adhesion molecules (CAMs), integrins, tetraspanins, and major histocompatibility complex (MHC) proteins are mutual amongst varied Exo, fusion and transferring-associated proteins such as Rab2, Rab7, annexins, and ALG2-interacting protein X (Alix) are nonspecific

exosomal proteins [11]. The connections between Exo and target cells comprise surface proteins of exosomes including integrins, tetraspanins, and ICAMs, enabling interaction between exosomes and recipient cell by receptor-ligand binding [54]. In this regard, integrins play pivotal roles, for instance, integrin $\alpha\beta 5$ provides communication between tumors-derived exosomes and Kupffer cells in the fibronectin-rich liver, and also integrin $\beta 3$ -abundant exosomes could fuse with CD31-positive brain endothelial cells [55]. Besides, exosomes enclosing tetraspanin8 and its related CD49d shapes an interaction with endothelial cells by CD106, a ligand for CD49d, which in turn, stimulates angiogenesis-associated gene expression in endothelial progenitors [56]. Meanwhile, ICAM-1-enriched exosomes secreted from dendritic cells (DCs) are needed for effective naive T cell priming [57]. Especially, in pancreatic ductal adenocarcinoma, the tumor-adjacent normal pancreatic tissue could generate regenerating islet-derived 3 β (REG3 β) which diminishes exosomes secretion from malignant cells following binding to glycoproteins on the surface of circulating Exo [58]. Unlike proteins, exosomal lipid compositions are typically preserved and show cell type-specific characteristics. Lipids largely involve in producing and protecting exosomal construction, vesicle biogenesis, and also adjusting the homeostasis in the recipient cells [59]. Correspondingly, improved content of lysobisphosphatidic acid in the inner phospholipid layer of MVB membrane accompanying by Alix permits inward budding of MVBs and thus exosomes manufacturing [60]. As well, Exo also modify the homeostasis of the recipient cells by modulating their lipid components, in particular, cholesterol and sphingomyelin concentrations [60]. More importantly, it has been suggested that the regulation of several mechanisms and molecules by exosomes rely on their miRNAs cargo. The miR-21 stimulates angiogenesis by induction of protein kinase B (PKB or AKT) and the extracellular signal-regulated kinase (ERK), and also promoting the expression of VEGF and HIF-1 α in target cells [61]. Besides, miR-1246 could elicit angiogenesis by inducing Smad 1/5/8 signaling in human umbilical vein endothelial cells (HUVECs), and miR23a-3p expressed in endothelial cells supports angiogenesis by targeting Sprouty2 (SPRY2) and Semaphorin 6A (Sema6A), finally adjusting the angiogenesis by moderating vascular endothelial growth factor (VEGF) signaling [62]. On the other hand, Shao et al. found that MSC- exosomes could induce cardiomyocyte cell proliferation, suppress their apoptosis, and also target transforming growth factor-beta (TGF- β)-induced transformation of fibroblasts into myofibroblast in myocardial infarction (MI) murine model by

up-regulation of miR-29 and miR-24, positively regulating cardiac activities [63].

EVs proteome studies are novel strategies and are part of the rising interest in proteomics investigations. To date, a large number of proteomics studies have been carried out on EVs to clarify their varied roles. Today, mass spectrometry (MS) is a major method for the recognition and characterization of the protein content of EVs. As pronounced, the EVs can be generated by a spectrum of various cell types and they can be obtained from biological fluids and also conditioned medium (CM) [64, 65]. A distinctive procedure of MS-based proteomic examinations of EVs includes multiple stages, such as the separation of EVs from particular biofluids, the extraction of EV proteins utilizing lysis buffers, and the separation of the extracted proteins and digestion before the MS analysis [66]. Especially, the isolated EV proteins can be separated using gel electrophoresis and in-gel digestion. Further, they can be directly digested and then achieved peptides are fractionated by liquid chromatography (LC) before the MS analysis [66].

Although MSC-EVs derived from different sources mainly show remarkable therapeutic influences, they have various physiological functions, which can affect their therapeutic applications. For instance, EVs obtained from hBMMSCs show superior potential to treat cartilage defects or osteoarthritis which are in association with bone diseases [67, 68]. Also, reports have indicated that there are variances in uptake efficiency between the EVs derived from BM-MSCs and adipose tissue derived (A)-MSCs, while the corresponding mechanism for this phenomenon has not yet been elucidated [69]. In addition, human embryonic mesenchymal stem/stromal cells (ES-MSCs) could elicit better immunomodulatory effect than BM- and AT-MSCs [70]. Moreover, EVs derived from AMSCs could promote angiogenesis significantly more than EVs obtained from BMMSCs, as shown by study of their effects on human umbilical vein endothelial cell (HUVEC) tube formation and mitochondrial respiration [71]. In the context of tumor therapy, BM- and UC-MSC-EVs reduced cell growth, whereas an opposite impacts was detected with AMSC-EVs on U87MG glioblastoma cells line. Too, both BM- and UC-MSC-EVs stimulated the elimination U87MG cells; whereas AMSC-EVs had no significant effect [72]. Accordingly, the selection of the better cell sources for EVs therapy respecting the target tissue and disorders is of paramount importance.

MSCs-Exo isolations methods

Rendering literature, there are some challenges in the clinical application of exosomes, especially in regenerative medicine. Between various utilized isolation and

purification strategies, there is no general approach for exosomes separation from other micro-particles and also several exosomes subset's separations [73, 74]. Until, multiple accepted methods, including differential ultracentrifugation, density gradients, precipitation, filtration, and size exclusion chromatography, have been successfully used for Exo isolation purpose [75]. Traditional ultracentrifugation has been described as a most dependable method among investigators. It involves a run of centrifugation cycles of changing centrifugal force and period to separate exosomes based on their density and size variances [76]. Meanwhile, differential centrifugation (DC), enabling Exo purification by density and size, is one of the traditional and most broadly utilized methods for exosomal separation. This technique is simple and cost-effective; however, has a low output and specificity, and also exosomes may be contaminated with other EVs or be damaged during superspeed centrifuges. A combination of DC with a sucrose density gradient can improve the yield and purity of isolated exosomes [77, 78]. Another technique, filtration/ultrafiltration, separates exosomes according to the pore size of the filter and takes less time, and also is more simple than the DC. Nonetheless, as filtration/ultrafiltration is based on the size, the isolation of high-purity exosomes once contaminated with the same size's bodies, such as ApoBDs or microbubbles, is problematic [79, 80]. Likely, size exclusion chromatography (SEC) purifies high-purity exosomes based on the size using columns filled with pore beads. Despite the high-yields of high-purity exosomes purification, SEC has a time-consuming procedure and is also not fitting for the high-purity exosomes separation from large sample volumes [79, 81]. Moreover, the immunoaffinity-based isolation results in high-purity exosomes by the connection of exosomes-related antigens to antibodies in chromatography columns, magnetic beads, and micro-fluidic systems [82]. Besides, lower specificity and also co-precipitation of other micro-particles and also exosomes degradation because of the use of chemical components during the purification process may hinder the application of the precipitation method [79]. Overall, each purification technique has exclusive merits and shortcomings; its disadvantage could be restored by combining two or more purification methods and promoting both purity and quantity.

MSCs-Exo applications in regenerative medicine

As described, MSC- exosomes could serve several therapeutic benefits such as lower immunogenicity and upgraded safety profiles compared with MSC, and thereby are considered a rational substitute to whole-cell therapy to treat a wide spectrum of human disorders by delivering functional molecules to the target cells. In

exosomes, proteomic examinations have led to the recognition of hundreds of proteins participating in central biological events, including Exo biogenesis, cellular construction, tissue recovery, and also inflammatory response [83]. As well, mRNAs and miRNAs have been recognized in exosomes, which can be taken up by either adjacent or distant cells and then modify target cells [84].

Lung disorders

Various preclinical studies have been carried out to address the therapeutic efficacy of the MSC- exosomes in lung inflammatory disorders (Table 1). Investigations have revealed that MSC- exosomes could restore lung ischemia/reperfusion (I/R) by transporting miR-21-5p in a murine model [85]. Intratracheal injection of MSC- exosomes could diminish lung edema and deficits, alveolar macrophage's M1 polarization, and also hinder high mobility group box protein 1 (HMGB1), IL-8, IL-1 β , IL-6, IL-17, and tumor necrosis factor α (TNF α) expression in transplanted models. Moreover, MSC- exosomes could suppress pulmonary cell's intrinsic and extrinsic apoptosis pathway by miR-21-5p mediated by affecting phosphatase and tensin homolog (PTEN) and programmed cell death 4 (PDCD4) [85]. Similarly, BMMSCs- exosomes administration in halothane-induced liver injury (HILI) rat models resulted in the desired effect in the treated animal by inhibition of PTEN, which consequently led to the PI3K/AKT signaling pathway's activation. The observations indicated that miR-425 expression plays pivotal roles in PTEN down-regulation, thereby stimulating HILI recovery [86]. Also, miR-30b-3p-enriched MSC- exosomes caused acute lung injury (ALI) significant alleviation by a reduction in apoptosis of type II alveolar epithelial cells (AECs) mediated by targeting serum amyloid A (SAA), which commonly over-expressed in ALI and acts as an inducer of tumor metastasis [87]. Besides, it has been supposed that miR-451 in the UCMSC- exosomes reduced pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 levels in lung tissues and serum, and more importantly ameliorated burn-induced ALI by suppressing the toll-like receptor 4/nuclear factor kappa-light-chain-enhancer of activated B cells (TLR4/NF- κ B) pathway [88]. It has previously been found that reduced expression of NF- κ B leads to the promoted superoxide dismutase (SOD), glutathione, and myeloperoxidase (MPO), but diminishes malondialdehyde (MDA) and reactive oxygen species (ROS) in ALI, and thereby may exert protective effects against oxidative stress of lung tissue in ALI [89]. Another study has signified that intravenous infusion of BMMSC-Exo decreased levels of pro-inflammatory cytokines, prohibited apoptotic protein activation, and also TLR4/

Table 1 A brief overview of studies based on MSCs- exosomes in lung disorders

Condition	Sources	Main result	Refs.
IRI	BM	Inhibition of lung epithelial cells apoptosis stimulate by oxidative stress in mice model by anti-apoptotic competencies of miR-21-5p-enriched MSCs- exosomes	[85]
AAT-deficiency lung diseases	AT	Protection of lung epithelial cells by modifying protease/anti-protease balance in the damaged lungs in mice models by MSCs-exosomes	[148]
COVID-19	BM	Recovery of oxygenation, inhibition of cytokine storm, and reconstitution of immunity in human subjects by MSCs-exosomes (a clinical trial)	[95]
BPD	BM WJ	Amelioration of hyperoxia -related inflammation, development of lung function, reduction in fibrosis, and pulmonary vascular remodeling in mice models by MSCs-exosomes	[149]
ALI	BM	Reduction in TNF- α , IL-1 β , and IL-6 and promotion of the IL-10 level in BALF and plasma in association with inhibition of MMP-9 and improvement of the SP-C level in SD rats by MSCs-exosomes	[91]
IPF	BM	Amelioration of pulmonary fibrosis and lung inflammation by the promotion of an immunoregulatory, anti-inflammatory monocyte phenotype by MSCs-exosomes in rat models	[150]
HILI	BM	Suppression of HILI by affecting PTEN and induction of the PI3K/AKT signaling pathways by miR-425- enriched MSCs-exosomes in a murine model	[86]
PH	BM	Attenuation of ventricular hypertrophy and peripheral pulmonary vessel's muscularization in a murine model by MSCs-exosomes	[151]
ALI	BM	Protection toward LPS-induced ALI by suppressing SAA3 mediated via miR-30b-3p by MSCs-exosomes	[152]
ALI	BM	Inhibition of P2X7 expression resulted in oxidative stress rescue and suppressing inflammatory response by micro-RNA-30b-3p-overexpressing MSCs-exosomes in rat models	[153]
BPD	N.A	Boosting of the blood vessel number and size by pro-angiogenic mechanisms in rat models by MSCs-exosomes	[154]
BPD	UC	Significant recovery in the lung, cardiac and brain pathology in mice models by MSCs-exosomes	[155]
ALI	BM	Protection against IIR-induced ALI through suppression of TLR4/NF- κ B signaling in rat models by MSCs-exosomes	[90]
ALI	BM	Protection against endotoxin-induced ALI mediated by the down-regulation of TNF α and up-regulation of IL-10 in mice models by MSCs-exosomes	[156]
COPD	PL	Improvement of respiratory function, inhibition of TNF- α , IL-1 β , IL-12, and IFN- γ serum levels, promotion of IL-10 serum levels in cigarettes smoke-exposed mice by MSCs-exosomes	[157]
ALI	UC	Reduction in TNF- α , IL-1 β , and IL-6 levels in rat models by miR-451 enriched MSCs-exosomes	[88]
Asthma	N.A	Promotion of lung IL-10-producing interstitial macrophages (IMs) and inducing protection against allergic asthma in mice by MSCs-exosomes	[158]

Micro RNA (miR), Bone marrow (BM), Adipose tissue (AT), Wharton's jelly (WJ), Umbilical cord (UC), Placental (PL), Ischemia–reperfusion injury (IRI), Alpha-1 antitrypsin (AAT), Coronavirus disease 2019 (COVID-19), Bronchopulmonary dysplasia (BPD), Acute lung injury (ALI), Idiopathic pulmonary fibrosis (IPF), Hyperoxia-induced lung injury (HILI), Pulmonary hypertension (PH), Chronic obstructive pulmonary disease (COPD), Tumor necrosis factor α (TNF- α), Bronchoalveolar lavage fluid (BALF), Matrix metalloproteinase type 9 (MMP-9), Surfactant protein C (SP-C), Phosphatase and tensin homolog (PTEN), Phosphatidylinositol-3-kinase (PI3K)/AKT, Serum amyloid A3 (SAA3), Toll-like receptor 4/nuclear factor kappa-light-chain-enhancer of activated B cells (TLR4/NF- κ B), Not available (N.A)

NF- κ B signaling in ALI models, suggesting that Exo therapy can be a non-cellular alternative to MSC transplantation [90]. Further, Xu et al. have suggested that MSC- exosomes could elicit positive effects on phosgene-induced ALI in Sprague–Dawley (SD) rats by a decrease in TNF- α , IL-1 β , and IL-6, and promotion in IL-10 level in bronchoalveolar lavage fluid (BALF) and plasma concurrently constraining matrix metalloproteinase type 9 (MMP-9) synthesis and supporting surfactant protein C (SP-C) producing. These effects consequently improved pathological signs and diminished wet-to-dry proportion and total protein concentration in BALF of treated models [91]. The existence of an association between acute exacerbations and pulmonary insufficiency and SP-C-deficiency in patients with lung inflammatory diseases [92] highlights the importance of finding strategies to supports SP-C expression

and functions, as shown by MSC- exosomes injection in ALI murine models.

On the other hand, investigation of the therapeutic effects of exosomes in a neonatal mouse model of bronchopulmonary dysplasia (BPD) verified beneficial effects of human UCMSC- exosomes therapy which were closely linked to tumor necrosis factor alpha-stimulated gene-6 (TSG-6) [93]. The TSG-6 is a 35-kDa glycoprotein largely involved in modulating the inflammatory response through a variety of mechanisms such as inhibition of neutrophil recruitment into inflammation's zone and also averting TLR-stimulated NF- κ B pro-inflammatory signaling [94]. Correspondingly, systemic injection of UCMSC-Exo supported a robust recovery in the lung, cardiac and brain pathology in BPD mice models. In addition to the correction in pulmonary hypertension and right ventricular hypertrophy, cell death was diminished in the brain and the hypomyelination was reversed.

Importantly, knockdown of TSG-6 by specific small interfering RNA (siRNA) in Exo suppressed the positive effects of UCMSC- exosomes, signifying TSG-6 as an imperative therapeutic molecule [93].

In humans, a prospective nonrandomized open-label cohort study was carried out in April 2020, and verified the safety and efficacy of exosomes (ExoFlo™) derived from allogeneic BM-MSCs for treatment of 24 patients with severe 2019 coronavirus disease (COVID-19). Accordingly, a single intravenous injection of ExoFlo resulted in alleviated patient's clinical condition and oxygenation in the absence of any unwanted events during 2 weeks follow-up. Robust improvements in neutrophil count and reduction in acute phase reactants, such as C-reactive protein (CRP), ferritin, and D-dimer, described MSCs- exosomes as a capable therapeutic candidate for severe COVID-19 [95].

Liver disorders

Recent findings have suggested that MSCs- exosomes could transfer certain bioactive molecules and sustain repair and recovery of damaged liver. Correspondingly, investigation of the therapeutic capacity and underlying molecular mechanism for human BMMSCs- exosomes in carbon tetrachloride (CCl₄)-induced rat liver fibrosis indicated that Exo therapy effectively improved liver fibrosis, as shown by a lessening in collagen assembly, improved liver activities, suppressed inflammation, and also promotion of the hepatocyte regeneration. Moreover, exosome supported a decrease in MDA, IL-1, and IL-6 serum levels and also suppressed the Wnt/ β -catenin pathway axis in hepatic stellate cells (HSC), verifying that BMMSCs- exosomes could improve CCl₄-induced liver fibrosis [96]. Importantly, HSCs contribute to the onset, development, and regression of liver fibrosis by producing fibrogenic molecules that stimulate portal fibrocytes, fibroblasts, and bone marrow-derived myofibroblasts to make collagen and then progress fibrosis. Therefore, inhibition of HSCs functions and proliferation can be an effective therapeutic option in liver fibrosis [97]. As well, exosomes -derived from placenta-derived mesenchymal stem/stromal cells (PL-MSCs) because of the existence of the C-reactive protein (CRP) could alleviate acute liver failure (ALF) in rat models. Meanwhile, CRP promoted the expression of the factors in correlation to the Wnt signaling axis and also angiogenesis in transplanted models resulted in improved vascularization in rat hepatocytes through interrelating with endothelial cells [98]. Furthermore, exosomal miR-122 could modify the expression of insulin-like growth factor receptor 1 (IGF1R), Cyclin G (1) (CCNG1) and prolyl-4-hydroxylase α 1 (P4HA1) contributed to the proliferation of and collagen maturation in HSCs in CCl₄-induced liver fibrosis

models, and thereby could reduce collagen depositions and consequently alleviate liver fibrosis [99].

On the other hand, injection of UCMSCs- exosomes led to a reduction in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and pro-inflammatory cytokines levels and also inhibited NLR family pyrin domain containing 3 (NLRP3) activation in ALF animal models. Based on observations, exosomal miRNA-299-3p was responsible for the inhibition of inflammatory responses and also NLRP3 stimulation in transplanted models, enabling liver tissue repair [100]. Similarly, another study has revealed that down-regulation of NLRP3 activation and its succeeding inflammatory reactions may arise from exosomal miR-17, and could inhibit NLRP3 inflammasome stimulation by affecting thioredoxin-interacting protein (TXNIP) [101]. The TXNIP is an upstream partner to NLRP3 largely contributing to ALF, stroke, traumatic brain injury, diabetes, and Alzheimer's diseases (AD) pathogenesis, and also its correlations with NLRP3 are required for downstream inflammasome induction [102].

Besides, human menstrual blood-derived stem cells (MenSCs)-derived exosomes could express cytokines, such as intercellular adhesion molecule-1 (ICAM-1), angiopoietin-2, tyrosine kinase Axl, angiogenin, insulin-like growth factor-binding protein 6 (IGFBP-6), osteoprotegerin, IL-6, and IL-8, improving liver function and abrogating liver cell's apoptosis in mice model [103]. On the other hand, exosomes could mitigate liver mononuclear cells (MNCs) frequencies and cleaved caspase-3 levels in damaged livers, offering preliminary evidence for the anti-apoptotic potential of MenSCs- exosomes [103].

Kidney disorders

MSCs- exosomes have been described to recover kidney function, reduce kidney damage, and inhibit chronic kidney fibrosis by various mechanisms, such as inhibition of the pro-fibrotic genes TGF- β 1. Evaluation of whether miRNAs deregulation constrains the regenerative competencies of MSCs- exosomes in a model of glycerol-induced acute kidney injury (AKI) in severe combined immunodeficient mice showed that kidney genes deregulated following damage were alleviated via MSCs- exosomes treatment but not via Drosha-knockdown vesicles, thus signifying a crucial role of miRNAs in recovery after AKI [104]. Furthermore, MSCs engineered to overexpress miRNA-let7c (miR-let7c-MSCs), could specifically home to injured kidneys, restore kidney injury and considerably inhibit collagen IV α 1, MMP-9, transforming growth factor (TGF)- β 1, and its receptor (TGF- β R1) in unilateral ureteral obstruction (UUO) model, a model extensively used to evaluate

obstructive nephropathy. The analysis showed that the promoted expression of fibrotic genes in NRK-52E cells, a rat renal proximal tubular cell line, stimulated by TGF- β 1 was suppressed upon co-culture with exosomes derived from miR-let7c-MSCs in vitro, showing effective anti-fibrotic capabilities of modified MSCs-Exo to recover damaged kidneys [105]. As well, the examination of the potential protective effect of UC-MSCs-exosomes on cisplatin-induced AKI rat models showed that injected Exo elicited a substantial decline in blood urea nitrogen (BUN) and creatinine rates, suppressed destruction of proximal kidney tubules, and also modulated oxidative stress in treated rats. In vitro, UC-MSCs-exosomes reduced apoptotic NRK-52E-cells numbers which was in correlation with the inhibition of expression and activation of caspase 3 cells concurrently promotion of the activation of the extracellular-signal-regulated kinase (ERK)1/2 pathway in treated NRK-52E-cells compared with the control group [106]. Furthermore, He et al. found that MSCs secretome could reduce TGF- β 1-induced morphological alterations, and also hinder E-cadherin and α -smooth muscle actin (α -SMA) secretion, a marker of myofibroblastic phenotype described as a pathogenic event, in the proximal tubular epithelial cells (HK-2) cells in vitro [107]. Besides, MSCs secretome therapy resulted in alleviation of kidney damages in treated AKI murine models compared with control groups, as proved by reduced BUN and also serum creatinine (SCr) level [107].

Molecular analysis has demonstrated that miR-4516 expression commonly is down-regulated in the kidney cortex of chronic kidney disease (CKD) mice models and leads to the triggered cytoskeleton reorganization and mitochondrial dysfunction, and stimulates kidney fibrosis. Therefore, there is a hypothesis that improved expression of miR-4516 by melatonin or other molecules could decrease ROS formation and finally ameliorate mitochondrial function [108]. Collecting proofs have showing that patients with CKD display changed circadian rhythms of melatonin levels in the blood, and the manufacture of melatonin is lessened during CKD development, while melatonin supplementation may recover kidney function [109]. Correspondingly, possible potent chronic kidney disease (CKD) recovery has been confirmed by the use of melatonin-treated healthy MSCs-Exo mediated by the promotion of the expression of cellular prion protein (PrP^C) supported by the upregulation of miR-4516 [110]. PrP^C can bind and sustain mitochondrial kinase PTEN-induced kinase 1 (PINK1), playing important role in the protection of mitochondrial against oxidative stress-stimulated apoptosis and averting mitochondrial stress [111]. In vitro, treatment with melatonin-preconditioned MSCs-Exo protected

mitochondrial activities, cellular senescence, and proliferative capacity of CKD-MSCs, and also augmented the cellular rates of angiogenesis-related proteins in CKD-MSCs. Significantly, in CKD murine models, endogenous MSCs showed improved functional recovery and vessel repair following injection of melatonin-preconditioned MSCs-exosomes [110].

Neurodegenerative diseases

Recently, several studies in animal models have evidenced the potent capacities of the MSC-exosomes therapy in CNS-related disorders, ranging from neurodegenerative diseases to spinal cord injury (SCI) (Table 2). Rendering reports, MSC-exosomes could abrogate both neurodegenerative diseases onset and progress by inhibition of microglial and astrocyte activation mediated by inactivation of NF- κ B through miRNAs-enriched exosomes. In this part, we deliver an overview of the application of MSC-Exo in human neurodegenerative diseases, including AD, Parkinson's diseases (PD), multiple sclerosis (MS), etc. MSCs-exosomes could restore neurologic disorders through serving functional biomolecules to target cells. In vivo, studies in AD APP/PS1 mice models showed that infusion of normoxic MSCs-exosomes alleviated cognition and memory deficits concerning the Morris water maze test's consequences, decreased plaque assemblies, and also amyloid-beta (A β) levels in the brain. Furthermore, these Exo reduced astrocytes and microglia activation by inhibition of pro-inflammatory cytokines like TNF- α and IL-1 β concomitant with the promotion of anti-inflammatory cytokines such as IL-4 and -10 in AD mice [112]. Further, inhibition of both signal transducer and activator of transcription 3 (STAT3) and NF- κ B activations has been introduced as other corresponding mechanisms contributing to MSCs-exosomes elicited favorable outcomes in vivo. Molecular analysis showed that exosomal miR-21 plays a central role during the alleviation procedure and could support anti-inflammatory effects and also inhibitory effects on STAT3 and NF- κ B axis to restore the cognitive impairments in APP/PS1 mice [112]. Similarly, intracerebroventricularly (ICV) transplanted BMMSCs-Exo ameliorated cognitive deficits in the AD mice model by abrogation of astrocytic inflammation as well as the promotion of synaptogenesis. In vitro, observation delivered the proofs suggesting that exosomal miR-146a released from BM-MSCs induced a reduction in NF- κ B levels in astrocytes, which may recover astrocytic function and enable synaptogenesis and correction of cognitive deficits in AD model mice [113]. Besides, evaluation of the inhibitory influences of UCMSCs-exosomes on the proliferation of peripheral mononuclear blood cells (PBMC) in relapsing-remitting MS (RRMS) patients and healthy subjects displayed that

Table 2 A brief overview of studies based on MSCs-exosomes in CNS-related disorders

Condition	Sources	Main result	Refs.
MS	BM	Reduction in the mean clinical score of experimental autoimmune encephalomyelitis mice, attenuated demyelination, reduced neuroinflammation, and promotion of T regulatory cells frequencies by MSCs-exosomes	[159]
AD	BM	Boosted learning and memory competencies of APP/PS1 mice by amelioration of synaptic dysfunction and inhibition of inflammatory responses mediated via miR-21 by MSCs-exosomes	[112]
AD	BM	Improved learning and memory, suppressed reactive astrogliosis, diminished inflammation and microglial infiltration into the damaged hippocampus concomitant with supported blood–brain barrier integrity by MSCs-exosomes in a murine model	[160]
AD	WJ	Protection of hippocampal neurons from damage stimulated by amyloid- β oligomers (A β O) resulting from enzymatically active catalase presented in MSCs-exosomes in vitro	[9]
AD	BM	A diminished level of NF- κ B by miR-146a secreted from BM-MSCs in vitro	[113]
PD	BM	Recovery of dopaminergic neurons, and animal's behavioral functions in the staircase test in rat models by MSCs-exosomes	[116]
MS ALS	BM	Modulation of the pro-inflammatory phenotype of activated N9 microglia cells in SOD1G93A mice models by inhibition of TNF- α and IL-1 β expression by miR-467f and miR-466-enriched MSCs-exosomes	[161]
SCI	BM	Induction of neurogenesis and suppression cell apoptosis in SCI rat models by miR-126-modified MSCs-exosomes	[162]
SCI	BM	Hindrance of microglial polarization affecting TLR4/NF- κ B/PI3K/AKT signaling cascades by miR-216a-5p-enriched MSCs-exosomes in vitro	[163]
SCI	BM	Inducing the M2-type macrophages in SCI rat models by MSCs-exosomes	[164]
SCI	BM	Reduced lesion size and amended functional recovery and also cellular apoptosis and inflammation in the injured spinal cord in murine models by MSCs-exosomes	[165]
SCI	BM	Suppression of the migration of pericytes, improvement of the integrity of the blood-spinal cord barrier via NF- κ B p65 signaling in pericytes, attenuated brain cell death, reinforced neuronal survival and regeneration and boosted motor function by MSCs-exosomes	
SCI	BM	Inhibition of the apoptosis of neuron cells by inhibition of the expression of PTEN in a rat model by miR-21/miR-19b-enriched MSCs-exosomes	[166]

Micro RNA (miR), Bone marrow (BM), Wharton's jelly (WJ), Multiple sclerosis (MS), Alzheimer's diseases (AD), Parkinson's diseases (PD), Spinal cord injury (SCI), Amyotrophic lateral sclerosis (ALS), Amyloid precursor protein (APP)/Presenilin-1 (PS1), Nuclear factor kappa-light-chain-enhancer of activated B cells, (NF- κ B), Tumor necrosis factor α (TNF- α), Phosphatase and tensin homolog (PTEN)

the proliferation of PBMCs reduced in the existence of MSCs and also inhibition was more remarkable by MSC-exosomes than MSCs [114]. On the other hand, injection of exosomes derived from human periodontal ligament stem cells (hPDLSCs) caused a significant decrease in NALP3, cleaved caspase 1, IL-1 β , and IL-18 in the spinal cord of experimental autoimmune encephalomyelitis (EAE) mice model, a common MS animal model. Moreover, inhibition of TLR4/NF- κ B axis was verified in EAE mice following administration of hPDLSCs-exosomes, which sustain robust immunosuppressive potential of the injected exosomes in EAE mice [115]. Furthermore, administration of hBMSCs-Exo exhibited superiority over hBMSCs in the 6-hydroxydopamine (6-OHDA) rat PD model in terms of the functional deficit's rescue. Moreover, hBMSCs-exosomes stimulated higher rates of in vitro neuronal differentiation, providing a premise of the potential use of hBMSCs secretome for treating PD [116].

Arthritis

Investigations of MSC-exosomes therapy in musculoskeletal disorders have resulted in promising consequences in pre-clinical models (Table 3). It seems that

inhibition of inflammatory responses in concomitant with down-regulation of matrix-degrading enzymes plays a central role in this course. In this section, we discuss finding concerning the advantages of MSC-exosomes in arthritis, as the most common musculoskeletal disorder.

Osteoarthritis (OA)

Current reports have shown that the exosomes derived from MSCs could sustain chondrocyte homeostasis and improve the pathological sternness of OA in vivo, making them a potent strategy to treat OA. Investigation of the possible beneficial effects of the infrapatellar fat pad (IPFP) MSCs-derived exosomes (MSCIPFP-exosomes) on OA revealed that Exo could alleviate the OA severity by inhibition of chondrocyte cell apoptosis, improvement of matrix production, and a reduction in the presentation of a catabolic factor in vitro. Also, MSCIPFP-exosomes boosted autophagy rates in chondrocytes by negative regulation of mechanistic target of rapamycin (mTOR) because of the existence of high-levels of miR-100-5p in MSCIPFP-exosomes. In OA murine model, MSCIPFP-exosomes restored articular cartilage's damages and gait abnormality by sustaining cartilage homeostasis, which relied on miR100-5p-mediated modulation of

Table 3 A brief overview of studies based on MSCs-exosomes in musculoskeletal disorders

Condition	Sources	Main result	Refs.
OA	BM	OA rescue by suppressing inflammatory cytokines and also NLRP3 inflammasome activation by MSCs-exosomes in a rabbit model	[167]
IDD	BM	Modulation of endoplasmic reticulum stress and inhibition of excessive nucleus pulposus cell apoptosis by inducing AKT and ERK signaling in rat models by MSCs-exosomes	[168]
Osteoporosis	iPS-MSCs	Recovery of critical-sized bone defects by improved angiogenesis and osteogenesis by MSCs-exosomes in osteoporotic murine models	[169]
ONFH	iPS-MSCs	Inhibition of ONFH by exerting angiogenesis and inhibition of bone loss through the activation of PI3K/ AKT signaling pathway on endothelial cells elicited by iPS-MSCs-exosomes in ONFH mice model	[170]
OA	BM	Promotion of cartilage development and homeostasis through targeting WNT5A in mice by miR-92a-3p-enriched MSCs-exosomes	[122]
OA	ESCs-MSCs	OA rescue by regulation of the synthesis and degradation of chondrocyte ECM in mice models by ESCs-MSCs-exosomes	[118]
RA	BM	Reduction in joint destruction by inhibiting synovioyte hyperplasia and angiogenesis by MSCs-exosomes in murine models	[124]
Cartilage defect	BM	Induction of cartilage repair in a rabbit model after combination therapy with MSCs-exosomes and hyaluronic acid	[171]
OA	Synovial	Improvement of the proliferation and migration of chondrocytes by Wnt5a and Wnt5b-enriched MSCs-exosomes mediated by YAP activation	[172]
DMD	PL	Reduction in creatine kinase and TGF- β levels and also promotion in utrophin levels in mice models by MSCs-exosomes	[173]
IVD	BM	Alleviation of IVD degeneration by miR-21 enriched MSC-exosomes through inhibition of PTEN and consequently activation of PI3K/ AKT pathway	[174]
OA	BM	Alleviation of damage of the synovial fibroblasts via blocking PTGS2 elicited by miR-26a-5p enriched MSCs-exosomes in OA rat models	[175]
OA	BM	Re-induction of the expression of type II collagen, aggrecan, and also inhibition of MMP-13, ADAMTS, and iNOS along with inhibition of macrophage activation in mice models by MSCs-exosomes	[176]
OA	AT	Protection of articular cartilage and alleviation of gait abnormalities by inhibition of mTOR in OA mice models by miR-100-5p enriched MSCs-exosomes	[117]

Table 3 (continued)

Condition	Sources	Main result	Refs.
RA	BM	Suppression of fibroblast-like synoviocytes activation, migration, and invasion in vitro and recovery of arthritis and bone lesions in murine models in vivo by miR-320a-enriched MSCs-exosomes	[177]

Micro RNA (miR), Bone marrow (BM), Adipose tissue (AT), Placental (PL), Embryonic stem cells (ESCs), Induced pluripotent stem cells (iPSCs), Osteoarthritis (OA), Intervertebral disc degeneration (IDD), Osteonecrosis of the femoral head (ONFH), Rheumatoid arthritis (RA), Duchenne muscular dystrophy (DMD), Intervertebral disk (IVD) diseases, NLR family pyrin domain containing 3 (NLRP3), Extracellular signal-regulated kinase (ERK), Phosphatidylinositol-3-kinase (PI3K)/AKT, Wnt family member 5A (WNT5A), Extracellular matrix (ECM), Yes-associated protein (YAP), Transforming growth factor-beta (TGF-β), Phosphatase and tensin homolog (PTEN), Prostaglandin-endoperoxide synthase 2 (PTGS2), Matrix metalloproteinase type 13 (MMP-13), A disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5), Inducible nitric oxide synthase (iNOS), Mammalian target of rapamycin (mTOR)

the mTOR-autophagy pathway [117]. Similarly, human embryonic stem cell (ESC)-induced MSCs- exosomes alleviated OA in C57BL/6 J mice OA model by regulation of the production and degradation of chondrocyte ECM, thereby suppressing cartilage destruction [118]. In vitro, exosomes sustained the chondrocyte phenotype by promoting collagen type II synthesis concomitant with a reduction in expression of a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) [118], an ECM degrading enzyme demonstrating proteolytic functions against the hyaluronan group of chondroitin sulfate proteoglycan. The importance of the ADAMTS5 inhibitions to achieve a positive outcome in OA can be deduced by a variety of in vivo studies showing that deletion of ADAMTS5 may improve protection against proteoglycan degradation and diminish the severity of murine OA [119, 120].

Previous studies have shown that Wnt5a exists in human OA cartilage and could boost chondrocyte catabolic functions by non-canonical Wnt signaling, which proposes a possible role in OA progress by various mechanisms, such as the promotion of MMP1 and MMP13 expression [121]. Accordingly, assessment of the molecular relation between exosomal miR-92a-3p and WNT5A in OA models showed that exosomal miR-92a-3p adjusts cartilage development and homeostasis, promotes cartilage proliferation and matrix genes expression by directly binding to the 3'-UTR and inhibition of WNT5A in OA mice models. These findings suggest that miR-92a-3p enriched MSCs-Exo may perform as WNT5A inhibitor leading to OA recovery [122].

Rheumatoid arthritis (RA)

MSCs- exosomes also could exert functional recovery in RA murine models by induction of inhibitory effect on effector immune cells. For instance, MSCs-derived exosomes in collagen-induced arthritis (CIA) models inhibited T lymphocyte proliferation and also reduced the frequencies of CD4-positive and CD8-positive T cell subsets. On the other hand, MSCs-exosomes augmented Treg cell percentage, and decreased plasmablast differentiation, thus documenting the positive effects of MSCs-exosomes in RA by suppressing immune responses [123]. Besides, miR-150-5p enriched MSCs- exosomes (MSCs-Exo-150) obstructed migration and motility of RA-fibroblast-like synoviocytes (FLS) and also inhibited tube formation in human umbilical vein endothelial cells (HUVECs) through negative regulating MMP-14 and also VEGF. Likewise, in the mouse CIA model, MSCs-exosomes -150 administration decreased hind paw thickness, and concurrently reduced joint devastation through suppressing synoviocyte hyperplasia and angiogenesis [124]. Similarly, miRNA-124a-abundant

MSCs- exosomes have been suggested that could inhibit proliferation and motility of FLS cell line in vitro and improve these cells apoptosis in co-culture condition, facilitating RA recovery possibly in vivo [125].

Recently, studies have demonstrated that tumor necrosis factor- α -induced-protein 3 (TNFAIP3) gene polymorphisms (rs2230926 and rs5029937) are allied with the promoted risk of RA [126], and also revealed that TNFAIP3 expression is significantly lessened in RA patients compared with the healthy controls. Moreover, the expression rates of the TNFAIP3 gene have a negative association with the RA score, anti-cyclic citrullinated peptide (CCP) antibody, and also CRP levels [127]. Correspondingly, a study showed that long non-coding RNA heart and neural crest derivatives expressed 2-antisense RNA 1 (HAND2-AS1)-abundant MSCs- exosomes inhibited the proliferation, migration, and inflammation and also exerted the apoptosis in RA-FLS cells by the suppression of the NF- κ B pathway [128]. The central role of TNFAIP3 in the regulation of the NF- κ B axis was elucidated when Lee et al. supposed that TNFAIP3^{-/-} mice quickly expired because of the systemic inflammation elicited by spontaneously activated NF- κ B [129].

Myocardial infarction (MI)

Respecting recent studies, MSCs-exosomes are known to contribute to myocardial repair following myocardial infarction (MI), while the underlying mechanism is not entirely elucidated. Sphingosine-1-phosphate (S1P) is an important regulator of the immune-inflammatory response; thereby its role in MI has been widely confirmed. Owing to this fact, it has been found that MSCs derived from adipose tissue (AMSCs) and their exosomes may stimulate a recovery in MI by repressing cardiac dysfunction, apoptosis, and fibrosis, accompanied with inhibition of inflammatory reactions in vitro and in vivo. Despite the macrophage M2 polarization induced by these Exo, it seems that S1P/ sphingosine kinase 1 (SK1)/ sphingosine-1-phosphate receptor-1 (S1PR1) axis is responsible for AMSCs- exosomes -mediated myocardial recovery because S1PR1 silencing mitigated the suppressive influences of Exo on MI-stimulated cardiac apoptosis and fibrosis in vitro. Therefore, observations indicated that AMSCs-Exo could alleviate MI mechanistically through activating S1P/SK1/S1PR1 signaling and promoting M1- to M2-phenotype shift [130]. In this regard, another study has signified that MSCs- exosomes due to the presence of the miR-182 could decrease infarct size degree and also inflammation responses in heart and serum of MI mice models. Observations introduced miR-182 as a mediator of macrophage polarization and TLR-4 as its downstream target [131]. Other findings have shown that P53 signaling plays a fundamental role

in the development of pathological remodeling and heart failure after MI [132], while Bari et al. showed that miR-125b enriched MSCs- exosomes could suppress the expression of the pro-apoptotic genes p53 and Bcl2-antagonist/killer 1 (BAK1) in cardiomyocytes (CMC) in vitro, and also ameliorate ischemic damages in the injured heart and induce noticeable CMC activities post-MI after systemic injection into MI mice models [133]. Also, Exo derived from genetically modified MSCs to overexpress AKT resulted in alleviated cardiac function in the MI models five weeks after injection. Moreover, AKT-MSCs- exosomes considerably enhanced endothelial cell growth and motility, boosted the creation of tube-like construction in vitro, and also improved blood vessel development in vivo possibly mediated by induction of platelet-derived growth factor D (PDGF-D) expression and activation, highlighting the prominent functions of PDGF-D in AKT-MSCs- exosomes -induced angiogenesis in MI models [134].

Besides, Exo derived from macrophage migration inhibitory factor (MIF)-overexpressed MSCs restored heart function, decreased heart remodeling, supported mitochondrial functions, and suppressed ROS generation in MI murine models [135]. These findings provide a potent method for MI treatment concerning cardioprotective activities of MIF throughout ischemia, as evidenced by elevated infarct size in MIF-/- deficient mice compared with control groups [136].

Wound healing

Chronic wounds are mutual and endure to be the one cause of morbidity and mortality, while therapeutic options for these conditions are missing and often unsuccessful. Collecting proofs have showed the efficacy of MSCs- exosomes to repair and regenerate the damaged area, and thereby sustain cutaneous wound healing. MSCs- exosomes activates numerous axis contributing to wound healing, such as AKT, ERK, and STAT3, and also trigger the expression of hepatocyte growth factor (HGF), IGF1, nerve growth factor (NGF), and stromal-derived growth factor-1 (SDF1), providing an opportunity for wound healing cell-free based therapies [137]. Also, promotion of macrophage M2 polarization resulting from direct targeting of *pknox1*, a critical regulator for macrophage polarization, by exosomal miR-223 was found that supports cutaneous wound healing stimulated by MSCs- exosomes in vivo [138]. As well, in vivo study in streptozotocin-induced diabetic rats revealed that exosomes derived from BMMSCs-preconditioned with deferroxamine, an angiogenic stimulator, could induce the PI3K/AKT axis by miR-126 mediated phosphatase and tensin homolog (PTEN) suppression leading to the induction of angiogenesis in treated rats [139]. Also,

other studies showed that regardless of the improvement of M2 polarization and activation of PI3K/AKT pathways, MSCs- exosomes induced wound healing process in diabetic models by suppression of the pro-inflammatory factors IL-1 β and TNF- α and inducible nitric oxide synthase (iNOS), and conversely through promoting the anti-inflammatory factor IL-10 and arginase-1 (Arg-1) [140]. The observed effect was mediated by the increased M2 polarization through up-regulation of the expression of PTEN and inhibiting the phosphorylation of AKT [140]. Importantly, melatonin-induced MSC- exosomes considerably sponsored the healing of diabetic wounds via constraining immune cell activation, thus further enabling angiogenesis and collagen production in vivo [140]. Likewise, atorvastatin-induced BMMSCs- exosomes could exhibit restored pro-angiogenic competencies in diabetic wound healing achieved via increasing the biological activities of endothelial cells via AKT/ endothelial nitric oxide synthase (eNOS) pathway following up-regulating the miR-221-3p [141]. In the AKT/eNOS axis, AKT mediates eNOS phosphorylation at Ser1177, leading to an elevation in eNOS activity. Then, activated eNOS induces NO production and supports NO-mediated VEGF activation and also endothelial cell proliferation and migration, which in turn, facilitates tissue repair [142]. Likely, UCMSCs- exosomes treated wounds displayed augmented re-epithelialization, with improved cyokeratin 19 (CK19), proliferating cell nuclear antigen (PCNA), and collagen I expression in rat skin burn model in addition to the promotion of proliferation and inhibition of apoptosis of skin cells upon heat-stress in vitro. Observations revealed that induction of Wnt/ β -catenin by injected exosomes largely contributed to wound re-epithelialization and cell proliferation, while knockdown of *Wnt4* in UCMSCs- exosomes suppressed β -catenin activation and skin cell proliferation and motility in vitro. Consistently, AKT activation by UCMSCs-Exo was showed that participated in the decrease of heat stress-stimulated apoptosis in skin cell [143].

Conclusion and future prospect

MSCs- exosomes are now being broadly applied to advance innovative regenerative approaches for several disorders as they serve most of the therapeutic attributes of MSCs. Exosomes provide a chance for cell-free therapy, which diminishes safety issues concerning the use of viable cells [144]. During last years, preclinical data have proven the beneficial effects of the MSCs- exosomes in various disorders mediated mainly by inhibition of inflammatory responses, targeting of immune cell's pivotal signaling axis, inhibition of PTEN activation, suppression of expression of MMPs, promotion of generation of pro-angiogenic factors, and also inhibition of

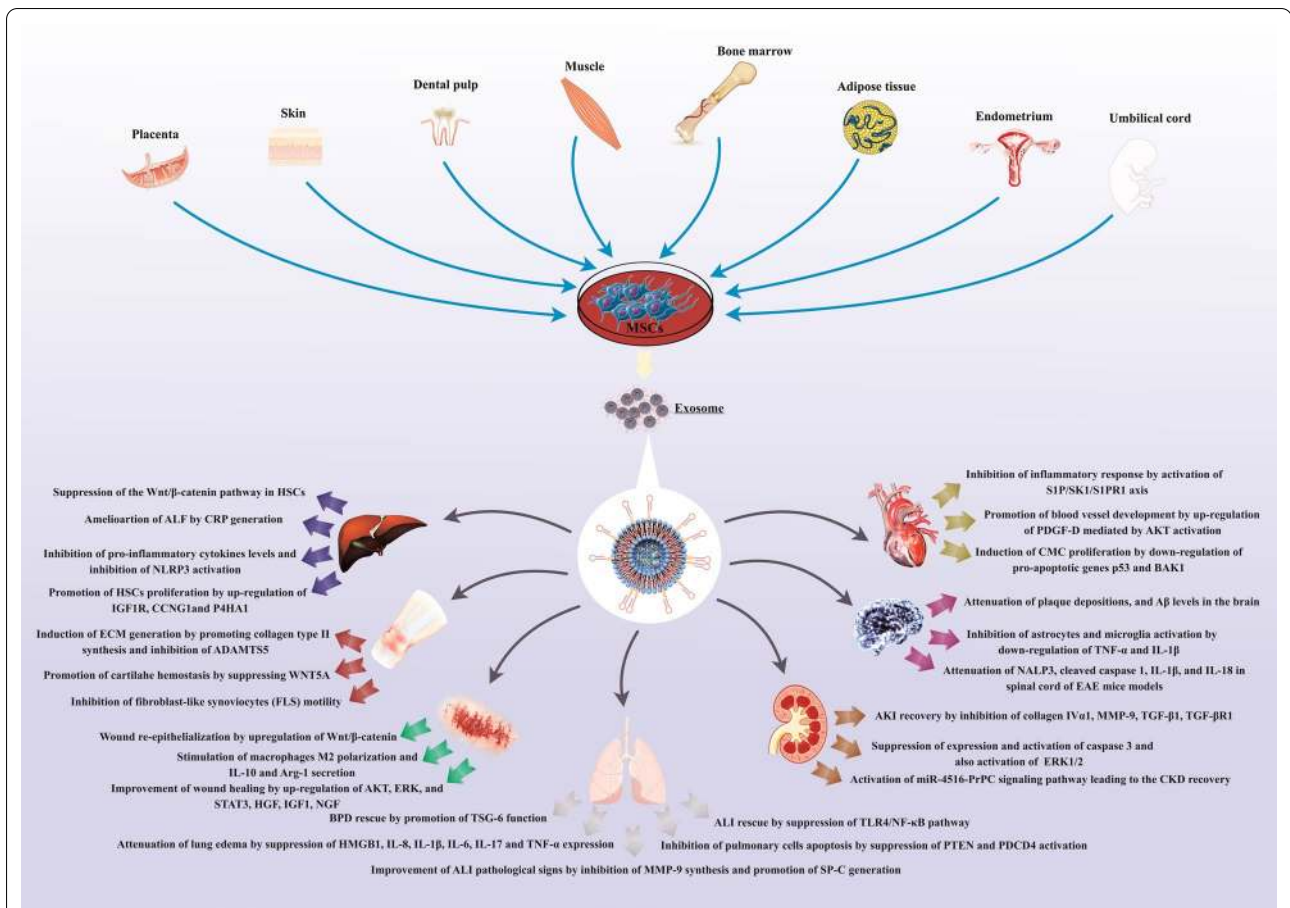


Fig. 2 Application of MSCs-exosomes in regenerative medicine. In vivo reports have evidenced the valuable belongings of the MSCs-exosomes, following separation from the parental cell, for treating several disorders, such as lung, kidney, liver, neurodegenerative, cardiac, musculoskeletal diseases, and cutaneous wounds. Inhibition of secretion of pro-inflammatory cytokines and pro-fibrotic molecules, inhibition of survival and proliferation-related pathways in immune cells, and also improvement of the secretion of pro-angiogenic factors may be responsible for MSCs-exosomes-elicited desired effects. Micro RNA (miR), Acute liver failure (ALF), C-reactive protein (CRP), Hepatic stellate cells (HSCs), NLR family pyrin domain containing 3 (NLRP3), Insulin-like growth factor receptor 1 (IGF1R), Cyclin G (1) (CCNG1), Prolyl-4-hydroxylase α1 (P4HA1), A disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTSS), Extracellular matrix (ECM), Extracellular signal-regulated kinase (ERK), Hepatocyte growth factor (HGF), Nerve growth factor (NGF), Signal transducer and activator of transcription 3 (STAT3), Arginase 1 (Arg-1), Bronchopulmonary dysplasia (BPD), High mobility group box protein 1 (HMGB1), Tumour Necrosis Factor alpha (TNF-α), Phosphatase and tensin homolog (PTEN), Acute lung injury (ALI), Programmed cell death 4 (PDCD4), Toll-like receptor 4/nuclear factor kappa-light-chain-enhancer of activated B cells (TLR4/NF-κB), Chronic kidney disease (CKD), Cellular prion protein (PrPC), Matrix metalloproteinases (MMPs), Transforming growth factor beta (TGF-β), NACHT, LRR and PYD domains containing protein (NALP3), Experimental autoimmune encephalomyelitis (EAE) mice, Cardiomyocytes (CMC), BCL2-antagonist-killer 1 protein (BAK1), Platelet-derived growth factors (PDGFs), Sphingosine-1-phosphate (S1P), Sphingosine kinase 1 (SK1), Sphingosine-1-phosphate receptor 1 (S1PR1), Interleukin (IL)

pro-fibrotic molecules expression and activation (Fig. 2). It seems that exosomal miRNAs, in particular, miR-21-5p, miR-425, miR-30b-3p, miR-21, miR-146a, miR-466, miR-126, miR-216a-5p, miR-19b and miR-26a-5p, are responsible for MSCs-Exo elicited desired outcome in vivo (Tables 1, 2 and 3). Also, several clinical trials have been conducted or are ongoing (Table 4); however, the use of MSC-Exo in the clinic is restricted because of lacking established cell culture conditions and generally accepted procedures for manufacture, separation, and

storage of exosomes, optimum therapeutic dose, and injection schedule, and dependable potency analyzes to address the efficacy of Exo therapy. Although, the MSCs have been approved as the first “off-the-shelf” stem cell pharmaceutical drug, there is no reliable study supporting that the effective EVs yields can be obtained from “off-the shelf” MSCs [145]. In sum, though the examination on the MSC- exosomes applications still faces various challenges, the benefits and capability of MSC- exosomes are appealing growing attention. On the other hand, to

Table 4 Clinical trials based on MSCs-exosomes registered in ClinicalTrials.gov (May 2021)

Condition	Source	Study Phase	Participant Number	Route	Study Location	NCT Number
ARDS	BM	1/2	169	Inhalation	China	NCT04602104
Lung diseases	N.A	1	27	Inhalation	China	NCT04313647
COVID-19	N.A	1/2	30	Inhalation	Russian	NCT04491240
BPD	BM	1	18	Intravenous	USA	NCT03857841
COVID-19	BM	1	24	Inhalation	China	NCT04276987
MODS	UC	N.A	60	Intravenous	China	NCT04356300
Drug-Resistant	AT	1/2	60	Intravenous	China	NCT04544215
Chronic wounds	WJ	1	38	Topical	Indonesia	NCT04134676
Ischemic stroke	N.A	1/2	5	Intravenous	Iran	NCT03384433
Dry eye	UC	1/2	27	Eye Drops	China	NCT04213248
Macular holes	UC	Early 1	44	Eye Drops	China	NCT03437759
AD	AT	1/2	9	Nasal Drops	China	NCT04388982
EB	BM	1/2	10	Topical	NA	NCT04173650

Bone marrow (BM), Adipose tissue (AT), Wharton's jelly (WJ), Umbilical cord (UC), Coronavirus disease 2019 (COVID-19), Bronchopulmonary dysplasia (BPD), Alzheimer's diseases (AD), Acute respiratory distress syndrome (ARDS), Epidermolysis bullosa (EB), Multiple organ dysfunction syndromes (MODS), Not available (N.A)

promote our comprehension of the possibly ambiguous influences of MSC- exosomes, a better comprehension of their targeting and bio-distribution is of paramount importance. In spite of the development of EVs studies, only a few reports have evaluated EVs biodistribution in animal models for EVs trafficking. For broader utility of EVs, addressing the fate of EV in vivo is of paramount significance. Evaluation of EV's biodistribution in mice upon administration by intravenous route signified that although administered EVs largely collected mainly in liver, spleen, gastrointestinal tract and lungs, alterations based on the EV cell origin were documented. As well, it seems that delivery route and dose of infused EVs affect the biodistribution pattern [146]. Moreover, study of the biodistribution and kidney localization of EVs in AKI showed that the labeled EVs gathered specially in the kidneys of AKI mice model, and were measurable in whole body images and also in dissected kidneys [147].

Abbreviations

MSCs: Mesenchymal stem/stromal cells; Exo: Exosomes; EVs: Extracellular vesicles; BM: Bone marrow; UC: Umbilical cord; OA: Osteoarthritis; miRNAs: micro-RNAs; ALI: Acute lung injury; NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; TNF- α : Tumor necrosis factor alpha; PTEN: Phosphatase and tensin homolog; MMPs: Matrix metalloproteinases; ILs: Interleukins; NLRP3: NLR family pyrin domain containing 3; ERK: Extracellular signal-regulated kinase; PI3K: Phosphatidylinositol-3-kinase.

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Authors' contributions

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