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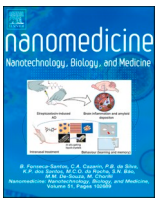

Mesenchymal stem cell-derived exosomes as cell free nanotherapeutics and nanocarriers

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ABSTRACT

Many strategies for regenerating the damaged tissues or degenerating cells are employed in regenerative medicine. Stem cell technology is a modern strategy of the recent approaches, particularly the use of mesenchymal stem cells (MCSs). The ability of MSCs to differentiate as well as their characteristic behaviour as paracrine effector has established them as key elements in tissue repair. Recently, extracellular vesicles (EVs) shed by MSCs have emerged as a promising cell free therapy. This comprehensive review encompasses MSCs-derived exosomes and their therapeutic potential as nanotherapeutics. We also discuss their potency as drug delivery nano-carriers in comparison with liposomes. A better knowledge of EVs behaviour in vivo and of their mechanism of action are key to determine parameters of an optimal formulation in pilot studies and to establish industrial processes.

Mesenchymal stem cells

MSCs are multipotent adult stem cells of great interest in tissue engineering and regenerative medicine owing to their differentiation potential. Their capacity to differentiate mainly into chondrocytes, osteoblasts, and adipocytes[3] make them distinctive along with self-renewal properties. They express surface markers including CD 29, CD73, CD90, CD105, without the expression profile of HLA- II, CD11b, CD14, CD19, CD34 and CD45.[4] Morphologically, MSCs appear like fibroblasts[5] and easily adhere to plastic substrates[6] with simple ex vivo expansion conditions.[7] Initially discovered in the bone marrow,[9] MSC have been isolated from a variety of tissues, such as dental pulp, adipose tissue, amniotic fluid, placenta, Wharton's jelly, umbilical cord blood, liver, spleen, kidney, thymus, lung, and pancreas.[10]

Therapeutical Applications in the regeneration of damaged tissues span a variety of chronic diseases such as cardiovascular, musculoskeletal, renal, hepatic, autoimmune disorders like systemic lupus erythematosus, rheumatoid arthritis (RA), and diabetes type 1, neurodegenerative diseases such as Parkinson's, multiple sclerosis, vitiligo, and Alzheimer's.[11] MSCs can migrate to inflammation sites and damaged tissues, producing their immunomodulatory and trophic effects through secretion of cytokines, chemokines, growth factors including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β) and epithelial growth factor (EGF).[8] Therapeutic effects would rely on the ability of MSC to migrate to damaged tissues, engraft, and differentiate and replace the damaged cell for tissue regeneration. However, investigators also underline that the therapeutic efficacy of MSCs also relies on their secretion of paracrine mediators.[1,12,13] Indeed, MSCs-secreted molecules operate as efficient mediators, either directly by activating the target cells or indirectly by stimulating by-standers for the secretion of trophic factors Aside from truly soluble mediators, Extracellular vesicles secreted by MSC constitute another storage pool of mediators that cargo active molecules in body fluids.[14] However, it has recently been discovered that MSCs generate many extracellular vesicles (EVs) that contribute to tissue regeneration by communicating with damaged tissue or cells and exerting therapeutic activity similar to the MSCs.[2,14,15]

Extracellular vesicles

Extracellular vesicles (EVs) are membrane-enveloped vesicles that are bio-generated by different cell types, such as B cells, platelets, T cells, mast cells, dendritic cells, endothelial cells, Schwann cells,

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epithelial cells, neuronal cells, cancerous cells, embryonic cells, oligodendrocytes, and MSCs. EVs have been found in different physiological fluids including blood, saliva, breast milk, cerebrospinal fluid, breast milk, amniotic fluid, normal urine, semen, bronchial lavage fluid, bile, synovial fluid, and ascites fluid.[10] The different terms have been coined for the sub-populations of EVs including micro-particles, ectosomes, apoptotic bodies, oncosomes, microvesicles, exosomes, prostasomes, and membrane particles.[16] Microvesicles (MVs) and exosomes are the two most significant EVs. Since years, paracrine and autocrine secreted mediators have been evidenced in intercellular communication in addition to the cell-cell contact. The last two decades, EVs have emerged as new actors in intercellular communication in several physiopathological processes involving homeostasis, inflammation, immune responses, hemostasis, angiogenesis and cancer pathogenesis.[17]

Main types of EVs

Exosomes

Exosomes are the most significant sub-category of EVs released by different types of cells. These membrane vesicles are nanosized (30–120 nm) with an endosomal origin. The development of early endosomes occurs through invagination of the plasma membrane due to the absorption of intracellular fluid. This early endosome maturation and expansion results in the late endosome formation; then the internal budding of the endosomal membrane generates multivesicular bodies (MVBs) containing intraluminal vesicles (ILV). The MVBs are then released into the extracellular micro-environment after fusing to the cell membrane. The vesicles are now known as exosomes[18,19] (Fig. 1A). In a sucrose gradient, exosomes exhibit a density between 1.13 and 1.19 g/mL. They can be separated by centrifugation at 100,000*g* and kept functionally intact for more than 6 months when stored at −80 °C without the use of hazardous cryoprotectants.[20] Their membranes are rich in sphingomyelin, ceramide and cholesterol collectively known as lipid rafts.[21] Exosomes are also abundant in annexins and tetraspanins like CD9, CD63, and CD81. They also contain heat shock proteins such as Hsp60, Hsp70, and Hsp90. Most exosomes also express evolutionary set of proteins including clathrin, Alix, and tumor susceptibility gene 101 (Tsg101)[22] (Table 1). They are also immuno compatible due to their cell-based origin and immunomodulatory properties. In addition, exosomes are protected by a bilayer membrane that protects their contents and permit them to travel far inside tissues. The outer face of membrane contains considerable amounts of phosphatidylserine. Exosomes carry a cargo of genetic information through pre-miRNA, miRNA, mRNA and noncoding RNA.[23] These genetic materials and proteins interact with the recipient cells by three distinct mechanisms including endocytic uptake, direct fusion with plasma membrane, and ligand binding to the receptor[24] (Fig. 1A). These interactions suggest that exosomes play crucial roles in immunological regulation and cell-to-cell communication under various physiological and pathological conditions.[25] Currently, there are several different used methods for isolation and characterization of exosomes[20,26,109] (Fig. 1B).

Microvesicles

Microvesicles also known as shedding vesicles and are generated by budding of the plasma cell membrane (Fig. 1A). This process is dependent on the raise in intracellular calcium following cell stimulation, calpain activity and cytoskeleton reorganization. These vesicles have a 100–1000 nm diameter and can be separated by centrifugation having a density of 1.04–1.07 g/mL. MVs are enriched with surface markers, ceramide, sphingomyelin, phosphatidylserine, selectins and integrins, depending on the cell origin (Table 1). Their cargo includes lipids, proteins, microRNAs and mRNAs. MVs interact directly with target cells through ligand-receptor interactions.[23] They are irregular in shape and can change the functionality of target cells by transferring proteins. For example, microvesicles released by endothelial cells can transfer proangiogenic molecules promoting angiogenesis.[27]

Therapeutic effects of EVs derived from MSCs

In recent years, the paracrine effects of MSCs have attracted the focus of researchers for their regenerative potential. In contrast to cell-based therapies, cell-free strategies circumvent the risks and the difficulties caused by MSC engraftment and differentiation post-transplantation, among which, tumorigenicity, uncontrolled proliferation or differentiation.[28] Exosome-based therapies would also cargo small amounts membrane-bound MHC molecules.[29] The therapeutic potential of MSC-EVs was first explored in a mouse model for myocardial ischemia.[30] Since then, they have been studied in many diseases. MSC-derived exosomes are the most studied of MSC's EVs, we summarize the therapeutic potential of MSC-exosomes in cardiovascular, kidney, liver, neurological and degenerative diseases.

Therapeutic potential of MSC-derived exosomes in cardiovascular diseases

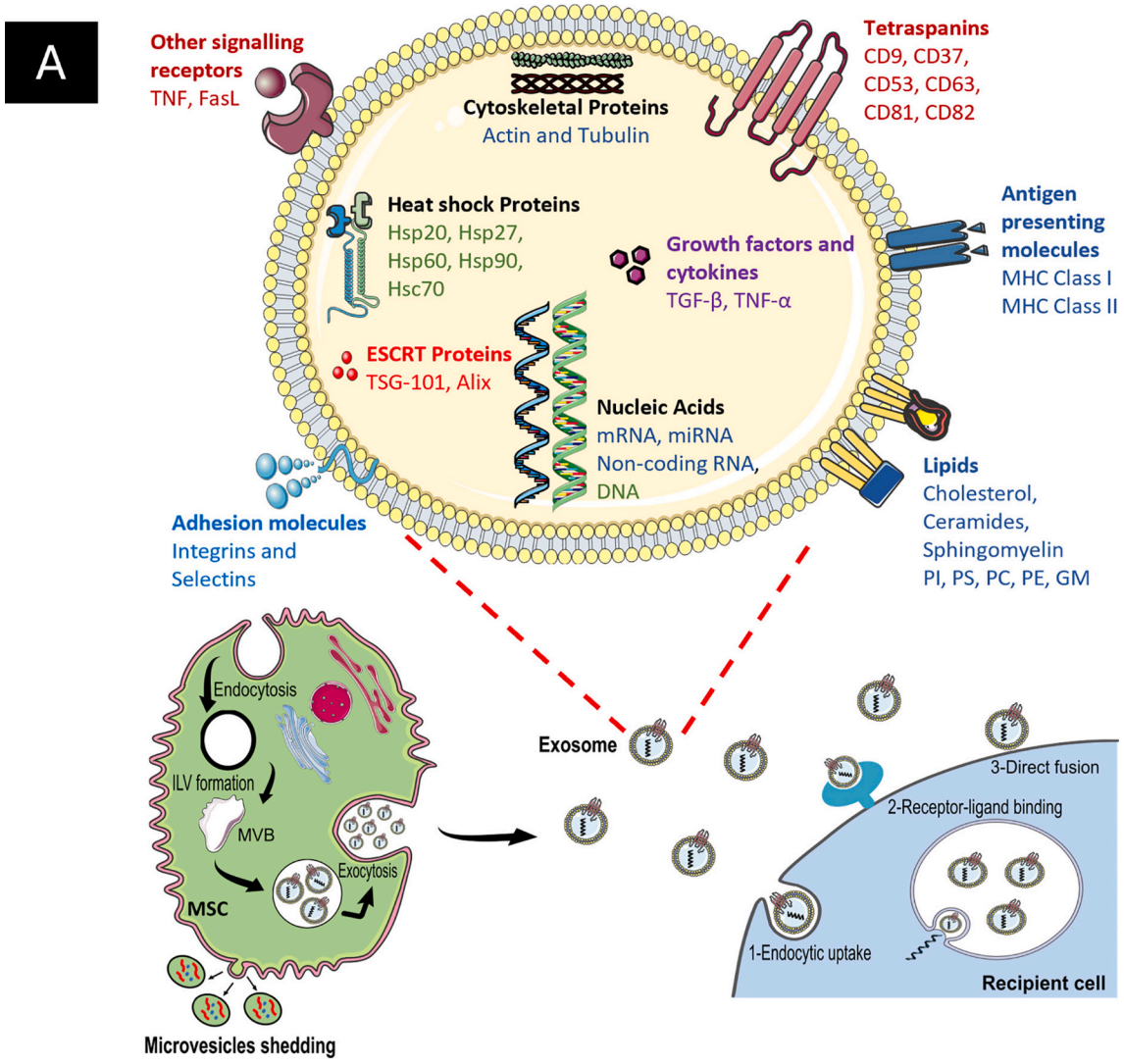
MSC-exosomes are key factors for cardioprotection by reducing cardiomyocyte apoptosis and promoting angiogenesis. In a mouse heart model of ischemia/reperfusion injury, Lai et al.[30] explored that MSC-exosomes decreased infarct size, resulting in cardioprotection. Exosomes from (HIF-1)-modified MSCs have also been shown to promote neoangiogenesis by upregulating proangiogenic factors that mediate cardioprotection.[31] Exosomes obtained from UCBMSCs, BMMSCs and ADMSCs promoted angiogenesis by upregulation of bFGF, HGF and VEGF. Furthermore, three kinds of MSC-based exosomes increased microvascular density MVD, decreased infarction area and inhibited apoptosis in cardiomyocytes. However, ADMSCs exosomes upregulated most of the cardioprotection factors.[32]

MicroRNAs derived from MSC-based exosomes play vital role in cardioprotection. For instance, Kristin et al.[33] confirmed that, in a mice model, miR-21a-5 (sub-type of miRNA) is a major paracrine factor that mediates cardioprotection after transferring into the myocardium. Their results also showed that increase of miR-21a-5 in cardiomyocytes, downregulated the pro-apoptotic gene products PTEN, PDCD4, FasL and Peli 1 in the myocardium.

The therapeutic efficacy of exosomes can be enhanced by drug pretreatment of MSCs. Exosomes isolated from MSCs pretreated with atorvastatin, a drug that reduces the cholesterolemia through the inhibition of the HMG-CoA reductase, mediate cardioprotection in a model of acute cardiac infarction, angiogenesis and improved endothelial cell (EC) function via the MSC upregulation of LncRNA H19 (long noncoding RNA H19), its secretion in exosomes and delivery to the EC. The results also showed approximately 8 % reduction of infarcted area by the treatment group (MSC-atorvastatin-exosome) compared to control group (MSC-exosome).[34] MSC-exosomes have therapeutic efficacy by limiting pulmonary vascular remodeling. In a rat study, exosomes could significantly reduce the right ventricular hypertrophy (RVH) and inhibit apoptosis induced by hypoxia in pulmonary arterial endothelial cells. The mechanism involved the up regulation of Wnt5a expression.[35]

The anti-inflammatory and anti-fibrotic effects of MSC-exosomes are also well established. Intramyocardial transplantation of MSC-derived exosomes reduced inflammation[36] and inhibited cardiac fibrosis after ischemia reperfusion injury.[37] Previous studies also investigated that intravenous administration of exosomes attenuated inflammation[38] and reduced fibrosis.[39] Sun et al.[40] studied that MSC-exosomes changed macrophages' pro-inflammatory effects into anti-inflammatory effects through JAK2-STAT6 pathway.

MSC-exosomes are also cardioprotective via their antioxidant and anti-apoptotic properties. They may decrease oxidative stress and apoptosis of cardiomyocytes in the microenvironment of infarcted area. A recent study showed that treatment group (MIF-BM-MSC-exo) was associated with 33 % less generation of reactive oxygen species (ROS)



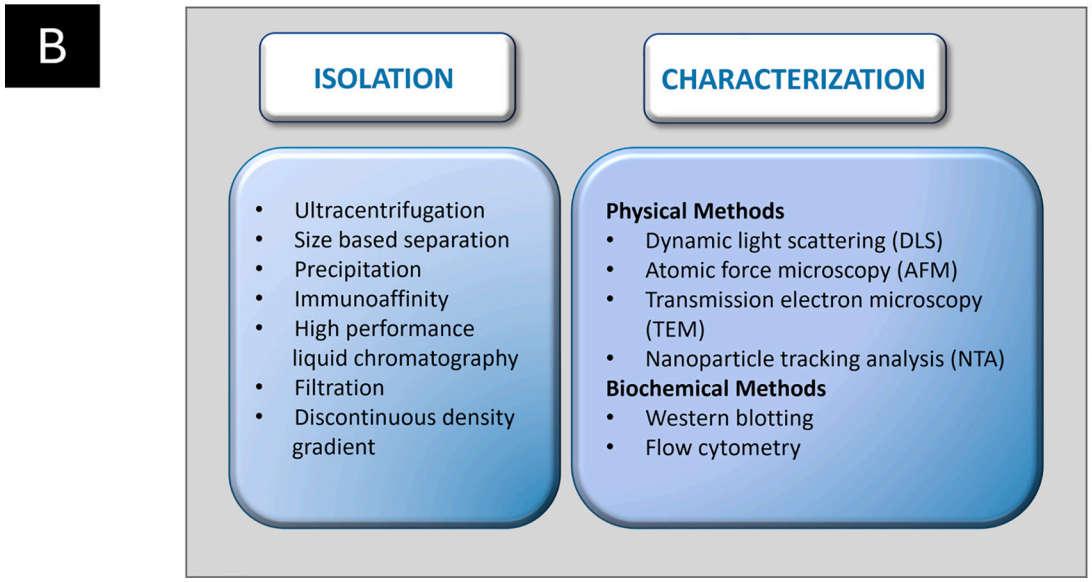


Fig. 1. (A) Biogenesis and secretion of two main subclasses of extracellular vesicles (exosomes and MVs) from MSCs. Exosomes are biogenerated from MVBs, while MVs are shed from the plasma membrane. Exosomes interact with recipient cells through endocytosis, receptor-ligand binding and direct fusion pathways. MVs, Microvesicles; MSCs, Mesenchymal Stem Cells; MVBs, Multivesicular Bodies; ILV, Intraluminal Vesicles. Created with inkscape. (B) Methods of exosome isolation and characterization.[20,26,109]

Table 1
Characteristics of exosomes and microvesicles.

	Exosomes	Microvesicles	Ref.
Origin	Endosomal origin	Plasma membrane budding	18,19
Size (nm)	30–120	100–1000	18,19
Density	1.13–1.19 g/mL	1.04–1.07 g/mL	20
Isolation techniques	Ultracentrifugation at 100,000 G, microfluidics, gelfiltration	Ultracentrifugation at 10,000–20,000 G 60–90 min Magnetic beads	20
Composition	Proteins, lipids, nucleic acids, and metabolites	Proteins, lipids, nucleic acids, and metabolites	19,23
Major components	Tetraspanins (CD9, CD63, CD81), ESCRT proteins (Tsg 101, Alix, clathrin) Heat shock proteins (Hsp60, Hsp70, Hsp90)	Plasma membrane CDs Phosphetidylserine, selectins, Integrins, Enzymes Receptors caspases	21,22
Function	Immunomodulation (NK cells, beta cells, T-cells etc), Cell-cell communication	Cell-cell communication	25,27

and 12 % reduction in infarction compared to control group. There was also improvement in left ventricular ejection fraction (LVEF) up to 10 % in a rat model of MI treated with MIF-BM-MSC-exo along with reduced apoptosis and decreased cardiomyocyte mitochondrial fragmentation after cardiac infarction.[41] In a similar study, exosomes encapsulated in alginate (2 %) with 0.5 % calcium chloride released 100 % exosomes while alginate (2 %) with 2 % calcium chloride released 60 % exosomes within 10 days. These released exosomes ameliorated cardiac apoptosis, and promoted angiogenesis.[42] In a myocardial infarction (MI) rat model, exosomes alleviated oxidative stress and extracellular matrix remodeling possibly via Akt/Sfrp2 signaling pathway.[43] Many studies investigated those miRNAs including miR-22,[44] miR-214,[39] miR-19a,[45] miR-199a[46] play roles in exosome-triggered anti-apoptotic reparative effects. In another study of rodents, miR-210 mediated cardiac regeneration and reduced apoptosis via increased expression of Bcl-2 and β-catenin while suppressing the expression of caspase-3, p16 and adenomatous polyposis coli (APC).[47]

In summary, MSC-exosomes offer cardioprotection through multiple pathways involved in fibrosis, inflammation, apoptosis, and remodeling and thereby reduce the infarct size, (Fig. 2). Thus, exosomes derived from MSCs could be an alternative to cardioprotective drugs and cell-based therapies for cardiovascular diseases. However, extensive research is required for translational application. Continuous attempts are also needed to evaluate and compare the various routes of administration, their biodistribution and pharmacokinetics, their optimal dose in different acute and chronic cardiovascular pathologies.

Therapeutic potential of MSC-derived exosomes in kidney diseases

Renal fibrosis (RF), especially tubulointerstitial fibrosis, is the outcome of progressive chronic kidney disease (CKD). The deposition of extracellular matrix (ECM) within the walls of glomerular capillaries and in the interstitium is associated with fibroblast activation, expansion of the peritubular microvasculature and tubular cell necrosis resulting in

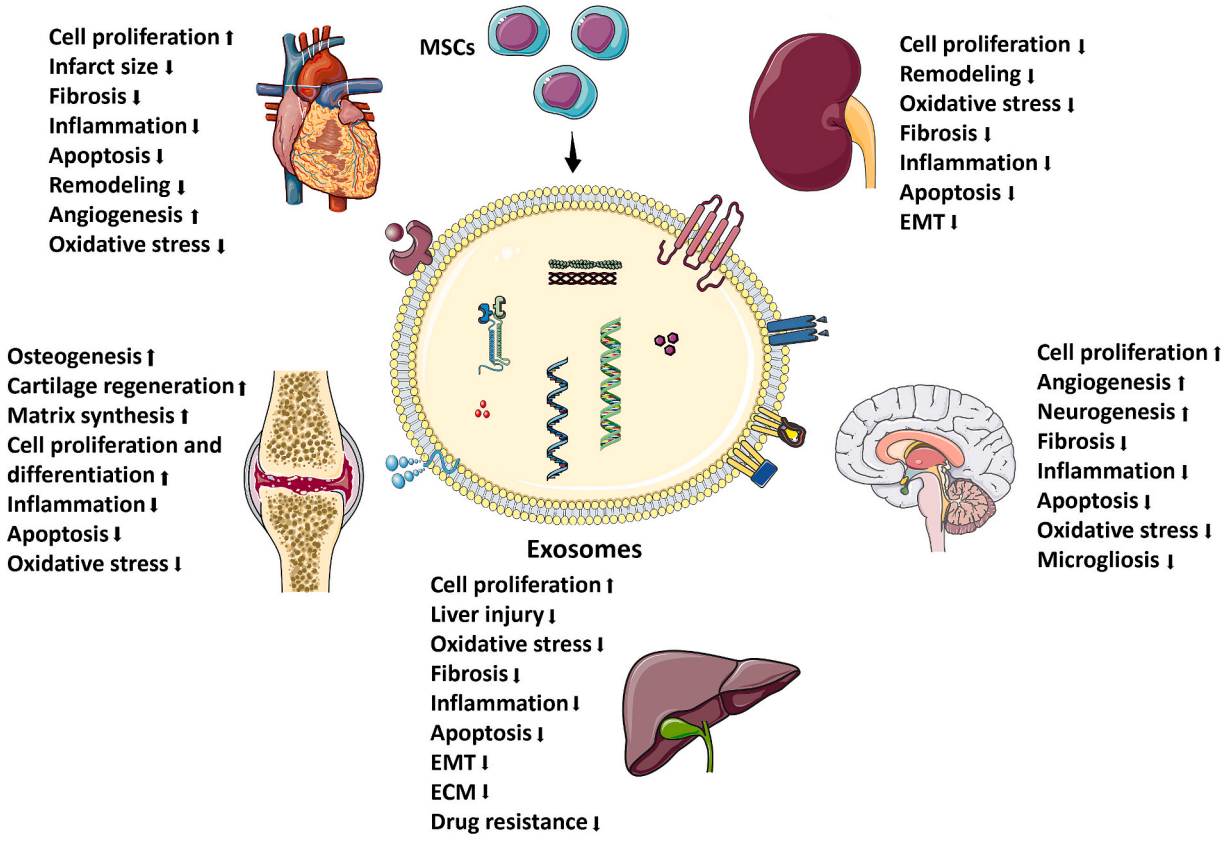


Fig. 2. Therapeutic effect of mesenchymal stem cells derived exosomes in different disease models. EMT, Epithelial Mesenchymal Transition; ECM, Extracellular Matrix. Portions of the figure utilized images from Servier Medical Art, licenced under Creative Common Attribution 4.0.

nephron demise.[48] Several studies have investigated the anti-fibrotic therapeutic potential of MSC derived exosomes. Recently, in vivo, and in vitro study showed that MSC-exosomes can protect against renal fibrosis by upregulating the expression of SIRT6 and downregulating the expression of β-catenin.[49] Epithelial mesenchymal transition type-2 is a process where epithelial cell changes their phenotype to myofibroblasts with significant pro-inflammatory and profibrotic characteristics resulting in tissue damage and fibrotic events.[50] BMMSC-exosomes may prevent this epithelial mesenchymal transition (EMT) of renal tubular epithelial cells by upregulating and transporting Nedd4L.[51]

Another mechanism of renal fibrosis is oxidative stress due to high generation of reactive oxygen species (ROS).[52] Over-production of ROS suppress the antioxidant capacity of enzymes resulting in cell lysis via DNA fragmentation, protein damage and lipid peroxidation. Consequently, ROS can accelerate renal interstitial fibrosis by the infiltration of macrophages and monocytes.[53] UCMSC-exosomes mitigated renal interstitial fibrosis and restored kidney function by inhibition of ROS, apoptosis (2.1 %), and ROS-mediated P38MAPK/ERK signaling pathways.[54] Several other studies have also evaluated the anti-fibrotic effects of exosomes by different mechanisms including the downregulation of collagen-1 and TGF-β and upregulation of IL-10,[55] decreased α-SMA and PDGFR-β,[56] increased expression of E-cadherin and decreased expression of α-SMA and collagen-1.[57]

Hypertension is also one of the main causes of CKD because of the harmful effects of increased blood pressure (BP) on the kidney. Persistent hypertension alters the systemic and renal micro and macrovasculature, impairing renal autoregulation, raising glomerular capillary pressure, and inflicting tubular damage from hyperfiltration.[58] Hyperfiltration leads to glomerular proteinuria, which encourages the release of growth factors and inflammatory cytokines.[59] Additionally, chronic hypertension causes vascular stretching, endothelial dysfunction, and subsequent activation of renin-angiotensin system (RAS), which amplifies the release of growth factors and cytokines, enhances the production of ECM, and ultimately results in progressive glomerular and interstitial fibrosis.[60] According to Lindoso et al., AMSC-exosomes provided renal protection against hypertension-mediated kidney damage by the downregulation of the pro-inflammatory markers like plasminogen activating inhibitor-1 (PAI1) and monocyte chemoattracting protein-1 (MCP-1) and reduced infiltration of macrophages in the kidney. Moreover, the administration of exosomes significantly altered the miRNA-200-TGF-β axis resulting in reprogramming of EMT signaling pathway and preventing renal fibrosis (100 % reduction in collagen deposition) and inflammation.[61] Recently, Feng et al.[62] explored that exosome from iPS-MSCs inhibited structural remodeling in old mice kidney. They also investigated that administration of exosomes could attenuate upregulation of MMP2 and MMP9 levels resulting in inhibition of collagen deposition in glomeruli and mitigation of tubulointerstitial nephritis.

To conclude, MSC-exosomes provide renal protection through different pathways including prevention of EMT, reduction in remodeling, oxidative stress, fibrosis, inflammation, and apoptosis (Fig. 2). However, additional in vivo studies are required to better understand the role of exosomes in other kidney diseases like renal cancer and acute tubular necrosis (ATN). It may also be interesting to explore if renal protective drugs (angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB) and some loop diuretics) can enhance the therapeutic efficacy of MSC-derived exosomes in acute and chronic renal pathologies.

Therapeutic potential of MSC-derived exosomes in osteoarthritis

Osteoarthritis (OA) is one of the degenerative joint diseases that affects almost all tissues in a joint including the cartilage, subchondral bone, meniscus, and ligament. The pathogenesis of OA is complicated and involves many factors, including senescence, biomechanics, inflammation, and metabolism.[63] Recently, exosomes isolated from mesenchymal stem cell, have attracted much attention of researchers in OA therapy. MSC-derived exosomes have been employed in basic and clinical research to investigate their cartilage repair therapeutic potential for osteoarthritis.[64] The exosomes carry a cargo of miRNAs that are capable of mediating cell-cell communication including cartilage formation and gene regulation. For instance, a study revealed that miR-29a and miR-29b targeted directly the 3′UTR of col2α1, that encodes type II collagen regulated by SOX9 protein.[65] Additionally, miRNAs in exosomes can inhibit arthritic inflammation limiting OA progression. Studies showed that miR-129-5p in exosomes isolated from human synovial MSCs (HSMSCs) could relieve OA by the downregulation of COX2, MMP13, iNOS and NF-κB and simultaneously inhibition of HMGB1 release.[66] miR-140-5p, miR-210 and miR-135b are some of the other exosomal miRNAs associated to chondrocyte proliferation and cartilage regeneration.[67–69]

Long noncoding RNAs (Lnc-RNAs) are similar to mRNA having more than 200 nucleotides and considered as significant component of mammalian transcriptome. They play integral roles in cell growth, differentiation, and apoptosis.[70] These Lnc-RNAs can be employed as potential biomarkers and therapeutic targets for a variety of disorders.[71] It has been demonstrated that exosomal Lnc-RNAs including Lnc-RNA MALAT1,[72] Lnc-RNA MEG3[72] and Lnc-RNA H19[73] have been investigated for their significant roles to mitigate bone and joint disorders. The BMMSC-derived exosomal Lnc-RNA MALAT1 also increases activities of osteoblast.[74]

Exosomal proteins have also been extensively investigated due to their vital roles. A comprehensive proteomic analysis of MSC-derived exosomes revealed a total of 1927 proteins.[75] Some of these exosomal proteins like CD9, CD63 and TSG101 can stimulate the migration and proliferation of chondrocytes thus attenuating OA.[76]

Biomaterials are useful auxiliary tools employed for retention and controlled release of MSC-derived exosomes.[77] For example, Yang et al.[78] used Diels-Alder cross-linked hyaluronic acid/PEG hydrogel to control the sustained release of MSC-derived exosomes with the help of hyaluronidase enzyme. The cumulative release rates were 33.44 %, 56.06 % and 78.44 % respectively in 0.1 μg/mL, 1 μg/mL and 10 μg/mL hyaluronidase treated groups. Their results of in vivo study also showed the improvement in therapeutic efficacy and bioavailability of exosomes for OA treatment. The MSC-derived exosomes also revealed enhanced therapeutic effects by extending their retention time and enhanced stability when encapsulated in injectable chitosan hydrogel.[79] Other studies also explored that exosome exerted a longer reparative effect for bone defects when embedded in Hydroxyapatite-embedded hyaluronic acid-Alginate hydrogel[80] and PLGA based scaffolds (MSCs migrated 33 % more in presence of exosomes).[81] Previous researches indicated that scaffolds modified by MSC-derived exosomes could increase osteogenic differentiation potential of human bone marrow MSCs (hBMSCs) and enhance regeneration of bone and tissue. For instance, exosome/β-TCP scaffold showed greater osteogenic differentiation (80 % bone stained by alizarin red) as compared to unmodified β-TCP only (only 20 % bone stained by alizarin red). Similarly, bone mass density (BMD) was also 40 % higher in exosome treated group as compared to β-TCP only.[82] Recently, another study demonstrated that immobilizing MSC-derived exosomes on the surface of titanium (Ti) can improve MSCs adhesion and spreading after 24 h and proliferation after 3 and 6 days.[83] Wang et al. reported that the modification of PCL scaffold by MSC-derived exosomes and GSNO could promote the osteogenic differentiation in hBMSCs while concurrently suppressing pro-inflammatory genes in macrophages.[84] In a recent study, 3D printing technique was applied to create a scaffold made up of exosome/gelatin methacrylate and extracellular matrix (ECM). This radially oriented scaffold restored the mitochondrial dysfunction of chondrocytes and enhanced their migration resulting in OA improvement. Moreover, the expression of collagen-II also upregulated upto 8 % in exosome containing group as compared to the control group.[85]

In a nutshell, MSC-derived exosomes can improve osteoarthritis

through cartilage regeneration by increasing cell proliferation and differentiation (MSCs to chondrocytes) and decreasing inflammation and oxidative stress. Furthermore, MSC-derived exosomes are also biocompatible with biomaterials employed to tune their retention and release. Therefore, it is of great interest to explore the behaviour of exosomes following encapsulation in multiple biomaterials (natural and synthetic) for OA treatment. It might also be interesting to investigate whether anti-osteoarthritis drug-loaded exosomes have better therapeutic effects for cartilage regeneration than either exosomes or the drug alone.

Therapeutic potential of MSC-derived exosomes in liver diseases

MSC-derived exosomes have been reported to exert their reparative effect in different hepatic injuries including acute liver failure, liver fibrosis, and hepatocellular carcinoma. Hepatocyte death is the primary feature of most hepatic injuries triggering inflammatory cell infiltrations resulting in apoptosis and necrosis.[86] Several studies have reported the anti-apoptotic features of MSC-derived exosomes. In an in vitro study, Zhao et al.[87] investigated the anti-apoptotic effects of BMSC-derived exosomes on D-galactosamine and lipopolysaccharide (D-GalN/LPS) induced apoptosis. They demonstrated that exosomes could attenuate hepatocyte apoptosis via the upregulation of autophagy proteins LC3 and Beclin-1 and downregulation of proapoptotic proteins Bax and cleaved caspase-3. Simultaneously, the expression of autophagosomes and antiapoptotic protein Bcl-2 was upregulated. Another study evaluated the antioxidant and hepatoprotective effects of MSC-derived exosomes in mouse models of hepatic injury induced by CCL4.[88] Similarly, Du et al.[89] found that MSC-derived exosomes could attenuate hepatic ischemia reperfusion injury through the activation of sphingosine kinase and sphingosine-1-phosphate (S1P) signaling pathways with significant reduction in AST (57 % after 24 h) and ALT (50 % after 24 h) hepatocyte injury markers in a murine ischemia/reperfusion injury model.

Hepatic stellate cells (HSCs) play critical role in the development of hepatic fibrosis, characterized by excessive and abnormal accumulation of extracellular matrix proteins (ECM), particularly collagens..[90] A recent study suggested that MSC-derived exosomal circDIDO1 (circular DNA that forms a closed loop and has no ends) could suppress and inhibit the proliferation of hepatic stellate cells by miR-141-3p/PTEN/AKT signaling pathway in human hepatic fibrotic cells.[91] It has been demonstrated that exosomal miRNA-125b reduced fibrosis by suppressing Hedgehog (Hh) signaling via the downregulation of Smo expression resulting in liver regeneration.[92] Another study showed that MSC-derived exosomes expressing miRNA-122 could decrease the activation and proliferation of hepatic stellate cells (HSCs) thereby reducing fibrosis in liver.[93]

Hepatocellular carcinoma (HCC) is the fourth fatal cancer worldwide.[94] Exosomes showed strong anti-HCC effects in vitro as well as in vivo. For example, hBMSC-exosomes induced apoptosis and inhibited cell cycle progression in HepG2 cells. Moreover, in vivo intra-tumor administration of exosomes in established tumors generated by subcutaneous injection of HepG2 cancer cell lines in SCID mice substantially inhibited tumor growth.[95] In another study, hBMSC-exosomes interacted with cancer cell lines resulting in significant inhibition of tumor site angiogenesis, metastasis, and invasiveness in rats.[96] MSCs and secreted exosomes can be modified to enhance their anti-HCC effects. Exosomes isolated from BMSCs silenced by the SiRNA to the Glucose regulatory protein 78 (GRP78), a key chaperone of the unfolded protein response in the ER inhibited the growth of cancer cells (HepG2, PLC) in vitro. Additionally, siGRP78 -secreted exosomes also significantly increased the sensitivity (approximately 20 %) of resistant cancer cells to Sorafenib, a multi kinase inhibitor for advanced kidney cancer.[97] According to another study, exosomes rich in miRNA-451a isolated from human umbilical cord MSCs when co-cultured with SMMC-7721 and Hep3B cell lines, reversed the resistance to paclitaxel, an antimitotic drug, promoted apoptosis of HCC cells, and inhibited epithelial-mesenchymal transition (EMT), a response promoting lung tissue remodeling and ultimately fibrosis.[98]

The therapeutic role of MSC-derived exosomes in treating acute and chronic hepatic diseases is in full swing. The therapeutic molecular mechanism in HCC and liver fibrosis is also of research highlight. However, several key signaling mechanisms involved in therapeutic exosomes remain undiscovered. On the contrary, disease promoting exosomes are also secreted as the liver disease progresses. Little research has been done for their identification and characterization. Large scale animal studies are still required to understand their role in liver pathogenesis and to inhibit their formation.

Current investigations have found that some chemotherapeutics when loaded in exosomes, showed improved cellular uptake, decreased toxicity and less resistance. This indicates the possibility of loading multiple chemotherapeutics in MSC-derived exosomes to obtain synergistic effects against liver cancer. Likewise, hepatoprotective drugs (such as *N*-acetylcysteine and silymarin) can be loaded in MSC-derived exosomes in the future to achieve better efficacy than free drug.

Therapeutic potential of MSC-derived exosomes in neurological diseases

Recently, in a mouse model of Parkinson's disease, MSC-derived exosomes showed therapeutic effect by promoting ICAM1-mediated angiogenesis mediated by the activation of SMAD3 and P38MAPK signaling pathways in microvascular endothelial cells of brain, resulting in a possible therapeutic potential for Parkinsonism.[99] Similarly, −modified exosomes enriched in miRNA-17-92 could provide neuroprotection of peripheral nerves by the activation of PI3K/protein kinase B/mechanistic target of rapamycin/glycogen synthase kinase 3β signaling pathway.[100] MSC-derived exosomes can also restore traumatic brain injury. In a LPS-induced inflammatory model of rat, exosomes suppressed the NFkB and P38 mitogen-activated protein kinase signaling in the microglia/macrophages resulting in reduced inflammation and rapid recovery of traumatic brain injury.[101]

Alzheimer's disease (AD) is characterized by dementia and cognitive dysfunction due to the deposition of β-amyloid (Aβ) in and around neurons with neurofibrillary tangles (NFT) of abnormal protein called hyperphosphorylated tau.[102] A study indicated that ADMSC-derived exosomes carry neprilysin (neprilysin specific activity level of 1 μg protein obtained from exosomes was equivalent to 0.3 ng of recombinant neprilysin), Zn metalloproteinase that can degrade Aβ of the brain. Exosomes significantly decreased levels of both secreted and intracellular Aβ in vitro.[103] Recently, MSC-derived exosomes injected intravenously, reduced Aβ expression in transgenic mice model of the disease. The exosomes also inhibited the downregulation of neuronal memory/synaptic plasticity-related genes including brain-derived neurotrophic factor (BDNF exon IV, 53.7 %) and synaptophysin (Syp, 16.2 %).[104] Similarly, MSC-derived exosomes relieved Aβ-mediated cognitive impairment and promoted neurogenesis in the subventricular zone in another mouse model of Alzheimer's disease.[105] In a murine model of the disease, MSC-based RVG-modified exosomes reduced the pro-inflammatory action of various mediators such as TNF-α (20 %), IL-β (10 %), and IL-6 (10 %) with significant elevation in anti-inflammatory factors including IL-10 (20 %), IL-4 (10 %), and IL-13 (8 %).[106]

Interestingly in a stroke rat model, intravenous administration of MSC-derived exosomes could improve post stroke neurological recovery by promoting neurogenesis and angiogenesis.[107] MSC-derived exosomes enriched with the miR-17-92 via transfection electroporation markedly enhanced neurogenesis, oligodendrogenesis, neural plasticity/neurite remodeling, and functional recovery when compared to control MSC exosomes, possibly via inhibiting PTEN and increasing the phosphorylation of phosphatase resulting in the activation of PI3K/protein kinase B/mechanistic target of rapamycin/glycogen synthase kinase 3β signaling pathway.[108]

In summary, MSC-derived exosomes have been mainly studied in the context of Parkinsonism and Alzheimer's disease. For example, they have been shown to provide neuroprotection and reduce Aβ expression

in animal models of both the diseases respectively. However, their role in other neurodegenerative diseases (e.g., Amyotrophic lateral sclerosis and Lewy body dementia) raises several unanswered questions. Further studies are also required to identify the contribution of natural exosomes to the neurodegenerative diseases and provide insights on the best therapeutical strategy to target these crippling diseases in future.

MSC-derived exosomes as drug delivery nanocarriers

MSC-derived exosomes are also claimed a new approach for drug delivery in the treatment of various disorders including cardiovascular diseases, neurological diseases, and malignant tumors. Drugs could be loaded onto exosomes by several methods including incubation, electroporation, sonication, extrusion, freeze-thawing and pH-gradient. These drug loaded exosomes can be characterized by transmission electron microscopy (TEM), confocal microscopy, magnetic resonance imaging (MRI), western blotting, low speed centrifugation, ultracentrifugation, size exclusion chromatography (SEC), ultrafiltration and sucrose gradient ultracentrifugation etc..[109] Compared with synthetic drug delivery carriers, exosomes own lower toxicity, better drug-target communication, stable drug release profile, tissue specific targeting, and higher biocompatibility with prolonged blood circulation[110] (Fig. 3).

In addition to encapsulate different therapeutic genetic elements (such as proteins and multiple RNA types), MSC-derived exosomes could also wrap different small therapeutical agents to augment their efficacy. Several studies have compared the efficacy of exosome encapsulated drugs to the drug alone and/or to empty exosomes. Liang et al.[111] showed that the encapsulation of norcantharidin (NCTD) in BMMSC-exosomes could promote the uptake of NCTD by HepG2 cells, reduce HepG2 proliferation, increase HepG2 apoptosis and facilitated HepG2 cycle arrest. Similarly, Bagheri et al.[112] also developed a drug delivery system to encapsulate doxorubicin (DOX) in MSC-derived exosomes with 35 % encapsulation efficiency against colorectal cancer. Their results showed that this engineered DOX-exosome-apt system inhibited tumor growth (65 %) as compared to DOX-exosome (25 %) and free DOX (16 %). This controlled drug delivery system was found to be non-immunogenic, biocompatible along with targeted ability and desirable biodistribution. Another investigation demonstrated that exosomes loaded with paclitaxel induced apoptosis and suppressed EMT signaling in Hela cervical cancer cells. The effective nanocarrier system of paclitaxel suppressed the proteins responsible for EMT in Hela cells.[113] Recently, MSC-derived exosomes could be excellent nanocarriers to deliver doxorubicin against osteosarcoma with decreased toxicity in cardiac tissue. The results of this study also showed that the targeting ability of exosomes was due to the chemotaxis of exosomes to osteosarcoma cells mediated through SDF1-CXCR4 axis.[114] In another study, the engineered exosomes loaded with curcumin and indocyanine green showed better chemo-phototherapy effect as compared to chemotherapy or phototherapy alone. It was found that engineered *exo*-CUR + ICG can cause cancer cell inhibition by triggering apoptosis along with cell arrest in G2/M phase against glioma mice model.[115] Yang et al. found that desialylated exosomes loaded with doxorubicin significantly improved cellular uptake, enhanced targeting efficacy, and had a better inhibiting effect against HepG2 cancer cell lines when compared to free doxorubicin.[116]

Most of the drugs are unable to cross the blood–brain barrier (BBB) and represent major problem for drug delivery of modern neuropharmacology. In this regard, exosomes own a significant advantage, because they can cross BBB[117] (Fig. 3). These new advancements provide possibilities to administer drugs intravenously or even intranasally to deliver them at desired neural site via exosomes. In a mice model of Parkinsonism, the combination of engineered exosomes with curcumin crossed the complex membrane barriers including blood brain barrier (BBB) resulting in direct release of drug into the cytoplasm of target cells following intranasal administration. This promising synergistic combination also reduced α-synuclein aggregates, promoted neuron function recovery, and alleviated the neuroinflammation. Consequently, there

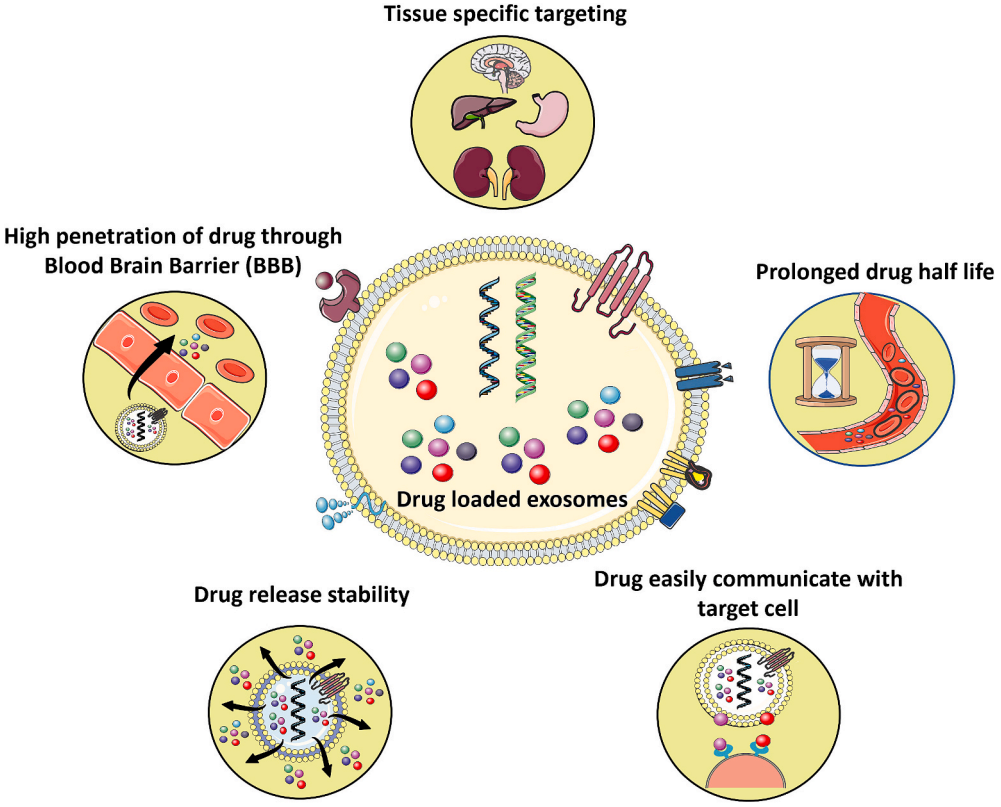


Fig. 3. Therapeutic advantages of MSC-derived exosomes as drug delivery nanocarriers.

was significant improvement in the movement and coordination.[118] Similarly, another study revealed that blood derived exosomes loaded with dopamine can cross the BBB because of interaction between transferrin and its receptor. The findings of this study proved that blood exosomes are powerful nanocarriers for targeted therapy against Parkinsonism in mice and possibly for other central nervous system diseases as well.[119]

Currently, liposomes are the preferred vehicles for drug delivery. They have been used for the delivery of multiple drugs including analgesics,[120] anti-fungal drugs,[121] anti-cancer drugs[122–124] etc. Liposomes with a bilayer phospholipid membrane, could encapsulate hydrophobic drugs within the hydrophobic membranes and hydrophilic drugs in their aqueous core (Fig. 4A). They may also break the plasma membrane to release the drug. Considering the similar composition of phospholipid membrane of exosomes and liposomes (Fig. 4A), exosomes are termed as natural liposomes. Being nature-derived, exosomes could potentially circumvent some of the limitations presented by synthetic liposomes such as toxicity of the synthetic phospholipid membranes. Exosomes also own many better key features when compared to synthetic liposomes including longer circulating half-life, more specific internalization by the target cells and greater evasion by the host immune system (Fig. 4B).

Exosomes have several features that make them an ideal drug delivery vehicle. Firstly, exosomes have wide biodistribution in body fluids such as breast milk,[125] blood[126] and urine.[127] Therefore, these nanovehicles have longer circulating half-life resulting in improved

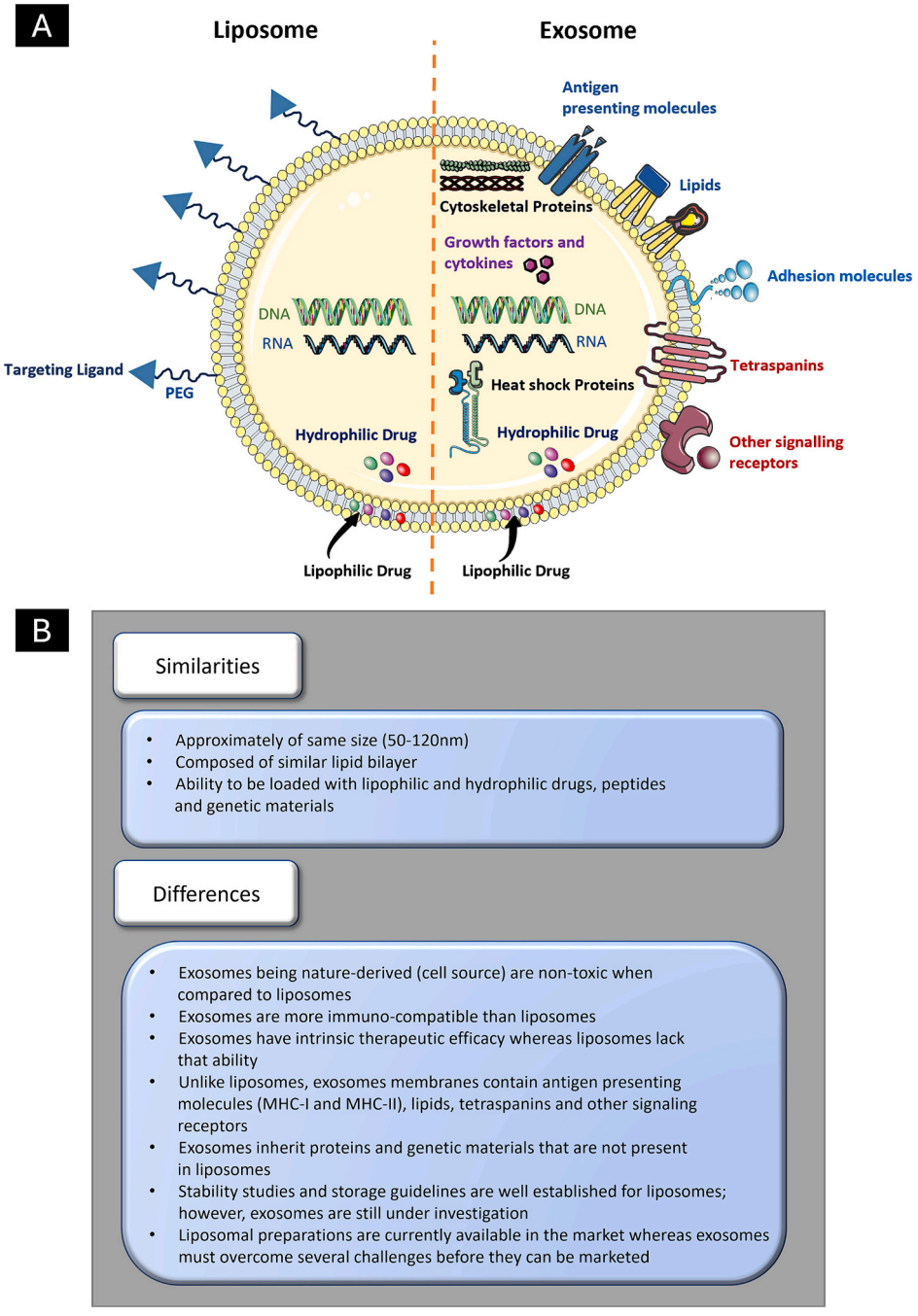


Fig. 4. (A) Comparative schematic illustration of exosome (right part) and liposome (left part) structures. (B) The main similarities and differences of exosomes and liposomes. PEG, polyethylene glycol; MHC, major histocompatibility complex.

efficacy. Secondly, the abundance of genetic materials and proteins in exosomes implies that such biological materials could be encapsulated into exosomes. Thirdly, exosomes also own homing ability to target tissues with preferential homing targets based on their cell source. For example, Hood et al.[128] showed that homing of melanoma exosomes to sentinel lymph nodes promoted tumor metastasis. Fourthly, exosomes can be up taken through endocytosis unload their cargo into target cells. For example, Andrey et al.[129] revealed that dendritic cell-derived exosomes can transfer MHC Class I/Peptide complexes to other dendritic cells to tune immune response. Finally, modifications to exosome membranes could be made to enable cell type-specific targeting. For example, Alvarez-Erviti et al.[130] reported that in dendritic cells over-expressing of LAMP2B (an exosomal membrane protein), secreted exosomes expose RVG peptide on their membranes. When loaded with exogenous siRNA and injected intravenously these exosomes crossed the blood brain barrier, delivered siRNA to target specific gene in neurons, microglia and oligodendrocytes, resulting in a target gene knockdown.

The stability of nanocarriers is a key factor in drug delivery therapeutic applications. Indeed, the chemical integrity and the therapeutic effect of the encapsulated drugs depend on the stability of the liposomal preparations. A stable liposomal formulation should preserve its physical and chemical stability. Generally, physical stability includes the determination of size and visual inspection of liposomes to check the tendency of aggregation. While chemical stability can be considered the ability of liposomes to preserve their encapsulation efficiency (EE) when exposed to pH changes, oxidizing agents, and surfactants.[131] However, fusion and breakage of liposomes due to instability may result in drug leakage.[132] Furthermore, liposomal stability also includes the resolution of some other parameters such as the impact of biological fluids on the liposomes, conservation of their size and structure and the interactions between phospholipids and the encapsulated drugs.[133] The presence of unsaturated fatty acids in liposomes can lead to oxidation triggered by light and metal ions. The removal of microbial contaminants from parenteral liposomal products is also important to control the microbial stability.[134] Liposomal products can be stored in dry powder form or in an aqueous solution.[135] However, the storage of liposomes in dry powder form offers long term stability.[136] Among the available methods, lyophilization is the most applied technique for water removal to overcome the instabilities of liposomes in an aqueous solution. In summary, liposomal behaviour and stability have been deeply studied under various conditions to evaluate the impact of multiple external parameters including pH, UV exposure and temperature, in relation to the intrinsic parameters of the carrier (composition, charge and size). Retention time of liposomal cargo can vary from hours to months depending on these parameters and surface modifications have also been developed to tune these properties, with as a classical example the use of PEG coating to strengthen plasma lifetime.[137] In contrast, natural vesicles storage guidelines are still under investigations and their storage under −80 °C seems preferable if fresh use (within few hours) is not possible.[138] As for all cell-based therapies, expensive bioreactors costs are required (for instance, a lab scale bioreactor can cost tens of thousands of euros). The process of vesicle production requires the stimulation of cells through chemical (e.g. sulfhydryl blocking agents or cytochalasins) or physical (e.g. shear stress or through acoustic exposition) stress, or even via specific culture conditions such as hypoxia or starvation.[139] The production method applied has also an impact on the produced EVs (e.g. size) and their therapeutic potential. In comparison, liposomes are produced using physicochemical based principles (reverse phase evaporation, thin-film hydration, freeze-drying, cross-flow filtration and membrane contractor)[140] and the impact of the process parameters is well understood and optimized.

Liposomal formulations are now well mastered with dozens of commercial products on the market (AMBISOME®, VISUDYNE®, LIPIREX®…)[140,141] but the higher therapeutic potential of natural EVs should promise them a robust avenir.

Challenges in marketing

There are several challenges to introduce the exosomes as a therapeutic moiety and nano-carrier to the clinic and market. Currently, clinical trials are being conducted focusing on the application of MSC-derived exosomes as therapeutic tools. However, the resolution of parameters such as exosome mass production, optimal dose, standard testing methods, stability studies at different conditions and storage standardization still requires preclinical to clinical transition. The complex biochemical nature of nanoscale drugs makes them vulnerable to instability and storage complexity. These promising nanovesicles also face other challenges, including batch-to-batch variation, costs, and the profit/risk ratios, and extensive research is still required to master the impact of their production method. Likewise, formulations containing drug loaded exosomes should be tailored to offer the optimal dose with a desired delivery profile to target the specific pathological sites. The drug loading method should be robust enough not to affect the delicate structure of the exosomal membranes.

Moreover, as exosomes are derived from cells, some ethical issues may need to be addressed. The therapeutic efficacy, minimum batch-to-batch variation, long-term biocompatibility, and stability of nano pharmaceutical should also be confirmed to pass some regulatory assessments. Further in vivo investigations and clinical trials should clarify the in vivo behaviour of MSC-derived exosomes and their formulations.

Conclusion and perspective

MSC-derived exosomes have gained significant potential regarding their use as nano-therapeutics in regenerative medicine. Unique properties of MSC-derived exosomes such as low immunogenicity, cell-origin nature, engineering capacity, nano-sized scale, high biocompatibility, and crossing through BBB make them superior to MSCs. As a cell-free therapy, MSC-exosomes carry biologically active molecules capable to exert their therapeutic effects like immunomodulation, tissue regeneration, and many other significant beneficial effects. Moreover, owing to many features of an ideal drug delivery nanocarrier, exosomes can serve better than liposomes in the treatment of various disorders. However, large scale production of exosomes is a challenge regardless of whether they are used as exosomes therapy or drug delivery nanocarrier. Hence, even though many studies revealed promising results, many of them were conducted in a preclinical setting, requiring extensive investigations for the translational application.

Abbreviations

HIF-1α	hypoxia inducuble factor 1-alpha
PDGFR-b	platelet derived growth factor receptor beta
α-SMA	alpha-smooth muscle actin
HSMSCs	human synovial mesenchymal stem cells
HMGB1	high mobility group protein -1
NFkB	nuclear factor kappa B
GSNO	S-Nitrosoglutathion
β-TCP	tricalcium phosphate
UCBMSCs	umbilical cord blood derived mesenchymal stem cells
BMMSCs	bone marrow mesenchymal stem cells
ADMSCs	adipose tissue derived mesenchymal stem cells
MVD	microvascular density
MIF	macrophage migration inhibitory factor

Ethics approval and consent to participate

No clinical experiment was done for this article.

Consent for publication

All of the authors consent for this article publication.

CRediT authorship contribution statement

Ali Imran Abid: Writing – original draft. **Guillaume Conzatti:** Writing – review & editing. **Florence Toti:** Writing – review & editing. **Nicolas Anton:** Writing – review & editing. **Thierry Vandamme:** Writing – review & editing.

Declaration of competing interest

All of the authors declare no conflict of interests.

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References

1. Shaer A, Azarpira N, Aghdaie MH, Esfandiari E. Isolation and characterization of human mesenchymal stromal cells derived from placental decidua basalis; umbilical cord Wharton's jelly and amniotic membrane. *Pakistan journal of medical sciences*. 2014;30(5):1022.
2. {Citation}Rani, S., Ryan, A. E., Griffin, M. D., & Ritter, T.. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. *Mol Ther*. 2015;23(5):812–823.
3. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *J Cell Physiol*. 1999;181(1):67–73.
4.. Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., ... & Marshak, D. R. (1999). Multilineage potential of adult human mesenchymal stem cells. *science*, *284*(5411), 143–147.
5. Dominici MLBK, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315–317.
6. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood, The Journal of the American Society of Hematology*. 2001;98(8):2396–2402.
7. Crapnell K, Blaesius R, Hastings A, Lennon DP, Caplan AI, Bruder SP. Growth, differentiation capacity, and function of mesenchymal stem cells expanded in serum-free medium developed via combinatorial screening. *Exp Cell Res*. 2013;319(10):1409–1418.
8.. Phan J, Kumar P, Hao D, Gao K, Farmer D, Wang A. Engineering mesenchymal stem cells to improve their exosome efficacy and yield for cell-free therapy. *Journal of extracellular vesicles*. 2018;7(1):1522236.
9. Charbord P. Bone marrow mesenchymal stem cells: historical overview and concepts. *Hum Gene Ther*. 2010;21(9):1045–1056.
10. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol*. 2013;200(4):373–383.
11.. Farini, A., Sitzia, C., Erratico, S., Meregalli, M., & Torrente, Y. (2014). Clinical applications of mesenchymal stem cells in chronic diseases. *Stem cells international*, *2014*.
12. Madrigal M, Rao KS, Riordan NH. A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. *J Transl Med*. 2014;12(1):1–14.
13. Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells *in vitro*: effects of dexamethasone and IL-1α. *J Cell Physiol*. 1996;166(3):585–592.
14. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem*. 2006;98(5):1076–1084.
15. Xin H, Li Y, Liu Z, Wang X, Shang X, Cui Y, et al. MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. *Stem Cells*. 2013;31(12):2737–2746.
16. Duijvesz D, Luider T, Bangma CH, Jenster G. Exosomes as biomarker treasure chests for prostate cancer. *Eur Urol*. 2011;59(5):823–831.
17. Konala VBR, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. *Cytotherapy*. 2016;18(1):13–24.
18. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol*. 2002;2(8):569–579.
19. Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and α-granules. *Blood, The Journal of the American Society of Hematology*. 1999;94(11):3791–3799.
20. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol*. 2006;30(1):3–22.
21. Wubbolts R, Leckie RS, Veenhuizen PT, Schwarzmann G, Möbius W, Hoernschemeyer J, et al. Proteomic and biochemical analyses of human B cell-derived exosomes: potential implications for their function and multivesicular body formation. *J Biol Chem*. 2003;278(13):10963–10972.
22. Simpson RJ, Jensen SS, Lim JW. Proteomic profiling of exosomes: current perspectives. *Proteomics*. 2008;8(19):4083–4099.
23. Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: from biogenesis to uptake and intracellular signalling. *Cell Communication and Signaling*. 2021;19(1):1–19.
24. Keshtkar S, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. *Stem cell research & therapy*. 2018;9:1–9.
25. Corrado C, Raimondo S, Chiesi A, Ciccia F, De Leo G, Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. *Int J Mol Sci*. 2013;14(3):5338–5366.
26. González-Cubero E, González-Fernández ML, Gutiérrez-Velasco L, Navarro-Ramírez E, Villar-Suárez V. Isolation and characterization of exosomes from adipose tissue-derived mesenchymal stem cells. *J Anat*. 2021;238(5):1203–1217.
27. Merino-González C, Zuñiga FA, Escudero C, Ormazabal V, Reyes C, Nova-Lamperti E, et al. Mesenchymal stem cell-derived extracellular vesicles promote angiogenesis: potencial clinical application. *Front Physiol*. 2016;7:24.
28. Herberts CA, Kwa MS, Hermsen HP. Risk factors in the development of stem cell therapy. *J Transl Med*. 2011;9:1–14.
29. Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. *Int J Mol Sci*. 2014;15(3):4142–4157.
30. Lai RC, Arslan F, Lee MM, Sze NSK, Choo A, Chen TS, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2010;4(3):214–222.
31.. Sun J, Shen H, Shao L, Teng X, Chen Y, Liu X, et al. HIF-1α overexpression in mesenchymal stem cell-derived exosomes mediates cardioprotection in myocardial infarction by enhanced angiogenesis. *Stem Cell Research & Therapy*. 2020;11:1–13.
32. Xu H, Wang Z, Liu L, Zhang B, Li B. Exosomes derived from adipose tissue, bone marrow, and umbilical cord blood for cardioprotection after myocardial infarction. *J Cell Biochem*. 2020;121(3):2089–2102.
33. Luther KM, Haar L, McGuinness M, Wang Y, Lynch IV TL, Phan A, et al. Exosomal miR-21a-5p mediates cardioprotection by mesenchymal stem cells. *J Mol Cell Cardiol*. 2018;119:125–137.
34. Huang P, Wang L, Li Q, Tian X, Xu J, Xu J, et al. Atorvastatin enhances the therapeutic efficacy of mesenchymal stem cells-derived exosomes in acute myocardial infarction via up-regulating long non-coding RNA H19. *Cardiovasc Res*. 2020;116(2):353–367.
35. Zhang S, Liu X, Ge LL, Li K, Sun Y, Wang F, et al. Mesenchymal stromal cell-derived exosomes improve pulmonary hypertension through inhibition of pulmonary vascular remodeling. *Respir Res*. 2020;21:1–12.
36. Zhao J, Li X, Hu J, Chen F, Qiao S, Sun X, et al. Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization. *Cardiovasc Res*. 2019;115(7):1205–1216.
37.. Shao, L., Zhang, Y., Lan, B., Wang, J., Zhang, Z., Zhang, L., ... & Li, Y. (2017). MiRNA-sequence indicates that mesenchymal stem cells and exosomes have similar mechanism to enhance cardiac repair. *BioMed research international*, *2017*.
38. Mao Q, Liang XL, Zhang CL, Pang YH, Lu YX. LncRNA KLF3-AS1 in human mesenchymal stem cell-derived exosomes ameliorates pyroptosis of cardiomyocytes and myocardial infarction through miR-138-5p/Sirt1 axis. *Stem cell research & therapy*. 2019;10(1):1–14.
39. Wang X, Chen Y, Zhao Z, Meng Q, Yu Y, Sun J, et al. Engineered exosomes with ischemic myocardium-targeting peptide for targeted therapy in myocardial infarction. *J Am Heart Assoc*. 2018;7(15), e008737.
40. Sun X, Shan A, Wei Z, Xu B. Intravenous mesenchymal stem cell-derived exosomes ameliorate myocardial inflammation in the dilated cardiomyopathy. *Biochem Biophys Res Commun*. 2018;503(4):2611–2618.
41. Liu X, Li X, Zhu W, Zhang Y, Hong Y, Liang X, et al. Exosomes from mesenchymal stem cells overexpressing MIF enhance myocardial repair. *J Cell Physiol*. 2020;235(11):8010–8022.
42.. Lv K, Li Q, Zhang L, Wang Y, Zhong Z, Zhao J, et al. Incorporation of small extracellular vesicles in sodium alginate hydrogel as a novel therapeutic strategy for myocardial infarction. *Theranostics*. 2019;9(24):7403.
43.. Ni, J., Liu, X., Yin, Y., Zhang, P., Xu, Y. W., & Liu, Z. (2019). Exosomes derived from TIMP2-modified human umbilical cord mesenchymal stem cells enhance the repair effect in rat model with myocardial infarction possibly by the Akt/Sfrp2 pathway. *Oxidative medicine and cellular longevity*, *2019*.
44. Feng Y, Huang W, Wani M, Yu X, Ashraf M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. *PloS One*. 2014;9(2), e88685.
45. He JG, Li HR, Han JX, Li BB, Yan D, Li HY, et al. GATA-4-expressing mouse bone marrow mesenchymal stem cells improve cardiac function after myocardial infarction via secreted exosomes. *Sci Rep*. 2018;8(1):9047.
46. Zhu G, Pei L, Lin F, Yin H, Li X, He W, et al. Exosomes from human-bone-marrow-derived mesenchymal stem cells protect against renal ischemia/reperfusion injury via transferring miR-199a-3p. *J Cell Physiol*. 2019;234(12):23736–23749.
47. Arif M, Pandey R, Alam P, Jiang S, Sadayappan S, Paul A, et al. MicroRNA-210-mediated proliferation, survival, and angiogenesis promote cardiac repair post myocardial infarction in rodents. *J Mol Med*. 2017;95:1369–1385.
48. Duffield JS. Cellular and molecular mechanisms in kidney fibrosis. *J Clin Invest*. 2014;124(6):2299–2306.
49. Liu L, Wu Y, Wang P, Shi M, Wang J, Ma H, et al. PSC-MSC-derived exosomes protect against kidney fibrosis *in vivo* and *in vitro* through the SIRT6/β-catenin signaling pathway. *Int J Stem Cells*. 2021;14(310–319):10–15283.

50. Rout-Pitt N, Farrow N, Parsons D, Donnelley M. Epithelial mesenchymal transition (EMT): a universal process in lung diseases with implications for cystic fibrosis pathophysiology. *Respir Res*. 2018;19(1):1–10.
51. Yin S, Zhou S, Ren D, Zhang J, Xin H, He X, et al. Mesenchymal stem cell-derived exosomes attenuate epithelial–mesenchymal transition of HK-2 cells. *Tissue Eng Part A*. 2022;28(13–14):651–659.
52. Liu Y. Cellular and molecular mechanisms of renal fibrosis. *Nat Rev Nephrol*. 2011;7(12):684–696.
53. Ratliff BB, Abdulmahdi W, Pawar R, Wolin MS. Oxidant mechanisms in renal injury and disease. *Antioxid Redox Signal*. 2016;25(3):119–146.
54.. Liu B, Hu D, Zhou Y, Yu Y, Shen L, Long C, et al. Exosomes released by human umbilical cord mesenchymal stem cells protect against renal interstitial fibrosis through ROS-mediated P38MAPK/ERK signaling pathway. *American Journal of Translational Research*. 2020;12(9):4998.
55.. Ishiy, C. S. R. A., Ormanji, M. S., Maquigussa, E., Ribeiro, R. S., da Silva Novaes, A., & Boim, M. A. (2020). Comparison of the effects of mesenchymal stem cells with their extracellular vesicles on the treatment of kidney damage induced by chronic renal artery stenosis. *Stem cells international*, *2020*.
56. Ji C, Zhang J, Zhu Y, Shi H, Yin S, Sun F, et al. Exosomes derived from hucMSC attenuate renal fibrosis through CK1δ/β-TRCP-mediated YAP degradation. *Cell Death Dis*. 2020;11(5):327.
57. Zhang ZY, Hou YP, Zou XY, Xing XY, Ju GQ, Zhong L, et al. Oct-4 enhanced the therapeutic effects of mesenchymal stem cell-derived extracellular vesicles in acute kidney injury. *Kidney and Blood Pressure Research*. 2020;45(1):95–108.
58. Griffin KA. Hypertensive kidney injury and the progression of chronic kidney disease. *Hypertension*. 2017;70(4):687–694.
59.. Chagnac A, Zingerman B, Rozen-Zvi B, Herman-Edelstein M. Consequences of glomerular hyperfiltration: the role of physical forces in the pathogenesis of chronic kidney disease in diabetes and obesity. *Nephron*. 2019;143(1):38–42.
60. López-Novoa JM, Martínez-Salgado C, Rodríguez-Peña AB, Hernández FJL. Common pathophysiological mechanisms of chronic kidney disease: therapeutic perspectives. *Pharmacol Ther*. 2010;128(1):61–81.
61. Lindoso RS, Lopes JA, Binato R, Abdelhay E, Takiya CM, de Miranda KR, et al. Adipose mesenchymal cells-derived EVs alleviate DOCA-salt-induced hypertension by promoting cardio-renal protection. *Molecular Therapy-Methods & Clinical Development*. 2020;16:63–77.
62.. Feng R, Ullah M, Chen K, Ali Q, Lin Y, Sun Z. Stem cell-derived extracellular vesicles mitigate ageing-associated arterial stiffness and hypertension. *Journal of extracellular vesicles*. 2020;9(1):1783869.
63. Abramoff B, Caldera FE. Osteoarthritis: pathology, diagnosis, and treatment options. *Medical Clinics*. 2020;104(2):293–311.
64. Bao C, He C. The role and therapeutic potential of MSC-derived exosomes in osteoarthritis. *Arch Biochem Biophys*. 2021;710, 109002.
65. Yan C, Wang Y, Shen XY, Yang G, Jian J, Wang HS, et al. MicroRNA regulation associated chondrogenesis of mouse MSCs grown on polyhydroxyalkanoates. *Biomaterials*. 2011;32(27):6435–6444.
66. Qiu M, Liu D, Fu Q. MiR-129-5p shuttled by human synovial mesenchymal stem cell-derived exosomes relieves IL-1β induced osteoarthritis via targeting HMGB1. *Life Sci*. 2021;269, 118987.
67. Tao SC, Yuan T, Zhang YL, Yin WJ, Guo SC, Zhang CQ. Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model. *Theranostics*. 2017;7(1):180.
68.. He L, Chen Y, Ke Z, Pang M, Yang B, Feng F, et al. Exosomes derived from miRNA-210 overexpressing bone marrow mesenchymal stem cells protect lipopolysaccharide induced chondrocytes injury via the NF-κB pathway. *Gene*. 2020;751, 144764.
69.. Wang R, Xu B, Xu H. TGF-β1 promoted chondrocyte proliferation by regulating Sp1 through MSC-exosomes derived miR-135b. *Cell Cycle*. 2018;17(24):2756–2765.
70. Chen LL, Zhao JC. Functional analysis of long noncoding RNAs in development and disease. *Systems Biology of RNA Binding Proteins*. 2014:129–158.
71. Sun Z, Yang S, Zhou Q, Wang G, Song J, Li Z, et al. Emerging role of exosome-derived long non-coding RNAs in tumor microenvironment. *Mol Cancer*. 2018;17(1):1–9.
72. Pan L, Liu D, Zhao L, Wang L, Xin M, Li X. *Retracted: Long Noncoding RNA MALAT1 Alleviates Lipopolysaccharide-Induced Inflammatory Injury by Upregulating microRNA-19b in Murine Chondrogenic ATDC5 Cells*. 2018.
73.. Li B, Luan S, Chen J, Zhou Y, Wang T, Li Z, et al. The MSC-derived exosomal lncRNA H19 promotes wound healing in diabetic foot ulcers by upregulating PTEN via MicroRNA-152-3p. *Molecular Therapy-Nucleic Acids*. 2020;19:814–826.
74.. Yang X, Yang J, Lei P, Wen T. LncRNA MALAT1 shuttled by bone marrow-derived mesenchymal stem cells-secreted exosomes alleviates osteoporosis through mediating microRNA-34c/SATB2 axis. *Aging (Albany NY)*. 2019;11(20):8777.
75. Anderson JD, Johansson HJ, Graham CS, Vesterlund M, Pham MT, Bramlett CS, et al. Comprehensive proteomic analysis of mesenchymal stem cell exosomes reveals modulation of angiogenesis via nuclear factor-kappaB signaling. *Stem Cells*. 2016;34(3):601–613.
76.. Zhu Y, Wang Y, Zhao B, Niu X, Hu B, Li Q, et al. Comparison of exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells and synovial membrane-derived mesenchymal stem cells for the treatment of osteoarthritis. *Stem cell research & therapy*. 2017;8(1):1–11.
77. Akbari A, Jabbari N, Sharifi R, Ahmadi M, Vahhabi A, Seyedzadeh SJ, et al. Free and hydrogel encapsulated exosome-based therapies in regenerative medicine. *Life Sci*. 2020;249, 117447.
78. Yang Y, Zhu Z, Gao R, Yuan J, Zhang J, Li H, et al. Controlled release of MSC-derived small extracellular vesicles by an injectable Diels-Alder crosslinked hyaluronic acid/PEG hydrogel for osteoarthritis improvement. *Acta Biomater*. 2021;128:163–174.
79. Zhang K, Zhao X, Chen X, Wei Y, Du W, Wang Y, et al. Enhanced therapeutic effects of mesenchymal stem cell-derived exosomes with an injectable hydrogel for hindlimb ischemia treatment. *ACS Appl Mater Interfaces*. 2018;10(36):30081–30091.
80. Yang S, Zhu B, Yin P, Zhao L, Wang Y, Fu Z, et al. Integration of human umbilical cord mesenchymal stem cells-derived exosomes with hydroxyapatite-embedded hyaluronic acid-alginate hydrogel for bone regeneration. *ACS Biomater Sci Eng*. 2020;6(3):1590–1602.
81. Li W, Liu Y, Zhang P, Tang Y, Zhou M, Jiang W, et al. Tissue-engineered bone immobilized with human adipose stem cells-derived exosomes promotes bone regeneration. *ACS Appl Mater Interfaces*. 2018;10(6):5240–5254.
82.. Zhang J, Liu X, Li H, Chen C, Hu B, Niu X, et al. Exosomes/tricalcium phosphate combination scaffolds can enhance bone regeneration by activating the PI3K/Akt signaling pathway. *Stem cell research & therapy*. 2016;7(1):1–14.
83. Wang X, Shah FA, Vazirisani F, Johansson A, Palmquist A, Omar O, et al. Exosomes influence the behavior of human mesenchymal stem cells on titanium surfaces. *Biomaterials*. 2020;230, 119571.
84.. Wang, X., Ao, J., Lu, H., Zhao, Q., Ma, Y., Zhang, J., ... & Zhang, Y. (2020). Osteoimmune modulation and guided osteogenesis promoted by barrier membranes incorporated with S-nitrosoglutathione (GSNO) and mesenchymal stem cell-derived exosomes. *International Journal of Nanomedicine*, 3483–3496.
85.. Chen P, Zheng L, Wang Y, Tao M, Xie Z, Xia C, et al. Desktop-stereolithography 3D printing of a radially oriented extracellular matrix/mesenchymal stem cell exosome bioink for osteochondral defect regeneration. *Theranostics*. 2019;9(9):2439.
86. Wang K. Molecular mechanisms of liver injury: apoptosis or necrosis. *Exp Toxicol Pathol*. 2014;66(8):351–356.
87.. Zhao, S., Liu, Y., & Pu, Z. (2019). Bone marrow mesenchymal stem cell-derived exosomes attenuate D-GaIN/LPS-induced hepatocyte apoptosis by activating autophagy *in vitro*. *Drug design, development and therapy*, 2887-2897.
88.. Jiang, W., Tan, Y., Cai, M., Zhao, T., Mao, F., Zhang, X., ... & Yan, Y. (2018). Human umbilical cord MSC-derived exosomes suppress the development of CCl4-induced liver injury through antioxidant effect. *Stem cells international*, *2018*.
89. Du Y, Li D, Han C, Wu H, Xu L, Zhang M, et al. Exosomes from human-induced pluripotent stem cell–derived mesenchymal stromal cells (hiPSC-MSCs) protect liver against hepatic ischemia/reperfusion injury via activating sphingosine kinase and sphingosine-1-phosphate signaling pathway. *Cell Physiol Biochem*. 2017;43(2):611–625.
90. Schuppan D. Liver fibrosis: common mechanisms and antifibrotic therapies. *Clin Res Hepatol Gastroenterol*. 2015;39:S51–S59.
91. Ma L, Wei J, Zeng Y, Liu J, Xiao E, Kang Y, et al. Mesenchymal stem cell-originated exosomal circDIDO1 suppresses hepatic stellate cell activation by miR-141-3p/PTEN/AKT pathway in human liver fibrosis. *Drug Deliv*. 2022;29(1):440–453.
92. Hyun J, Wang S, Kim J, Kim GJ, Jung Y. MicroRNA125b-mediated hedgehog signaling influences liver regeneration by chorionic plate-derived mesenchymal stem cells. *Sci Rep*. 2015;5(1):14135.
93. Lou G, Yang Y, Liu F, Ye B, Chen Z, Zheng M, et al. MiR-122 modification enhances the therapeutic efficacy of adipose tissue-derived mesenchymal stem cells against liver fibrosis. *J Cell Mol Med*. 2017;21(11):2963–2973.
94. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol*. 2019;16(10):589–604.
95. Bruno S, Collino F, Deregibus MC, Grange C, Tetta C, Camussi G. Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth. *Stem Cells Dev*. 2013;22(5):758–771.
96.. Alzahrani, F. A., El-Magd, M. A., Abdelfattah-Hassan, A., Saleh, A. A., Saadeldin, I. M., El-Shetry, E. S., ... & Alkarim, S. (2018). Potential effect of exosomes derived from cancer stem cells and MSCs on progression of DEN-induced HCC in rats. *Stem Cells International*, *2018*.
97. Li H, Yang C, Shi Y, Zhao L. Exosomes derived from siRNA against GRP78 modified bone-marrow-derived mesenchymal stem cells suppress Sorafenib resistance in hepatocellular carcinoma. *J Nanobiotechnol*. 2018;16:1–13.
98. Xu Y, Lai Y, Cao L, Li Y, Chen G, Chen L, et al. Human umbilical cord mesenchymal stem cells-derived exosomal microRNA-451a represses epithelial–mesenchymal transition of hepatocellular carcinoma cells by inhibiting ADAM10. *RNA Biol*. 2021;18(10):1408–1423.
99. Xue C, Li X, Ba L, Zhang M, Yang Y, Gao Y, et al. MSC-derived exosomes can enhance the angiogenesis of human brain MECs and show therapeutic potential in a mouse model of Parkinson's disease. *Aging Dis*. 2021;12(5):1211.
100.. Dong, R., Liu, Y., Yang, Y., Wang, H., Xu, Y., & Zhang, Z. (2019). MSC-derived exosomes-based therapy for peripheral nerve injury: a novel therapeutic strategy. *BioMed research international*, *2019*.
101.. Chen Y, Li J, Ma B, Li N, Wang S, Sun Z, et al. MSC-derived exosomes promote recovery from traumatic brain injury via microglia/macrophages in rat. *Aging (Albany NY)*. 2020;12(18):18274.
102. Reiss AB, Arain HA, Stecker MM, Siegart NM, Kasselman LJ. Amyloid toxicity in Alzheimer's disease. *Rev Neurosci*. 2018;29(6):613–627.
103. Katsuda T, Tsuchiya R, Kosaka N, Yoshioka Y, Takagaki K, Oki K, et al. Human adipose tissue-derived mesenchymal stem cells secrete functional neprilysin-bound exosomes. *Sci Rep*. 2013;3(1):1197.

104. Chen YA, Lu CH, Ke CC, Chiu SJ, Jeng FS, Chang CW, et al. Mesenchymal stem cell-derived exosomes ameliorate Alzheimer's disease pathology and improve cognitive deficits. *Biomedicines*. 2021;9(6):594.
105. Reza-Zaldivar EE, Hernández-Sapiéns MA, Gutiérrez-Mercado YK, Sandoval-Ávila S, Gomez-Pinedo U, Márquez-Aguirre AL, et al. Mesenchymal stem cell-derived exosomes promote neurogenesis and cognitive function recovery in a mouse model of Alzheimer's disease. *Neural Regen Res*. 2019;14(9):1626.
106. Cui GH, Guo HD, Li H, Zhai Y, Gong ZB, Wu J, et al. RVG-modified exosomes derived from mesenchymal stem cells rescue memory deficits by regulating inflammatory responses in a mouse model of Alzheimer's disease. *Immunity & Ageing*. 2019;16(1):1–12.
107. Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. *Journal of Cerebral Blood Flow & Metabolism*. 2013;33(11):1711–1715.
108. Xin H, Katakowski M, Wang F, Qian JY, Liu XS, Ali MM, et al. MicroRNA-17–92 cluster in exosomes enhance neuroplasticity and functional recovery after stroke in rats. *Stroke*. 2017;48(3):747–753.
109. Mehryab F, Rabbani S, Shahhosseini S, Shekari F, Fatahi Y, Baharvand H, et al. Exosomes as a next-generation drug delivery system: an update on drug loading approaches, characterization, and clinical application challenges. *Acta Biomater*. 2020;113:42–62.
110. Ahmadi M, Mahmoodi M, Shoaran M, Nazari-Khanamiri F, Rezaie J. Harnessing Normal and engineered mesenchymal stem cells derived exosomes for Cancer therapy: opportunity and challenges. *Int J Mol Sci*. 2022;23(22):13974.
111. Liang L, Zhao L, Wang Y, Wang Y. Treatment for hepatocellular carcinoma is enhanced when norcantharidin is encapsulated in exosomes derived from bone marrow mesenchymal stem cells. *Mol Pharm*. 2021;18(3):1003–1013.
112. Bagheri E, Abnous K, Farzad SA, Taghdisi SM, Ramezani M, Alibolandi M. Targeted doxorubicin-loaded mesenchymal stem cells-derived exosomes as a versatile platform for fighting against colorectal cancer. *Life Sci*. 2020;261, 118369.
113. Abas BI, Demirbolat GM, Cevik O. Wharton jelly-derived mesenchymal stem cell exosomes induce apoptosis and suppress EMT signaling in cervical cancer cells as an effective drug carrier system of paclitaxel. *PloS One*. 2022;17(9), e0274607.
114.. Wei, H., Chen, F., Chen, J., Lin, H., Wang, S., Wang, Y., ... & Zhong, G. (2022). Mesenchymal stem cell derived exosomes as nanodrug carrier of doxorubicin for targeted osteosarcoma therapy via SDF1-CXCR4 Axis. *International Journal of Nanomedicine*, 3483–3495.
115. Li G, Wang J, Xu M, Zhang H, Tu C, Yang J, et al. Engineered exosome for NIR-triggered drug delivery and superior synergistic chemo-phototherapy in a glioma model. *Appl Mater Today*. 2020;20, 100723.
116. Yang C, Guan Z, Pang X, Tan Z, Yang X, Li X, et al. Desialylated mesenchymal stem cells-derived extracellular vesicles loaded with doxorubicin for targeted inhibition of hepatocellular carcinoma. *Cells*. 2022;11(17):2642.
117. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol*. 2011;29(4):341–345.
118. Peng H, Li Y, Ji W, Zhao R, Lu Z, Shen J, et al. Intranasal administration of self-oriented nanocarriers based on therapeutic exosomes for synergistic treatment of Parkinson's disease. *ACS Nano*. 2022;16(1):869–884.
119. Qu M, Lin Q, Huang L, Fu Y, Wang L, He S, et al. Dopamine-loaded blood exosomes targeted to brain for better treatment of Parkinson's disease. *J Control Release*. 2018;287:156–166.
120. Grant GJ, Barenholz Y, Bolotin EM, Bansinath M, Turndorf H, Piskoun B, et al. A novel liposomal bupivacaine formulation to produce ultralong-acting analgesia. *The Journal of the American Society of Anesthesiologists*. 2004;101(1):133–137.
121. Sundar S, Jha TK, Thakur CP, Mishra M, Singh VP, Buffels R. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: a multicenter study. *Clin Infect Dis*. 2003;37(6):800–804.
122. Lukyanov AN, Elbayoumi TA, Chakilam AR, Torchilin VP. Tumor-targeted liposomes: doxorubicin-loaded long-circulating liposomes modified with anti-cancer antibody. *J Control Release*. 2004;100(1):135–144.
123. Zhou J, Zhao WY, Ma X, Ju RJ, Li XY, Li N, et al. The anticancer efficacy of paclitaxel liposomes modified with mitochondrial targeting conjugate in resistant lung cancer. *Biomaterials*. 2013;34(14):3626–3638.
124. Joo KI, Xiao L, Liu S, Liu Y, Lee CL, Conti PS, et al. Crosslinked multilamellar liposomes for controlled delivery of anticancer drugs. *Biomaterials*. 2013;34(12):3098–3109.
125. Admyre C, Johansson SM, Qazi KR, Filén JJ, Lahesmaa R, Norman M, et al. Exosomes with immune modulatory features are present in human breast milk. *The Journal of immunology*. 2007;179(3):1969–1978.
126. Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C. Exosomal-like vesicles are present in human blood plasma. *Int Immunol*. 2005;17(7):879–887.
127. Zhou H, Pisitkun T, Aponte A, Yuen PS, Hoffert JD, Yasuda H, et al. Exosomal Fetuin-A identified by proteomics: a novel urinary biomarker for detecting acute kidney injury. *Kidney Int*. 2006;70(10):1847–1857.
128. Hood JL, San RS, Wickline SA. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. *Cancer Res*. 2011;71(11):3792–3801.
129. André F, Chaput N, Schartz NE, Flament C, Aubert N, Bernard J, et al. Exosomes as potent cell-free peptide-based vaccine. *I Dendritic cell-derived exosomes transfer functional MHC class I/peptide complexes to dendritic cells The Journal of Immunology*. 2004;172(4):2126–2136.
130. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol*. 2011;29(4):341–345.
131. Antimisiaris SG, Kallinteri P, Fatouros DG. Liposomes and drug delivery. *Pharmaceutical manufacturing handbook production and processes*. 2008:443–507.
132. Sopyan I, Gozali D. A review: A novel of efforts to enhance liposome stability as drug delivery approach. *Systematic Reviews in Pharmacy*. 2020;11(6).
133. Guimarães D, Cavaco-Paulo A, Nogueira E. Design of liposomes as drug delivery system for therapeutic applications. *Int J Pharm*. 2021;601, 120571.
134.. Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H. Preparation, characterization and applications of liposomes: state of the art. *Journal of colloid Science and Biotechnology*. 2012;1(2):147–168.
135. Sebaaly C, Greige-Gerges H, Stainmesse S, Fessi H, Charcosset C. Effect of composition, hydrogenation of phospholipids and lyophilization on the characteristics of eugenol-loaded liposomes prepared by ethanol injection method. *Food Biosci*. 2016;15:1–10.
136. Chen C, Han D, Cai C, Tang X. An overview of liposome lyophilization and its future potential. *J Control Release*. 2010;142:299–311. https://doi.org/10.1016/j.jconrel.2009.10.024.
137. Pasarin D, Ghizdareanu AI, Enascuta CE, Matei CB, Bilbie C, Paraschiv-Palada L, et al. Coating materials to increase the stability of liposomes. *Polymers*. 2023;15(3):782.
138. Wu JY, Li YJ, Hu XB, Huang S, Xiang DX. Preservation of small extracellular vesicles for functional analysis and therapeutic applications: a comparative evaluation of storage conditions. *Drug Deliv*. 2021;28(1):162–170.
139. Syromiatnikova V, Prokopeva A, Gomzikova M. Methods of the large-scale production of extracellular vesicles. *Int J Mol Sci*. 2022;23(18):10522.
140. Pasarin D, Ghizdareanu AI, Enascuta CE, Matei CB, Bilbie C, Paraschiv-Palada L, et al. Coating materials to increase the stability of liposomes. *Polymers*. 2023;15(3):782.
141. Andra VVSNL, Pammi SVN, Bhatraju LVKP, Ruddaraju LK. A comprehensive review on novel liposomal methodologies, commercial formulations, clinical trials and patents. *Bionanoscience*. 2022;12(1):274–291.