Therapeutic Potential of Mesenchymal Stem/Stromal Cell–Derived Exosomes

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Abstract

Mesenchymal stem/stromal cells (MSCs) have attracted a great deal of attention in the last decades, initially for their ability to differentiate into other various cell types, then later for their ability to release various biological factors with therapeutic effects, such as the mediation of cellular regeneration and protection and immunomodulation, highlighting their repertoire of attributes. These properties of MSCs have seen them used in various clinical trials to treat various human diseases. Recently, MSCs have been utilized as a factory of cells to produce and secrete extracellular vesicles (EVs), as it has been shown that MSC-derived EVs may carry part of the therapeutic effects of their parent cells. Obviously, this is advantageous as it would be mean that cell-free therapies laced with the therapeutic effects of MSCs could be possible. EVs can be termed as apoptotic bodies, microvesicle bodies, or exosomes, depending on their size. Interestingly and unsurprisingly, as it is common knowledge in the field that MSCs respond differently depending on their microenvironment, it has been found that MSCs can be pre-conditioned to produce and secrete EVs with different therapeutic properties. Herein, recent findings highlighting the therapeutic potential of MSC-derived EVs will be discussed, specifically MSC-derived exosomes.

Keywords

MSC · Exosomes · Immunomodulatory · Disease · Clinical trials · Pre-conditioning

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Mesenchymal Stem/Stromal Cells

Mesenchymal stem/stromal cells (MSCs) have attracted a great deal of interest in the last several decades due to their fascinating attributes. Initially they were seen as a potential route by which human diseases with cellular death could be treated due to their potential capacity to differentiate into several cell types, and hence allow cellular regeneration (Abumaree et al. 2017). However, it later seemed that MSCs were not found in vivo or were found but at low cellular numbers following their administration, albeit their therapeutic effects were still present, which suggested that their therapeutic effects might not be related to their differentiation capacity, but instead on their secreted factors through paracrine mechanisms (Abumaree et al. 2017). It has since been found that the secreted factors of MSCs can mediate cellular protection and regeneration, as well as display immunomodulatory properties (Abumaree et al. 2017). Although the exact mechanisms of action of MSCs are not yet currently known, it has become clear that they mediate most of their effects through paracrine mechanisms via factors that mediate both trophic and immunomodulatory effects (Abumaree et al. 2017). To date, no clinical trial of MSCs has shown that MSC use is associated with any side effects, which is a huge leap in safety compared to the once highly touted embryonic stem cells. However, cell-free therapies are always more ideal, especially if similar therapeutic effects can still be
established. Therefore, there has been an explosion in the field of MSC research, involving the secreted factors or the secretome of MSCs. This research has specifically targeted and examined the extracellular vesicles (EVs) secreted from MSCs, which can come in various sizes, and were first described as “platelet dust” by Peter Wolf in 1967 (Keshhtkar et al. 2018; Wolf 1967). EVs can be apoptotic bodies of at least 1000 nm in diameter, microvesicles of around 100–1000 nm in diameter, and exosomes of around 30–150 nm in diameter (Gurunathan et al. 2019). Exosomes contain microRNA (miRNA), messenger RNA (mRNA), cytokines, lipids, and growth factors that together bestow on exosomes their therapeutic effects on surrounding cells (Casado-Díaz et al. 2020).

### 7.2 Exosomes

In addition to being secreted by MSCs, exosomes can also be secreted by cells throughout the human body, and hence can be found in various physiological fluids, including urine, saliva, amniotic fluid, milk, and blood (Iraci et al. 2016). There are three pathways by which exosomes can be produced, including the Endosomal Sorting Complexes Required for Transport (ESCRT)-dependent pathway, the ESCRT-independent pathway, and the direct budding of the plasma membrane of cells (Baietti et al. 2012; Casado et al. 2017; Li et al. 2015). In the ESCRT-dependent pathway, which is considered the main route by which exosomes are produced, intraluminal vesicles are formed, and subsequently there is generation of multivesicular bodies (MVBs), which then can either fuse with lysosomes in order to be degraded or fuse with the plasma membrane to release the exosomes (Frankel and Audhya 2018). Thereafter, neighboring cells endocytose the exosomes or take them up via direct or ligand-receptor binding (Kahroba et al. 2019). However, blocking the ESCRT pathway does not inhibit the formation of MVB and hence exosome production and secretion from cells, indicating that an ESCRT-independent pathway also exists such as the syndecan-syntenin-ALIX pathway (Baietti et al. 2012).

There are various methods by which exosomes can be isolated. The standard isolation method is differential centrifugation, which allows the isolation of exosomes based on their size and density (Livshits et al. 2015). Although this method provides several advantages, including being straightforward to implement and affordable but effective, it may lead to the isolation of exosomes with other contaminants, which is why sometimes it is used in conjunction with cushions containing iodixanol or sucrose (Street et al. 2017; Yamashita et al. 2016). In addition, filtration can be used to isolate exosomes, using filters of a certain pore size, where contaminants such as cellular debris or other molecules are filtered, then ultrafiltration is carried out to remove contaminants that are smaller in size (Li et al. 2017). However, there is a risk that the exosomes might be contaminated with particles of a similar size (Yu et al. 2018). Similar to the filtration technique, size exclusion chromatography (SEC) also isolates exosomes based on size, but through the use of beads that contain pores which capture exosomes, while larger particles...
that cannot be captured pass through the column (Boing et al. 2014). However, because SEC is not scalable, it may be combined with initial ultrafiltration or tangential flow filtration (Benedikter et al. 2017; Nordin et al. 2019). The purity of the isolation of exosomes can be enhanced through the use of immunoaffinity chromatography—antibodies in a column bind to specific surface ligands on the exosomes; however, a drawback of this method is that only small sample volumes can be used (Xu et al. 2019b). Exosomes can be further concentrated using precipitation methods, however, because these involve treatments using chemicals, the exosomes may not be suitable for downstream functional assays (Doyle and Wang 2019). In a nutshell, it is recommended to use a combination of the aforementioned methods, in order to isolate exosomes at a high yield as well as purity.

Exosomes are essentially composed of a lipid bilayer that encapsulates various biomolecules, including mRNAs, miRNAs, and proteins, with the tetraspanin family of proteins being the most commonly found proteins (Dong et al. 2019). The membranes of exosomes are composed of various lipids such as cholesterol, ceramide, sphingomyelin that form lipid rafts, and phosphatidylserine and prostaglandin, among others (de Gassart et al. 2003). Moreover, proteins that function as transporters at the surface of exosomes and fusion proteins can be expressed in exosomes (Conde-Vancells et al. 2008; Subra et al. 2010). A significant number of studies have been performed on exosomes and their components, with all of the data compiled on online databases such as Vesiclepedia and ExoCarta (Kalra et al. 2012; Simpson et al. 2012). Altogether, the studies suggest that the nature of the exosomes depends on their cell source and whether they are active in a pathological or physiological state, with common features shared such as expression of the tetraspanin proteins CD-9, CD-63, and CD-81 in the membrane surface of exosomes, and which are routinely utilized as exosomal markers (Andreu and Yanez-Mo 2014).

Characterization of exosomes following their isolation is essential, in order to confirm their presence and to understand what their cargos exactly contain in terms of lipids, proteins, and RNAs (Thery et al. 2018). Exosomes can be physically characterized using various techniques such as iodixanol or sucrose gradients, transmission electron microscopy, atomic force microscopy, dynamic light scattering, nanoparticle tracking analysis, and fluorescence correlation spectroscopy if the exosomes are fluorescently labeled (Filipe et al. 2010; Harding et al. 1984; Parisse et al. 2017; Szatanek et al. 2017; Thery et al. 2006; Wyss et al. 2014). Moreover, exosomes can be molecularly characterized for their protein, RNA, and lipid content. Techniques such as western blotting and flow cytometry employing multiplexed beads coated with antibodies or using fluorescent antibodies, imaging flow cytometry, or high-throughput proteomic studies can be utilized to confirm the expression of exosome-related proteins or to investigate the type of proteins expressed in certain exosomal preparations (Gorgens et al. 2019; Kowal et al. 2016; Stoner et al. 2016; Thery et al. 2018; Wiklander et al. 2018). The RNA content can be investigated using techniques such as quantitative polymerase chain reaction, RNA, or DNA sequencing, while the lipid content can be examined using techniques such as mass spectrometry or gas liquid-chromatography (Llorente et al.
Although there are various methods by which exosomes can be characterized following their isolation, there are still challenges due to their heterogeneity, small size, presence of contaminating particles, and the low sensitivity of these current technologies (Mateescu et al. 2017).

Exosomes mediate their effects when they are taken up by recipient cells, and then their genetic information in the form of mRNA and miRNA subsequently influences the host cell’s protein expression (Sun et al. 2013). Exosomes in some ways represent the parent cell they are derived from, and they will most likely express similar biological molecules (Rabinowits et al. 2009). In addition, the biological molecules encapsulated within exosomes are protected from the surrounding microenvironment similarly to liposomes; RNAs are protected from degradation by RNase (Luan et al. 2017). Moreover, these potentially therapeutic biomolecules can be transported to distant locations in the human body via body fluids to allow the mediation of intercellular communication between cells without a simultaneous induction of immune responses (Luan et al. 2017). Therefore, exosomes have been investigated heavily as a potential therapeutic tool, especially for regions in the body that have been previously difficult to penetrate such as the blood-brain barrier (Chen et al. 2016). Although use of MSCs in various clinical trials has not been associated with side effects, there is always still a potential for unexpected events such as uncontrolled growth of the cells in ectopic sites with potential formation of tumors. In contrast, therapy involving exosomes holds several advantages, in that they do not induce metastasis, divide uncontrollably, or become mutated like cancer cells (Phinney and Pittenger 2017). In the following sections, the therapeutic potential of MSC-derived exosomes will be discussed, as well as the potential to pre-condition MSCs, in order to generate exosomes equipped with certain therapeutic properties.

### 7.3 Therapeutic Applications of Mesenchymal Stem/Stromal Cell–Derived Exosomes

MSC-derived exosomes have been investigated as a potential therapy in vitro and various in vivo animal models of disease. Generally, they have proven to be a possibly effective therapy for various diseases, including those effecting the heart, brain, spinal cord, liver, kidney, skin, muscles, and lungs.

Cardiovascular-related diseases could be positively affected by MSC-derived exosomes. Exosomes derived from the bone marrow MSC were examined in a rat myocardial infarction (MI) model. It was found that there was a reduction in the inflammatory response via an impairment of T-cell function, as well as various positive effects on the cardiovascular system; there was an increase in the tube formation of human umbilical vein endothelial cells (HUVECs) with an increased number of new functional capillaries, a reduction in the size of infarcts, improved recovery of blood flow, and a corresponding conservation of both cardiac diastolic and systolic performance (Teng et al. 2015). In another study, exosomes derived from the bone marrow MSCs were found to reduce cell apoptosis and the size of
myocardial infarcts in a rat model of ischemia-reperfusion injury (Liu et al. 2017). Likewise, exosomes derived from adipose tissue MSC were found to protect the myocardium of rats from ischemia-reperfusion injury through activation of the Wnt/β-catenin pathway (Cui et al. 2017). Interestingly, exosomes derived from MSCs isolated from the endometrium were found to be more cardioprotective compared to exosomes from the bone marrow and adipose tissue in a rat MI model by increasing the density of microvessels, which was found to be mainly attributed to the enhanced expression of miRNA-21 in the exosomes that seemed to mediate an increase in cell survival and angiogenesis (Wang et al. 2017).

Several studies have found that exosomes derived from MSCs may hold a therapeutic benefit for kidney-related diseases. Exosomes from umbilical cord MSCs were examined in a cisplatin-induced acute kidney injury model. It was found that they mediate a reduction in the levels of creatinine and nitrogen, and the necrotic death of proximal kidney tubules via mechanisms opposing apoptosis and oxidation (Zhou et al. 2013). In the same model, it was found that horizontal transfer of mRNA encoding the insulin-like growth factor-1 receptor (IGF-1R) to tubular cells of the kidney from exosomes derived from bone marrow MSCs enhanced their sensitivity to IGF-1 found locally, unravelling a potential mechanism by which bone marrow MSCs may mediate renal protection (Tomasoni et al. 2013).

MSC-derived exosomes have demonstrated therapeutic effects for various diseases and disorders related to the nervous system. In a traumatic brain injury (TBI) rat model, it was found that bone marrow MSC–derived exosomes improved the recovery of function and increased neuroplasticity (Zhang et al. 2015b). In another study using the same model and cell source of exosomes, it was found that lesion size was reduced and neurobehavioral performance was enhanced through a reduction in pathways promoting apoptosis and inflammation (Ni et al. 2019). In a spinal cord injury (SCI) rat model, bone marrow MSC–derived exosomes reduced the size of lesions and allowed improvement in functional recovery, by reducing activation of pathways associated with apoptosis and inflammation, but instead promoted cell survival and anti-inflammatory pathways, as well as angiogenesis (Huang et al. 2017). Other studies in an SCI rat model found that bone marrow MSC–derived exosomes associated with immunosuppressive M2 macrophages located in the injured spinal cord, and reduced the activation of SCI-induced astrocytes with therapeutic effects compared to when only MSCs are given intravenously (Lankford et al. 2018; Wang et al. 2018a). MSC-derived exosomes have also shown neuroprotective effects in stroke through an improvement of angiogenesis, axonal plasticity, and enhanced regeneration of neurons (Zhang and Chopp 2016). Another study examined the effects of adipose MSC–derived exosomes on brain microvascular endothelial cells, which were deprived of oxygen and glucose, and found that the exosomes increased their migration and ability to undergo angiogenesis, suggesting a potential therapeutic use for recovery following stroke (Yang et al. 2018). The potential therapeutic benefits of bone marrow MSC–derived exosomes were also shown in an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis via a reduction of the levels of inflammatory cytokines and promotion of regulatory T cells (Riazifar et al. 2019). Exosomes derived from
adipose tissue MSCs were found to contain large amounts of neprilysin and significantly more than exosomes derived from bone marrow MSCs; this enzyme is essential in the degradation of β-amyloid peptide in the brain (Katsuda et al. 2013). Transfer of these neprilysin-rich exosomes into neuroblastoma N2a cells leads to a reduction in the levels of both secreted and intracellular β-amyloid peptide, suggesting a potential therapy for Alzheimer’s disease (Katsuda et al. 2013). Moreover, in a mouse model of Alzheimer’s disease, MSC-derived exosomes reduced beta amyloid–induced impairment of mice cognition and enhanced neurogenesis (Reza-Zaldivar et al. 2019).

Animal models of liver disease or injury have also been used to investigate the potential benefits of MSC-derived exosomes. Umbilical cord MSC–derived exosomes were found to inhibit the production of collagen and epithelial-mesenchymal transition of hepatocytes, as well as reinstated the activity of serum aspartate aminotransferase in a carbon tetrachloride–induced mouse model of liver fibrosis (Li et al. 2013). In a mouse hepatitis model induced with concanavalin A, it was found that exosomes derived from adipose MSCs reduced the increased serum levels of aspartate transferase and alanine aminotransferase, various inflammatory cytokines, inflammasome activation, as well as liver inflammation and necrosis (Lou et al. 2017).

The aforementioned studies indicate that MSC-derived exosomes mediate their positive effects through the immune system as well as the various organ systems. The following studies also suggest that a part of the therapeutic effect of MSC-derived exosomes is mediated through the immune system. In an animal model of bronchopulmonary dysplasia, it was found that exosomes derived from both the umbilical cord and bone marrow reduce inflammation, fibrosis, pulmonary hypertension, and pulmonary vascular modelling, which altogether improved lung function (Willis et al. 2018). It was found that the mechanism might be through a modulation of the phenotype of macrophages with an increase in the number of immunosuppressive M2 macrophages (Willis et al. 2018). Peripheral blood mononuclear cells (PBMCs) were isolated from asthmatic patients and treated with bone marrow MSC-derived exosomes, which increased their expression of IL-10 and transforming growth factor beta 1 (TGF-β1) that enhanced the function of immunosuppressive regulatory T cells (Du et al. 2018). Exosomes derived from adipose tissue–derived MSCs were found to reduce atopic dermatitis in an in vivo mouse model, which was mediated through a reduction in the levels of inflammatory cytokines, eosinophils, infiltrated mast cells, IgE, CD-86+, and CD-206+ cells (Cho et al. 2018). In a skin-defect mouse model, exosomes from umbilical cord MSCs were found to reduce scar formation and accumulation of myofibroblasts (Fang et al. 2016). While in a rat skin burn model, it was found that exosomes from umbilical cord MSCs enhanced the ability of skin wounds to re-epithelize, as well as promoted the ability of skin cells to proliferate and survive (Zhang et al. 2015a). In another study using the rat skin burn model, it was found that umbilical cord MSC–derived exosomes reduced the burn-induced inflammation, and this was attributed to the expression of exosomal miRNA-181c (Li et al. 2016). Interestingly, in an in vivo model of skeletal muscle injury, bone marrow MSC–derived exosomes were found
to enhance the regeneration of skeletal muscle, which was attributed partly to miRNAs contained in the exosomes (Nakamura et al. 2015). The pathological damage due to the inflammation in a chronic graft-versus-host disease mouse model was found to be ameliorated, following treatment with exosomes from bone marrow MSC through a reduction in the activation and infiltration of CD-4+ T cells, suppression of T helper 17 cells, reduction in inflammatory cytokines, and an increase in the levels of regulatory T cells (Lai et al. 2018).

Although there has been a surge in the number of studies investigating the therapeutic potential of MSC-derived exosomes in vitro and various in vivo animal models of disease, there has not been a comparable increase in the number of clinical trials harnessing the therapeutic potential of MSC-derived exosomes. There are currently only nine international clinical trials that have been completed or are ongoing, examining the therapeutic potential of MSC-derived exosomes (Table 7.1) (clinicaltrial.gov). Interestingly, MSC-derived exosomes are being examined in a

| Condition/ s treated | Tissue source of MSC | Features | Country | Status | NCT number |
|----------------------|----------------------|----------|---------|--------|------------|
| Coronavirus          | Adipose              | Exosomes for treatment | China    | Not yet recruiting | NCT04276987 |
| Cerebrovascular disorders | Not stated           | Exosomes transfected with MiRNA-124 for treatment | Iran     | Completed     | NCT03384433 |
| Macular holes        | Umbilical cord       | Exosomes for treatment | China    | Recruiting    | NCT03437759 |
| Dry eye              | Umbilical cord       | Exosomes for reducing symptoms | China    | Recruiting    | NCT04213248 |
| Diabetes mellitus type 1 | Umbilical cord blood | Exosomes for treatment | Egypt    | Unknown       | NCT02138331 |
| Dystrophic epidermolysis bullosa | Not stated           | Exosomes for treatment | Not provided | Not yet recruiting | NCT04173650 |
| Chronic ulcer        | Wharton’s jelly      | Exosomes for treatment | Indonesia | Not yet recruiting | NCT04134676 |
| Pancreatic cancer    | Not stated           | Exosomes loaded with small interfering RNA against KrasG12D | United States | Not yet recruiting | NCT03608631 |
| Severe lung diseases | Adipose              | Aerosol inhalation of exosomes | China    | Recruiting    | NCT04313647 |
recently set up clinical trial as a potential therapy for the coronavirus, which has recently led to a worldwide pandemic in the last several weeks.

7.4 Pre-conditioning of Mesenchymal Stem/Stromal Cell–Derived Exosomes to Enhance Therapeutic Efficacy

As mentioned earlier, it is known in the field of MSC that these cells can modify their phenotypes and subsequent functional effects on surrounding cells based on the microenvironment they are exposed to. Therefore, it comes as no surprise that these properties of MSCs means that it is possible to pre-condition them, so that they produce exosomes with enhanced activities such as increased immunomodulation, cellular regeneration, and angiogenesis (Noronha et al. 2019). There are various ways by which MSCs can be pre-conditioned, including through the use of cytokines, hypoxia, and various biomolecules or chemicals.

It is widely known that inflammatory cytokines can enhance the therapeutic effects of MSCs. Interferon gamma (IFN-γ)-pre-conditioned bone marrow MSCs were found to secrete exosomes that reduced severe symptoms in an EAE mouse model through anti-inflammatory and neuroprotective effects (Riazifar et al. 2019). In addition, when PBMCs were cultured with the exosomes, their proliferation was reduced as well as their secretion of inflammatory cytokines, but they expressed more of immunosuppressive cytokines such as indoleamine-pyrolle 2,3-deoxygenase (IDO) (Riazifar et al. 2019). Pre-conditioning with tumor necrosis factor alpha (TNF-α) induced umbilical cord MSCs to secrete exosomes that prevented the activation of fibroblasts and reduced inflammation in an urethral fibrosis mouse model, which were effects attributed to the anti-inflammatory effects of miRNA-146a (Liang et al. 2019). Another study that carried out pre-conditioning with TNF-α found that adipose MSC–derived exosomes facilitated both the proliferation and differentiation of primary human osteoblasts, and this was attributed to the protein expression of Wnt-3a in the exosomes (Lu et al. 2017). The survival rate of a mouse model of sepsis was increased, following treatment with exosomes derived from umbilical cord MSC pre-conditioned with interleukin (IL)-1 beta (Song et al. 2017). The exosomes were found to contain miRNA-146a, which mediated anti-inflammatory effects by inducing differentiation of immunosuppressive M2 macrophages (Song et al. 2017). There are also studies where a combination of inflammatory cytokines is used in pre-conditioning. Exosomes derived from umbilical cord MSCs that were pre-conditioned with IFN-γ- and TGF-β-induced regulatory T cells, and this effect was attributed to IDO, suggesting a potential strategy for immune-mediated diseases (Zhang et al. 2018). Another study pre-conditioned adipose MSCs with IFN-γ and TNF-α and found that their exosomes induced immunosuppressive M2 macrophages, with the effects being attributed to the exosomal expression of the anti-inflammatory factors miRNA-34a and miRNA-146a (Domenis et al. 2018).

Hypoxia has also been found to modify MSCs and their exosomes, with the following studies showing that hypoxia pre-conditioning leads to exosomes that
promote angiogenesis, cardioprotection, immunomodulation, and neuroprotection. MSCs derived from the placenta were exposed to hypoxic conditions, and their secreted exosomes were found to promote the tube formation and migration of placental microvascular endothelial cells (Salomon et al. 2013). When exosomes from adipose MSCs pre-conditioned with hypoxia were used to treat HUVEC, it was found that the exosomes induced both tube formation and migration of the cells (Han et al. 2018). Moreover, the exosomes were found to reduce inflammation and increase the survival of fat grafts in a nude mouse model of subcutaneous fat grafting; mechanistically, it was found that there was increased protein expression of epidermal growth factor, fibroblast growth factor, angiopoietin-1, and the angiopoietin receptor that lead to an increase of angiogenesis in the fat grafts (Han et al. 2019). When HUVECs were treated with exosomes from adipose MSCs pre-conditioned with hypoxia, it was found that they more readily took up the exosomes, which enhanced their expression of vascular endothelial growth factor (VEGF) and the activation of the protein kinase A pathway that together increased their angiogenic capacity (Almeria et al. 2019; Xue et al. 2018). Cardioprotection has also been demonstrated in an MI mouse model where exosomes containing miRNA-125b-5p from bone marrow MSCs that were pre-conditioned with hypoxia mediated ischemic cardiac repair by reducing apoptosis of cardiomyocytes (Zhu et al. 2018). In a mouse model with acute lung injury induced by endotoxin, exosomes from bone marrow MSCs pre-conditioned with hypoxia reduced the levels of leukocytes, including neutrophils in the bronchoalveolar lavage fluid (Lee et al. 2016). Interestingly, MSC-derived exosomes that were pre-conditioned with hypoxia underwent reprogramming of glycolysis and accumulated metabolites that induced anti-inflammatory effects through the induction of regulatory T cells and M2 macrophage polarization (Showalter et al. 2019). Hypoxia pre-conditioned MSC exosomes have also been found to mediate neuroprotection in a mouse model of Alzheimer’s disease. The exosomes from bone marrow MSC were found to reduce deposition of amyloid plaque and the levels of beta amyloid reducing synaptic dysfunction, and increased the levels of anti-inflammatory cytokines to altogether significantly improve the learning and memory of the mice with the effects being attributed to the exosomal expression of miRNA-21 (Cui et al. 2018).

Various biomolecules have also been examined for their ability to modify the function of MSC-derived exosomes. Lipopolysaccharide (LPS)-pre-conditioned umbilical cord MSC exosomes displayed expression of miRNA let-7b that was shuttled to THP-1 cells and led to them increasing their secretion of anti-inflammatory cytokines and their polarization into M2 macrophages (Ti et al. 2015). Increased M2 macrophage polarization was also observed in a mouse model of MI that mediated a reduction of inflammation and apoptotic death of cardiomyocytes, following treatment with LPS-pre-conditioned bone marrow MSC exosomes (Xu et al. 2019a). The survival of a mouse model of acute radiation syndrome was increased, following treatment with bone marrow MSC exosomes that were pre-conditioned with LPS; clinical signs of radiation injury were reduced, and there was restoration of hematopoietic tissue in the bone marrow and spleen (Kink et al. 2019). Thrombin has also been used in pre-conditioning; exosomes
derived from Wharton’s jelly MSCs displayed increased anti-apoptotic and anti-inflammatory effects that mediated the reduction of brain infarctions in a rat model of hypoxic ischemic encephalopathy (Casado-Diaz et al. 2020). Moreover, bone marrow MSC exosomes pre-conditioned with melatonin reduced oxidative stress, apoptosis, inflammation, and increased cellular regeneration and angiogenesis, which altogether protected against renal ischemia-reperfusion injury in rats (Alzahrani 2019). Interestingly, advanced glycation end products-pre-conditioned bone marrow MSC exosomes displayed increased expression of miRNA-146a and were found to reduce calcification of vascular smooth muscle cells in vitro (Wang et al. 2018b). Deferoxamine was found to enhance the expression of miRNA-126a in exosomes derived from bone marrow MSCs, which induced vascularization and promoted recovery of skin wounds in a diabetic skin wound rat model (Ding et al. 2019). Suxiao Jiuxin pill, which is used to treat acute coronary syndrome in traditional Chinese medicine, was used to pre-condition cardiac MSC exosomes that were found to enhance the proliferation of cardiomyocytes (Ruan et al. 2018). Placental MSCs were pre-conditioned with nitric oxide, which induced their exosomes to enhance their expression of miRNA-126 and VEGF that increased the ability of HUVEC to undergo angiogenesis (Du et al. 2017). When hydrogen peroxide was used to pre-condition adipose MSCs, their exosomes were used to treat a rat model of ischemia-reperfusion injury, which reduced inflammation and apoptosis and increased vascularization that led to an improved recovery of skin flaps (Bai et al. 2018).

7.5 Concluding Remarks

Like the field of MSC, there has been an exponential growth in the field of exosomes. Together, both of these fields could hold the potential for future safe and efficacious therapies, targeting a wide range of diseases, including those that are associated with inflammation. Since exosomes carry part of the therapeutic effects of their parent cells, they could bypass any safety concerns associated with the injection of live MSCs. Although it is evident that exosomes like their MSC counterparts mediate their functions by inducing immunosuppressive effects on their surrounding cells, they may also possess other therapeutic effects, which require future studies to unearth. Moreover, pre-conditioning of MSCs, so that the exosomes that they secrete have altered therapeutic effects, may tailor the use of exosomes to target certain diseases. Importantly, since MSCs can exist in different physiological states, depending on the microenvironment they are exposed to, pre-conditioning will play an essential role in ensuring that the generated exosomes meet the desired needs of a specific therapeutic application. In addition, this lot-to-lot variation of MSCs could be overcome by using MSCs derived from induced pluripotent stem cells, which have been suggested to generate MSCs that behave consistently between preparations (Luzzani and Miriuka 2017).

Before MSC-derived exosomes can be applied to their full potential clinically, there needs to be standard procedures developed internationally for their isolation,
purification, and characterization, as well as guidelines for what level of purity is required. This is in order to prevent exosome preparations from being contaminated with other unwanted particles and to achieve the desired therapeutic effects. Furthermore, international standards for the quality control of MSC-derived exosomes also need to be formulated. Altogether, if the abovementioned points can be addressed, then it might lead to the accelerated emergence of MSC-derived exosomes for various future clinical applications.

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References

Abumaree MH, Abomaray FM, Alshabibi MA, ALAskar AS, Kalionis B (2017) Immunomodulatory properties of human placental mesenchymal stem/stromal cells. Placenta 59:87–95
Almeria C, Weiss R, Roy M, Tripisciano C, Kasper C, Weber V, Egger D (2019) Hypoxia conditioned mesenchymal stem cell-derived extracellular vesicles induce increased vascular tube formation in vitro. Front Bioeng Biotechnol 7:292
Alzahrani FA (2019) Melatonin improves therapeutic potential of mesenchymal stem cells-derived exosomes against renal ischemia-reperfusion injury in rats. Am J Transl Res 11:2887–2907
Andreu Z, Yanez-Mo M (2014) Tetraspanins in extracellular vesicle formation and function. Front Immunol 5:442
Bai Y, Han YD, Yan XL, Ren J, Zeng Q, Li XD, Pei XT, Han Y (2018) Adipose mesenchymal stem cell-derived exosomes stimulated by hydrogen peroxide enhanced skin flap recovery in ischemia-reperfusion injury. Biochem Biophys Res Commun 500:310–317
Baietti MF, Zhang Z, Mortier E, Melchior A, Degeest G, Geeraerts A, Ivarsson Y, Depoortere F, Coomans C, Vermeiren E et al (2012) Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. Nat Cell Biol 14:677–685
Benedikter BJ, Bouwman FG, Vajen T, Heinzmann ACA, Grauls G, Mariman EC, Wouters EFM, Savelkoul PH, Lopez-Iglesias C, Koenen RR et al (2017) Ultrafiltration combined with size exclusion chromatography efficiently isolates extracellular vesicles from cell culture media for compositional and functional studies. Sci Rep 7:15297
Boing AN, van der Poel E, Grootemaat AE, Coumans FA, Sturk A, Nieuwland R (2014) Single-step isolation of extracellular vesicles by size-exclusion chromatography. J Extracell Vesicles 3
Casado S, Lobo M, Paino CL (2017) Dynamics of plasma membrane surface related to the release of extracellular vesicles by mesenchymal stem cells in culture. Sci Rep 7:6767
Casado-Diaz A, Quesada-Gomez JM, Dorado G (2020) Extracellular vesicles derived from mesenchymal stem cells (MSC) in regenerative medicine: applications in skin wound healing. Front Bioeng Biotechnol 8:146
Chen CC, Liu L, Ma F, Wong CW, Guo YE, Chacko JV, Farhoodi HP, Zhang SX, Zimak J, Segaliny A et al (2016) Elucidation of exosome migration across the blood-brain barrier model in vitro. Cell Mol Bioeng 9:509–529
Cho BS, Kim JO, Ha DH, Yi YW (2018) Exosomes derived from human adipose tissue-derived mesenchymal stem cells alleviate atopic dermatitis. Stem Cell Res Ther 9:187

Conde-Vancells J, Rodriguez-Suarez E, Embade N, Gil D, Matthiesen R, Valle M, Elortza F, Lu SC, Mato JM, Falcon-Perez JM (2008) Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. J Proteome Res 7:5157–5166

Cui X, He Z, Liang Z, Chen Z, Wang H, Zhang J (2017) Exosomes from adipose-derived mesenchymal stem cells protect the myocardium against ischemia/reperfusion injury through Wnt/beta-catenin signaling pathway. J Cardiovasc Pharmacol 70:225–231

Cui GH, Wu J, Mou FF, Xie WH, Wang FB, Wang QL, Fang J, Xu YW, Dong YR, Liu JR et al (2018) Exosomes derived from hypoxia-preconditioned mesenchymal stromal cells ameliorate cognitive decline by rescuing synaptic dysfunction and regulating inflammatory responses in APP/PS1 mice. FASEB J 32:654–668

Ding J, Wang X, Chen B, Zhang J, Xu J (2019) Exosomes derived from human bone marrow mesenchymal stem cells stimulated by deferoxamine accelerate cutaneous wound healing by promoting angiogenesis. Biomed Res Int 2019:9742765

Domenis R, Cifu A, Quaglia S, Pistics C, Moretti M, Vicario A, Parodi PC, Fabris M, Niazi KR, Soon-Shiong P et al (2018) Pro inflammatory stimuli enhance the immunosuppressive functions of adipose mesenchymal stem cells-derived exosomes. Sci Rep 8:13325

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 Res Int 2019:6458237

Doyle LM, Wang MZ (2019) Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. Cell 8:727

Du W, Zhang K, Zhang S, Wang R, Nie Y, Tao H, Han Z, Liang L, Wang D, Liu J et al (2017) Enhanced proangiogenic potential of mesenchymal stem cell-derived exosomes stimulated by a nitric oxide releasing polymer. Biomaterials 133:70–81

Du YM, Zhuansun YX, Chen R, Lin L, Lin Y, Li JG (2018) Mesenchymal stem cell exosomes promote immunosuppression of regulatory T cells in asthma. Exp Cell Res 363:114–120

Fang S, Xu C, Zhang Y, Xue C, Yang C, Bi H, Qian X, Wu M, Ji K, Zhao Y et al (2016) Umbilical cord-derived mesenchymal stem cell-derived exosomal MicroRNAs suppress myofibroblast differentiation by inhibiting the transforming growth factor-beta/SMAD2 pathway during wound healing. Stem Cells Transl Med 5:1425–1439

Filipe V, Hawe A, Jiskoot W (2010) Critical evaluation of nanoparticle tracking analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. Pharm Res 27:796–810

Frankel EB, Audhya A (2018) ESCRT-dependent cargo sorting at multivesicular endosomes. Semin Cell Dev Biol 74:4–10

de Gassart A, Geminard C, Fevrier B, Raposo G, Vidal M (2003) Lipid raft-associated protein sorting in exosomes. Blood 102:4336–4344

Gorgens A, Bremer M, Ferrer-Tur R, Murke F, Tertel T, Horn PA, Thalmann S, Welsh JA, Probst C, Guerin C et al (2019) Optimisation of imaging flow cytometry for the analysis of single extracellular vesicles by using fluorescence-tagged vesicles as biological reference material. J Extracell Vesicles 8:1587567

Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH (2019) Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. Cell 8:307

Han YD, Bai Y, Yan XL, Ren J, Zeng Q, Li XD, Pei XT, Han Y (2018) Co-transplantation of exosomes derived from hypoxia-preconditioned adipose mesenchymal stem cells promotes neovascularization and graft survival in fat grafting. Biochem Biophys Res Commun 497:305–312

Han Y, Ren J, Bai Y, Pei X, Han Y (2019) Exosomes from hypoxia-treated human adipose-derived mesenchymal stem cells enhance angiogenesis through VEGF/VEGF-R. Int J Biochem Cell Biol 109:59–68

Harding C, Heuser J, Stahl P (1984) Endocytosis and intracellular processing of transferrin and colloidal gold-transferrin in rat reticulocytes: demonstration of a pathway for receptor shedding. Eur J Cell Biol 35:256–263
Huang JH, Yin XM, Xu Y, Xu CC, Lin X, Ye FB, Cao Y, Lin FY (2017) Systemic administration of exosomes released from mesenchymal stromal cells attenuates apoptosis, inflammation, and promotes angiogenesis after spinal cord injury in rats. J Neurotrauma 34:3388–3396.

Iraci N, Leonardi T, Gessler F, Vega B, Pluchino S (2016) Focus on extracellular vesicles: physiological role and signalling properties of extracellular membrane vesicles. Int J Mol Sci 17:171.

Kahroba H, Hejazi MS, Samadi N (2019) Exosomes: from carcinogenesis and metastasis to diagnosis and treatment of gastric cancer. Cell Mol Life Sci 76:1747–1758.

Kalra H, Simpson RJ, Ji H, Aikawa E, Altevogt P, Askenase P, Bond VC, Borras FE, Breakefield X, Budnik V et al (2012) Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. PLoS Biol 10:e1001450.

Katsuda T, Tsuchiya R, Kosaka N, Yoshioka Y, Takagaki K, Oki K, Takeshita F, Sakai Y, Kuroda M, Ochiya T (2013) Human adipose tissue-derived mesenchymal stem cells secrete functional nephrilysin-bound exosomes. Sci Rep 3:1197.

Keshtkar S, Azarpira N, Ghahremani MH (2018) Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. Stem Cell Res Ther 9:63.

Kink JA, Forsberg MH, Reshetylo S, Besharat S, Childs CJ, Pederson JD, Gendron-Fitzpatrick A, Graham M, Bates PD, Schmuck EG et al (2019) Macrophages educated with exosomes from primed mesenchymal stem cells treat acute radiation syndrome by promoting hematopoietic recovery. Biol Blood Marrow Transplant 25:2124–2133.

Kowal J, Arras G, Colombo M, Jouve M, Morath JP, Primald-Bengtson B, Dingli F, Loew D, Tkach M, Thery C (2016) Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc Natl Acad Sci U S A 113:E968–E977.

Lai P, Chen X, Guo L, Wang Y, Liu X, Liu Y, Zhou T, Huang T, Geng S, Luo C et al (2018) A potent immunomodulatory role of exosomes derived from mesenchymal stromal cells in preventing cGVHD. J Hematol Oncol 11:135.

Lankford KL, Arroyo EJ, Nazimek K, Bryniarski K, Askenase PW, Kocsis JD (2018) Intravenously delivered mesenchymal stem cell-derived exosomes target M2-type macrophages in the injured spinal cord. PLoS One 13:e0190358.

Lee TH, Chennakrishnaiah S, Meehan B, Montermini L, Garnier D, D’Asti E, Hou W, Magnus N, Gayden T, Jabado N et al (2016) Barriers to horizontal cell transformation by extracellular vesicles containing oncogenic H-ras. Oncotarget 7:51991–52002.

Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, Wang M, Zhou Y, Zhu W, Li W et al (2013) Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. Stem Cells Dev 22:845–854.

Li M, Rong Y, Chuang YS, Peng D, Emr SD (2015) Ubiquitin-dependent lysosomal membrane protein sorting and degradation. Mol Cell 57:467–478.

Li X, Liu L, Yang J, Yu Y, Chai J, Wang L, Ma L, Yin H (2016) Exosome derived from human umbilical cord mesenchymal stem cell mediates MiR-181c attenuating burn-induced excessive inflammation. EBioMedicine 8:72–82.

Li P, Kaslan M, Lee SH, Yao J, Gao Z (2017) Progress in exosome isolation techniques. Theranostics 7:789–804.

Liang YC, Wu YP, Li XD, Chen SH, Ye XJ, Xue XY, Xu N (2019) TNF-alpha-induced exosomal miR-146a mediates mesenchymal stem cell-dependent suppression of urethral stricture. J Cell Physiol 234:23243–23255.

Liu L, Jin X, Hu CF, Li R, Zhou Z, Shen CX (2017) Exosomes derived from mesenchymal stem cells rescue myocardial ischaemia/reperfusion injury by inducing cardiomyocyte autophagy via AMPK and Akt pathways. Cell Physiol Biochem 43:52–68.

Livshits MA, Khomyakova E, Evtushenko EG, Lazarev VN, Kulemin NA, Semina SE, Generozov EV, Govorun VM (2015) Isolation of exosomes by differential centrifugation: theoretical analysis of a commonly used protocol. Sci Rep 5:17319.
Llorente A, Skotland T, Sylvanne T, Kauhanen D, Rog T, Orlowski A, Vattulainen I, Ekroos K, Sandvig K (2013) Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. Biochim Biophys Acta 1831:1302–1309

Lou G, Chen Z, Zheng M, Liu Y (2017) Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases. Exp Mol Med 49:e346

Lu Z, Chen Y, Dunstan C, Roohani-Esfahani S, Zreiqat H (2017) Priming adipose stem cells with tumor necrosis factor-alpha preconditioning potentiates their exosome efficacy for bone regeneration. Tissue Eng Part A 23:1212–1220

Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D (2017) Engineering exosomes as refined biological nanoplatforms for drug delivery. Acta Pharmacol Sin 38:754–763

Luzzani CD, Miriuka SG (2017) Pluripotent stem cells as a robust source of mesenchymal stem cells. Stem Cell Rev Rep 13:68–78

Mateescu B, Kowal EJ, van Balkom BW, Bartel S, Bhattacharyya SN, Buzas EI, Buck AH, de Candia P, Chow FW, Das S et al (2017) Obstacles and opportunities in the functional analysis of extracellular vesicle RNA—an ISEV position paper. J Extracell Vesicles 6:1286095

Nakamura Y, Miyaki S, Ishitobi H, Matsuyama S, Nakasa T, Kamei N, Akimoto T, Higashi Y, Ochi M (2015) Mesenchymal-stem-cell-derived exosomes accelerate skeletal muscle regeneration. FEBS Lett 589:1257–1265

Ni H, Yang S, Siaw-Debrah F, Hu J, Wu K, He Z, Yang J, Pan S, Lin X, Ye H et al (2019) Exosomes derived from bone mesenchymal stem cells ameliorate early inflammatory responses following traumatic brain injury. Front Neurosci 13:14

Nordin JZ, Bostancioglu RB, Corso G, El Andaloussi S (2019) Tangential flow filtration with or without subsequent band-elute size exclusion chromatography for purification of extracellular vesicles. Methods Mol Biol 1953:287–299

Noronha NC, Mizukami A, Caliari-Oliveira C, Cominal JG, Rocha JLM, Covas DT, Swiech K, Malmegrim KCR (2019) Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. Stem Cell Res Ther 10:131

Parisse P, Rago I, Ulloa Severino L, Perissinotto F, Ambrosetti P, Paoletti R, Ricci M, Beltrami AP, Cessali D, Casalis L (2017) Atomic force microscopy analysis of extracellular vesicles. Eur Biophys J 46:813–820

Phinney DG, Pittenger MF (2017) Concise review: MSC-derived exosomes for cell-free therapy. Stem Cells 35:851–858

Rabinowits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH (2009) Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer 10:42–46

Rez-Zaldívar EE, Hernandez-Sapiens MA, Gutierrez-Mercado YK, Sandoval-Avila S, Gomez-Pinedo U, Marquez-Aguirre AL, Vazquez-Mendez E, Padilla-Camberos E, Canales-Aguirre AA (2019) Mesenchymal stem cell-derived exosomes promote neurogenesis and functional recovery in a mouse model of Alzheimer’s disease. Neural Regen Res 14:1626–1634

Riazifar M, Mohammadi MR, Pone EJ, Yeri A, Lasser C, Segaliny AL, McIntyre LL, Shelke GV, Hutchins E, Hamamoto A et al (2019) Stem cell-derived exosomes as nanotherapeutics for autoimmune and neurodegenerative disorders. ACS Nano 13:6670–6688

Ruan XF, Li YJ, Ju CW, Shen Y, Lei W, Chen C, Li Y, Yu H, Liu YT, Kim IM et al (2018) Exosomes from Suxiao Jiuxin pill-treated cardiac mesenchymal stem cells decrease H3K27 demethylase UTX expression in mouse cardiomyocytes in vitro. Acta Pharmacol Sin 39:579–586

Salomon C, Ryan J, Sobrevia L, Kobayashi M, Ashman K, Mitchell M, Rice GE (2013) Exosomal signaling during hypoxia mediates microvascular endothelial cell migration and vasculogenesis. PLoS One 8:e68451

Showalter MR, Wanczewicz B, Fiehn O, Archard JA, Clayton S, Wagner J, Deng P, Halmai J, Fink KD, Bauer G et al (2019) Primed mesenchymal stem cells package exosomes with metabolites associated with immunomodulation. Biochem Biophys Res Commun 512:729–735

Simpson RJ, Kalra H, Mathivanan S (2012) ExoCarta as a resource for exosomal research. J Extracell Vesicles 1
Song Y, Dou H, Li X, Zhao X, Li Y, Liu D, Ji J, Liu F, Ding L, Ni Y et al (2017) Exosomal miR-146a contributes to the enhanced therapeutic efficacy of interleukin-1beta-primed mesenchymal stem cells against sepsis. Stem Cells 35:1208–1221

Stoner SA, Duggan E, Condello D, Guerrero A, Turk JR, Narayanan PK, Nolan JP (2016) High sensitivity flow cytometry of membrane vesicles. Cytometry A 89:196–206

Street JM, Koritzinsky EH, Glispie DM, Yuen PST (2017) Urine exosome isolation and characterization. Methods Mol Biol 1641:413–423

Subra C, Grand D, Laulagnier K, Stella A, Lambeau G, Paillasse M, De Medina P, Monsarrat B, Perret B, Silvente-Poirot S et al (2010) Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. J Lipid Res 51:2105–2120

Sun D, Zhuang X, Zhang S, Deng ZB, Grizzle W, Miller D, Zhang HG (2013) Exosomes are endogenous nanoparticles that can deliver biological information between cells. Adv Drug Deliv Rev 65:342–347

Szatanek R, Baj-Krzyworzeka M, Zimoch J, Lecka M, Siedlar M, Baran J (2017) The methods of choice for extracellular vesicles (EVs) characterization. Int J Mol Sci 18

Teng X, Chen L, Chen W, Yang J, Yang Z, Shen Z (2015) Mesenchymal stem cell-derived exosomes improve the microenvironment of infarcted myocardium contributing to angiogenesis and anti-inflammation. Cell Physiol Biochem 37:2415–2424

Thery C, Amigorena S, Raposo G, Clayton A (2006) Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol Chapter 3:Unit 3.22

Thery C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK et al (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7:1535750

Ti D, Hao H, Tong C, Liu J, Dong L, Zheng J, Zhao Y, Liu H, Fu X, Han W (2015) LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. J Transl Med 13:308

Tomasoni S, Longaretti L, Rota C, Morigi M, Conti S, Gotti C, Capelli C, Introna M, Remuzzi G, Benigni A (2013) Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells. Stem Cells Dev 22:772–780

Turchinovich A, Drapkina O, Tonevitsky A (2019) Transcriptome of extracellular vesicles: state-of-the-art. Front Immunol 10:202

Wang K, Ji Jiang Z, Webster KA, Chen J, Hu H, Zhou Y, Zhao J, Wang L, Wang Y, Zhong Z et al (2017) Enhanced cardioprotection by human endometrium mesenchymal stem cells driven by exosomal MicroRNA-21. Stem Cells Transl Med 6:209–222

Wang L, Pei S, Han L, Guo B, Li Y, Duan R, Yao Y, Xue B, Chen X, Jia Y (2018a) Mesenchymal stem cell-derived exosomes reduce A1 astrocytes via downregulation of phosphorylated NF-kappaB P65 subunit in spinal cord injury. Cell Physiol Biochem 50:1535–1559

Wang Y, Ma WQ, Zhu Y, Han XQ, Liu N (2018b) Exosomes derived from mesenchymal stromal cells pretreated with advanced glycation end product-bovine serum albumin inhibit calcification of vascular smooth muscle cells. Front Endocrinol (Lausanne) 9:524

Wiklander OPB, Bostancioglu RB, Welsh JA, Zickler AM, Murke F, Corso G, Felldin U, Hagey DW, Evertsson B, Liang XM et al (2018) Systematic methodological evaluation of a multiplex bead-based flow cytometry assay for detection of extracellular vesicle surface signatures. Front Immunol 9:1326

Willis GR, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, Kwong A, Mitsialis SA, Kouroymbanas S (2018) Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation. Am J Respir Crit Care Med 197:104–116

Wolf P (1967) The nature and significance of platelet products in human plasma. Br J Haematol 13:269–288

Wubbolts R, Leckie RS, Veenhuizen PT, Schwarzmann G, Mobius W, Hoernschemeyer J, Slot JW, Geuze HJ, Stoorvogel W (2003) Proteomic and biochemical analyses of human B cell-derived...
exosomes. Potential implications for their function and multivesicular body formation. J Biol Chem 278:10963–10972
Wyss R, Grasso L, Wolf C, Grosse W, Demurtas D, Vogel H (2014) Molecular and dimensional profiling of highly purified extracellular vesicles by fluorescence fluctuation spectroscopy. Anal Chem 86:7229–7233
Xu R, Zhang F, Chai R, Zhou W, Hu M, Liu B, Chen X, Liu M, Xu Q, Liu N et al (2019a) Exosomes derived from pro-inflammatory bone marrow-derived mesenchymal stem cells reduce inflammation and myocardial injury via mediating macrophage polarization. J Cell Mol Med 23:7617–7631
Xu Y, Shen L, Li F, Yang J, Wan X, Ouyang M (2019b) microRNA-16-5p-containing exosomes derived from bone marrow-derived mesenchymal stem cells inhibit proliferation, migration, and invasion, while promoting apoptosis of colorectal cancer cells by downregulating ITGA2. J Cell Physiol 234:21380–21394
Xue C, Shen Y, Li X, Li B, Zhao S, Gu J, Chen Y, Ma B, Wei J, Han Q et al (2018) Exosomes derived from hypoxia-treated human adipose mesenchymal stem cells enhance angiogenesis through the PKA signaling pathway. Stem Cells Dev 27:456–465
Yamashita T, Takahashi Y, Nishikawa M, Takakura Y (2016) Effect of exosome isolation methods on physicochemical properties of exosomes and clearance of exosomes from the blood circulation. Eur J Pharm Biopharm 98:1–8
Yang Y, Cai Y, Zhang Y, Liu J, Xu Z (2018) Exosomes secreted by adipose-derived stem cells contribute to angiogenesis of brain microvascular endothelial cells following oxygen-glucose deprivation in vitro through MicroRNA-181b/TRPM7 axis. J Mol Neurosci 65:74–83
Yu LL, Zhu J, Liu JX, Jiang F, Ni WK, Qu LS, Ni RZ, Lu CH, Xiao MB (2018) A comparison of traditional and novel methods for the separation of exosomes from human samples. Biomed Res Int 2018:3634563
Zhang ZG, Chopp M (2016) Exosomes in stroke pathogenesis and therapy. J Clin Invest 126:1190–1197
Zhang B, Wang M, Gong A, Zhang X, Wu X, Zhu Y, Shi H, Wu L, Zhu W, Qian H et al (2015a) HucMSC-exosome mediated-Wnt4 signaling is required for cutaneous wound healing. Stem Cells 33:2158–2168
Zhang Y, Chopp M, Meng Y, Katakowski M, Xin H, Mahmood A, Xiong Y (2015b) Effect of exosomes derived from multipotential mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. J Neurosurg 122:856–867
Zhang Q, Fu L, Liang Y, Guo Z, Wang L, Ma C, Wang H (2018) Exosomes originating from MSCs stimulated with TGF-beta and IFN-gamma promote Treg differentiation. J Cell Physiol 233:6832–6840
Zhou Y, Xu H, Xu W, Wang B, Wu H, Tao Y, Zhang B, Wang M, Mao F, Yan Y et al (2013) Exosomes released by human umbilical cord mesenchymal stem cells protect against cisplatin-induced renal oxidative stress and apoptosis in vivo and in vitro. Stem Cell Res Ther 4:34
Zhu LP, Tian T, Wang JY, He JN, Chen T, Pan M, Xu L, Zhang HX, Qiu XT, Li CC et al (2018) Hypoxia-elicited mesenchymal stem cell-derived exosomes facilitates cardiac repair through miR-125b-mediated prevention of cell death in myocardial infarction. Theranostics 8:6163–6177