D J Med Sci 2023;9(1):24-38
doi: 10.5606/fng.btd.2023.128

Review

Exosomes derived from mesenchymal stem cells: Their content, obtaining methods, and therapeutic effects

Öznur Safgöl[1], Oytun Erbaş[2]

[1]*Bioengineering, Yıldız Technical University, Istanbul, Türkiye*
[2]*Institute of Experimental Medicine, Kocaeli, Türkiye*

ABSTRACT

Extracellular vesicles (EVs) are particles released by almost all types of cells into the extracellular space and are delimited by a lipid bilayer membrane, but they cannot duplicate themselves like cells. They can be divided into three subtypes: microvesicles, apoptotic bodies, and exosomes, according to their size, synthesis mechanism, and origin. Although the size of EVs varies between 50 to 1000 nm, exosomes are the smallest vesicles derived from the endosomal pathway and are typically 30-150 nm in size. Exosomes can be derived from various sources, such as dendritic cells and mesenchymal stem cells (MSCs). Among these sources, MSCs are the most convenient and efficient sources since they are biocompatible and offer potential as a therapeutic agent. Mesenchymal stem cell-derived exosomes contain a variety of biomolecules, including proteins, lipids, and carbohydrates, as well as nucleic acids such as deoxyribonucleic acid and micro-ribonucleic acid that have significant functions in transferring genetic material between cells. The MSCs-derived exosomes have emerged as an area of intense research interest in recent years due to their potential applications in various fields such as regenerative medicine, immunotherapy, and drug delivery. In this review, we not only emphasize exosomes and their biological functions but also examine MSC-derived exosomes and their obtaining methods in detail, as well as the current state of knowledge and research on their therapeutic strategies. Furthermore, we discuss new methodologies concerning the challenges of applying exosomes in healthcare and emphasize future perspectives to present effective and safe insights for exosome studies.

Keywords: Biogenesis, exosomes, isolation, mesenchymal stem cells, therapeutic effect.

Both prokaryotic and eukaryotic cells require the transfer of biological information throughout their cells. Therefore, a release mechanism occurs between cells, and the release of vesicles is beneficial for biological processes. Extracellular vesicles (EVs) are small, lipid bilayer and phospholipid-enclosed particles that play a pivotal role in releasing biological information as cargo.[1] They have an endocytic origin and typically circulate in bodily fluids. Body fluids such as blood and urine contain 10^{10} vesicles per mL, as cells release vesicles into their environment, and conditioned culture media usually include the same amount of cell-derived vesicles per mL.[2]

Received: March 16, 2023
Accepted: March 23, 2023
Published online: May 24, 2023

Correspondence: Öznur Safgöl.
E-mail: oznursafgol@gmail.com

Cite this article as:
Safgöl Ö, Erbaş O. Exosomes derived from mesenchymal stem cells: Their content, obtaining methods, and therapeutic effects. D J Med Sci 2023;9(1):24-38. doi: 10.5606/fng.btd.2023.128.

Extracellular vesicles are typically classified into three subtypes: microvesicles, apoptotic bodies, and exosomes, based on their origin, biogenesis, and size. Generally, their size ranges from 50 to 1000 nm.[3] These nanoscale particles are essential for both prokaryotic and eukaryotic cells, as they transfer specific compositions such as proteins, lipids, carbohydrates, and nucleic acids such as ribonucleic acid (RNA), micro-ribonucleic acid (miRNA), and deoxyribonucleic acid (DNA). All three types of vesicles play a significant role in various biological processes, such as homeostasis, coagulation, and intercellular signaling, as the recipient cells receive their components.[4-6] Although vesicles were previously known as waste management particles associated with the apoptosis process, recent studies suggest that EVs play a crucial role in cell-to-cell communication by carrying specific biological components. These studies point out that vesicles can be used for therapeutic purposes, and in the treatment of various diseases, depending on the methods used to obtain them.[7] Exosomes,

in particular, have gained much attention lately due to their several applications. However, their molecular mechanism and therapeutic activities require further investigation. This review will focus on the functional roles, sources, and biogenesis of exosomes in the body, isolation techniques, and recent developments in therapeutic studies. Additionally, we will discuss the promising aspects of mesenchymal stem cells (MSCs) derived exosomes, although they have some disadvantages, and groundbreaking new technological approaches by exosomes will be included.

Exosomes

Exosomes are well-known EVs that are nanosized and released by most cells, including in various body fluids such as urine, blood, and saliva. They are the smallest vesicles with sizes ranging from 30 to 150 nm and have a spherical shape that is limited by a lipid bilayer. Exosomes, when in a sucrose density gradient solution, float at a density of 1.15-1.19 g/mL^{-1}.[2] These vesicles transfer genetic materials such as messenger RNA (mRNA) and miRNA, and they also carry cargo molecules that include bioactive substances such as proteins, carbohydrates, lipids, and cytokines to recipient cells. The biochemical compositions of these recipient cells are altered by the transferred exosomes, affecting their signaling pathways. Exosomes can carry bioactive components and easily transfer externally given components to recipient cells through multiple pathways and regions. These active agents can serve therapeutic purposes, such as tissue repair, cancer diagnosis, and immune regulation.[8-10] In the past, exosomes were thought to be only particles that contribute to apoptosis, but research on their cell-to-cell communication feature and potential therapeutic applications in several diseases, such as neurodegenerative, cardiovascular, and lung disease, has dramatically increased in recent years.[11,12]

The concept of exosomes was first reported 42 years ago by Trams et al.[13] in 1981. They proposed the concept of exosomes, which are accepted as membrane vesicles with 5' nucleotide enzyme activity that may have physiological functions. These vesicles were arising from the exudation of various cell line cultures, and they are collectively called the vesicles produced in the plasma membrane as exosomes. The currently accepted concept of exosomes was introduced in 1983 by Johnstone et al.[14] who was working with maturing reticulocytes. During their study of transferrin receptors during maturation, they found that the loss of transferrin receptors that occurs in mature red blood cells is part of the exosome formation mechanism. They called it "exosomes" to distinguish it from EVs. In addition, in the Minimal Information for Studies of Extracellular Vesicles (MISEV) 2018 guidelines, it is stated that changing the concept of exosomes to small EVs is more appropriate due to the difficulties of separating exosomes.[15]

Although the structure of exosomes is specific to the cell in which they are secreted, some specific components are seen in each exosome, including heat shock proteins (Hsp 60, Hsp 70, and Hsp 90), certain tetraspanins (CD9, CD63, and CD81), proteins related to biogenesis such as tumor susceptibility gene (TSG) 101, fusion proteins (annexins, nucleotide guanosine triphosphate (GTP), and Rab proteins), nucleic acids (DNA, mRNA, miRNA, and long non-coding RNAs), lipids such as cholesterol, and membrane transport. Proteins in the structure of exosomes are divided into two. Among them, fusion proteins, Hsps, four-transmembrane protein superfamily, integrins, and endosomal sorting complex required for transport (ESCRT) complex-related proteins are proteins that are involved in membrane transport and fusion processes and participate in vesicle formation and secretion. Other proteins, such as CD45 and MHC-II, are closely associated with progenitor cells and are derived from antigen-presenting cells as well as cell-specific components. The difference in the content of exosomes ensures that parent cell signals are transmitted to recipient cells without direct contact between the cells. Furthermore, the diversity in the content of exosomes makes them a valuable tool in diagnosing and treating various diseases.[16] For example, by monitoring the components secreted by a specific type of tumor cell, one can gather information about the disease by examining intracellular communication and the release of exosomes. Tan et al.[17] focused on the numerous functions and mechanisms of tumor-derived exosomal components in breast cancer metastasis. The researchers addressed the

role of these exosomes in the development of a pre-metastatic niche and how they aid metastasis through several methods, such as promoting angiogenesis and suppressing the immune system. Exosomal components have the potential to serve as biomarkers and therapeutic targets for breast cancer metastasis, as demonstrated in the study. Overall, the researchers emphasized the importance of understanding the complex interactions between tumor-derived exosomal elements and the tumor microenvironment in the initiation and progression of breast cancer metastasis.

EXOSOME BIOGENESIS AND SECRETION

Exosomes are distinguished from other EVs not only by their size and origin but also by their biogenesis. In general, exosomes are formed by the inward folding of the plasma membrane during the endosomal maturation process, but microvesicles, for example, are formed by budding off the cell membrane. In the early stages of cell membrane infolding, initial endosomes are formed and bioactive substances accumulate in these early sorting endosomes. These endosomes are then mature into late sorting endosomes, which contain intraluminal vesicles (ILVs) formed by inward budding of the endosomal membrane, under the control of the ESCRT machinery and other related proteins.[18] Late sorting endosomes that have undergone a second indentation are called multiple vesicular bodies (MVBs), which contain multiple ILVs. Exosomes are secreted by cells through a process known as exocytosis, which involves the fusion of exosome-containing MVBs with the plasma membrane. Exosomes are released into the extracellular place to interact with other cells and tissues as a result of MVBs fusing with the plasma membrane.[19] In addition, MVBs can also be transported to a lysosome and digested as can be seen in Figure 1. The secretion of exosomes can be regulated by various factors such as environmental factors, signaling pathways, and cellular stress. The specific mechanisms of exosome biogenesis are still an area of interest for researchers, and the formation mechanism of exosomes can differ according to their origin. Moreover, there is debate about whether all vesicles released from cells are exosomes, and various studies have recently been conducted to isolate and characterize exosomes from other types of EVs, recently.[20]

The biogenesis of exosomes is divided into ESCRT-dependent and ESCRT-independent mechanisms. However, it is believed that components such as lipids and four-transmembrane domain proteins are involved in complex interactions in ESCRT-mediated vesicle formation and release. Although the effect on ILV formation is unknown, it has been observed that the expression of oncogenes activated through p52 regulation is induced by exosome secretion, much like hypoxia and genotoxic stress.[21] Additionally, the upregulation of six-transmembrane epithelial antigen of prostate 3, syndecan-4, and NadB have also been used to increase exosome production up to 10-fold in cell culture studies.[22] The mechanism of these processes is not fully understood due to knockdown procedures. The biogenesis of exosomes utilizes complexes with different names depending on whether it is in metazoans, protozoans, or other origins, but this article focuses on metazoans.

ESCRT-mediated pathway

The formation of ILVs from the limiting membrane of maturing endosomes plays an important role in the biogenesis of exosomes. Five different complexes such as ESCRT 0-III and Vps4 and two dozen proteins are involved in the formation of ILV called ESCRT. These complexes have functions such as cargo separation and membrane shaping.[21,22]

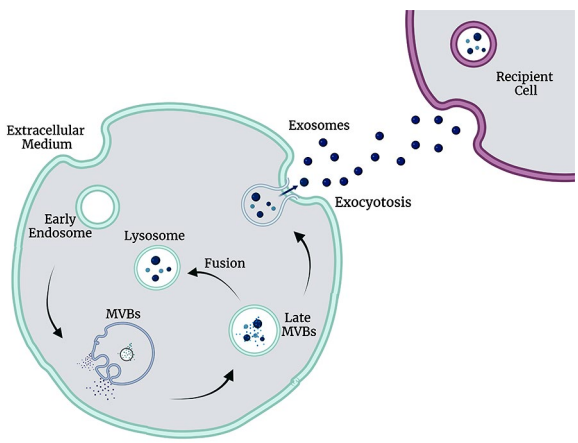

Figure 1. Biogenesis of exosomes, release and uptake mechanism.
The figure was drawn in Biorender.

The initial stage in the formation of ILVs is initiated by the ESCRT-0 complex. Comprised of signal-transducing adaptor molecule and Hrs proteins, which are continuously associated with each other as a heterodimer and heterotetramer, this complex interacts with membrane areas that are rich in phosphatidylinositol 3-phosphate. It also binds to general cargo via zinc finger domains and ubiquitin-interaction motifs, respectively.[23]

The ESCRT-I is a complex that binds to the C-terminus of the Hrs subunit of ESCRT-0 and is a heterotetramer of the TSG101, Vps37, MVB12, and Vps28 proteins and MVBs. The ESCRT-I complex forms a rod-like structure at opposite ends for ESCRT-0 and ESCRT-II. The ESCRT-I and ESCRT-II are also called supercomplexes, this supercomplex also induces endosome exit from the cytoplasm. In this process, the uploads move into the bud.[24]

The ESCRT-III complex is activated by the direct binding of CHMP6 to the ESCRT-II complex. This process is carried out after bud formation and cargo selection. The Vps4 complex consists of SKD1, LIP5, and CHMP5 proteins, and the ESCRT-III complex is disassembled by this complex.[23,24]

ESCRT-independent pathway

It has been suggested that the ESCRT-independent pathway cannot be considered independently from the ESCRT-mediated pathway and works together, but the molecular mechanisms of the ESCRT-independent pathway are not fully known. Besides, there may be several mechanisms in the ESCRT-independent pathway that involve sorting and budding processes, which do not rely on the commonly known ceramide-mediated membrane budding. However, the breakdown of sphingomyelin by neutral sphingomyelinase produces ceramide and forms raft-like structures. The reason for this is the self-assembly ability of ceramides. As a result of the assembly, it is said that the initial shape change of the membrane maintains.[25]

MESENCHYMAL STEM CELL BIOLOGY AND IMMUNOMODULATION

Mesenchymal stem cells belong to a unique category of stem cells that are characterized by their self-renewal and plastic adherent properties. These adult stem cells exhibit different properties depending on the tissue source from which they are obtained and play a crucial role in the regeneration and repair of various tissues. In addition to their unique features, MSCs possess multipotency, enabling them to differentiate into cells derived from all three embryonic germ lines.[26] Furthermore, the surface markers of MSCs distinguish them from other cell types. They express CD73, CD90, and CD105 at a rate of 95% and above on cell surfaces, while their expression of CD11β, CD14, CD19, CD34, CD45, CD79A, and HLA-DR markers is limited to 2% or less. Mesenchymal stem cells are typically isolated from various tissues, such as adipose tissue, bone marrow, cord blood, dental pulp, placenta, and skin. They can proliferate and renew themselves throughout the cell cycle and differentiate in response to various stimuli, such as environmental stressors. Researchers harness the potential of MSCs for the regeneration and repair of tissues. For instance, MSCs found in the bone marrow can differentiate into bone, cartilage, and adipose tissue cells, making them suitable for bone marrow transplantation procedures.[27,28] Moreover, their immunosuppressive and anti-inflammatory properties suggest that MSCs can potentially treat autoimmune and inflammatory diseases. However, further research is required to fully develop MSC-based therapies.

Recent studies have shown that MSC-derived exosomes may also be used as therapeutic agents due to their immunological functions. Zhang et al.[29] demonstrated that MSC-derived exosomes stimulated T cell proliferation and cytokine secretion, indicating that they possess immunologically active functions. This suggests that MSC-derived exosomes may play a role in modulating the immune response and treating immune-related disorders. Similarly, a study by Shabbir et al.[30] demonstrated that MSC-derived exosomes promote wound healing by enhancing cell proliferation, migration, and angiogenesis-related gene expression in both normal and chronic wound fibroblasts. In addition to their differentiation ability, the immunomodulatory potential of MSCs is also significant. Mesenchymal stem cells secrete various factors, including cytokines, chemokines, growth factors, and immunosuppressive molecules, which modulate

the function of the immune system. Mesenchymal stem cells influence the functions of immune cells, such as T cells, B cells, natural killer cells, and dendritic cells, in immune system-related diseases, including autoimmune diseases, allograft rejection, inflammatory diseases, and tissue damage.[31] The immunomodulatory ability of MSCs makes them a potential treatment modality for various diseases. The immunomodulatory effect of MSCs is based on several mechanisms that regulate the function of the immune system, including modulation of cytokine release, influencing the proliferation and differentiation of immune cells, triggering apoptosis of immune cells, and increasing the release of immunosuppressive molecules. Therefore, the immunomodulating ability of MSCs is a promising treatment modality for immune system-related diseases. However, more research and a better understanding of the mechanisms underlying the immunomodulating effect of MSCs are necessary to develop effective MSC-based therapies.

Mesenchymal stem cell-derived exosomes

Exosomes can be described as small, vesicle-like structures that contain information, such as protein and gene material which enables them to transfer information between cells. Of particular interest are exosomes that are secreted by MSCs due to their immunomodulatory functions. These exosomes can regulate the behavior of immune cells by binding to receptors on their surface, which changes their behavior through signal transduction. For example, MSC-derived exosomes can inhibit immune cells such as T cells and natural killer cells and can be used to treat autoimmune diseases and conditions associated with excessive immune response.[32] In addition to their immunomodulatory functions, MSC-derived exosomes can also regulate cytokines and chemokines that regulate inflammation. These exosomes can bind to cytokine and chemokine receptors on immune cells, inhibiting or activating signaling pathways dependent on these receptors. As a result, exosomes can regulate the inflammatory response, making them a promising therapeutic tool for immune system modulation.[33]

This can be particularly beneficial in conditions such as autoimmune diseases, inflammatory diseases, and transplantation. Exosomes can modulate the function of immune cells by inhibiting the release of pro-inflammatory cytokines, reducing the activation of immune cells, and inducing apoptosis in T cells. Hence, MSC-derived exosomes have the potential as an immunomodulatory therapeutic approach for the treatment of various diseases.[34,35]

These vesicles are an important communication tool that cells use to communicate with their environment. Mesenchymal stem cells-derived exosomes contain many different proteins, nucleic acids, lipids, and other biomolecules. These components, also, include growth factors, cytokines, miRNAs, mRNAs, lipids, enzymes, and extracellular matrix proteins. Their contents may vary according to the tissues from which MSCs originate, the age of MSCs, and environmental conditions. Mesenchymal stem cells-derived exosomes have several functions, including intercellular communication, wound healing, anti-inflammatory effects, tissue repair, promoting angiogenesis, inhibition of apoptosis, and neuroprotective effects.[36] Compared to MSCs, exosomes have the advantages of good stability and reduce the risk of cell transplantation and amplification. There are various exosome studies depending on the origin of MSCs, such as bone marrow mesenchymal stem cells (BM-MSCs), human umbilical cord mesenchymal stem cells (hUC-MSCs), adipose tissue-derived mesenchymal stem cells (AD-MSCs), and embryonic stem cell-derived human MSCs.[37,38] Overall, MSC-derived exosomes have the potential to be a useful tool for the treatment of various immune response-related conditions, including autoimmune diseases and inflammatory diseases.

OBTAINING TECHNIQUES OF EXOSOMES: ISOLATION AND PURIFICATIONS

Studies based on the release mechanisms of exosomes and related to their biogenesis are usually performed in cell lines *in vitro* by creating cell models. Different cell types release exosomes into the extracellular environment at different levels. It has been noted that in some cell lines, exosomes may have cell-specific molecular compounds for biogenesis. The collection of exosomes from cell cultures requires a separate sensitivity for each experiment.

The number of cells and the collection time of exosomes can also be adjusted according to different cell lines.[39] Exosome releases, unlike other particles, are studies whose kinetic studies are not frequently seen in the literature. Researchers' collections often range from a few hours to days. For example, in the research conducted by Llorente et al.,[40] it was observed that exosome release in PC-3 cells increased during the first 24 hours. Exosomes are grown in regions of fetal bovine serum as well as cell environments where exosomes congregate, which are of great importance as these environments simply affect results. To avoid this problem, media free of exosomes can be used, or collected in serum-free media depending on the tolerance of the cells. The bovine pituitary extract does not contain exosomes but may contain other proteins and vesicles during, for example, ultracentrifugation. After these cell culturing and collection procedures, various separation methods are applied for exosomes.

The separation of exosomes poses tremendous difficulty due to their nano size, low density, and mixing with similar components in body fluids such as proteins.[41,42] Additionally, according to the choice of various separation techniques, the biological activity of exosomes can also be affected.[43] Therefore, the separation methods of exosomes applied in standard protocols are of great importance in terms of separating exosomes and then adapting them to clinical studies. Some of the separation methods of exosomes contain ultrafiltration, polymer precipitation, immunoaffinity, ultracentrifugation, and size exclusion chromatography.[44-46] Ultracentrifugation is the most commonly used method for exosome isolation. It involves centrifuging MSC culture media or other biological fluids at high speeds to pellet the exosomes. The resulting pellet can then be resuspended and further purified by sucrose gradient centrifugation or other methods. On the other hand, the method of separating exosomes from other biological particles according to their sizes is called size-exclusion chromatography. While culture media or other biological components are passed through the column, exosomes can be collected in the void of the column. Additionally, the immunoaffinity technique can be used for the separation of exosomes, and it is the method that is specific to exosome surface markers and captures exosomes from other biological fluids and MSC culture media. The next steps involve purification, which can be performed by ultracentrifugation or other conventional methods.[47] It is important to note that each method has its advantages and limitations, and the choice of method depends on the specific application and the type of sample being used. Multiple methods can also be combined to increase the purity and yield of exosomes. The isolation and purification methods can be explained with more examples. However, in this review, common separation methods are given.

MSC-DERIVED EXOSOMES AS THERAPEUTIC AGENTS

Recent research focuses on the therapeutic efficacy of exosomes derived from MSCs. Exosomes are small vesicles that play a critical role in intercellular communication and are a reflection of the cell contents, rich in proteins, lipids, and nucleic acids. Exosomes obtained from MSCs can be used in the treatment of a wide variety of diseases, thanks to the different biomolecules they contain.[48,49] They contain many biological molecules that affect cells such as exosomes, growth factors, cytokines, RNA, and other bioactive molecules. Therefore, exosomes are considered an important mediator in intercellular communication. The therapeutic efficacy of exosomes derived from MSCs has been studied in various disease models such as cardiac diseases, neurological diseases, bone diseases, and cancer.[50-52] Exosomes derived from MSCs have much therapeutic efficacy. These include anti-inflammatory, immunomodulatory, regenerative, and neuroprotective effects. Exosomes can help reduce inflammation and play a role in regulating the immune system. It is also thought that exosomes can increase the regeneration of tissues and help repair damaged cells. Mesenchymal stem cell-derived exosomes can also be used as a promising therapeutic agent in the treatment of neurological diseases.[53] This is because the neuroprotective effects of exosomes are associated with reducing inflammation in brain tissue and helping repair damaged cells.

For instance, in their study Min et al.[54] investigate the effect of exosomes obtained from BM-MSCs on hypoxic-ischemic brain injury. The researchers modeled hypoxic-ischemic brain injury in newborn rats and administered exosomes from BM-MSCs intraventricularly to hypoxic-ischemic brain-damaged rats. The results showed that the cognitive and neurological functions of the rats treated with exosomes were significantly improved compared to the control group. Also, in this study, the molecular mechanism of the neuroprotective effects of exosomes was investigated. As a result of the examinations, it was observed that miRNA-124-3p released together with exosomes suppressed the tumor necrosis factor receptor-associated factor 6 gene, which reduces inflammation in brain tissue and protects neurons. These results suggest that exosomes derived from BM-MSCs can be used as a potential therapeutic agent in the treatment of hypoxic-ischemic brain injury.

Another study conducted by Lou et al.,[55] investigated the therapeutic efficacy of miR-199a-modified AD-MSC-derived exosomes that can be used in the treatment of liver cancer (hepatocellular carcinoma). In *in vitro* studies, miR-199a-modified exosomes reduced proliferation, function, and invasive properties of hepatocellular carcinoma cells. Moreover, miR-199a-modified exosomes increased the susceptibility of hepatocellular carcinoma cells treated with cytotoxic drugs. Mechanistically, the effects of miR-199a-modified exosomes are due to the inhibition of the mammalian target of rapamycin (mTOR) pathway in cells. miR-199a-modified exosomes suppress the mTOR signaling pathway in hepatocellular carcinoma cells, making cells more sensitive to chemotherapeutic agents. In addition, it was shown in this study that miR-199a-modified exosomes from AD-MSC can increase the sensitivity of hepatocellular carcinoma cells to doxorubicin (chemotherapy drug). The results of the study show that miR-199a-modified exosomes can be used in the treatment of hepatocellular carcinoma cells resistant to doxorubicin therapy. Exosomes can increase the efficacy of chemotherapeutic agents to reduce the development of drug resistance. These results show potential for the use of MSC-derived exosomes in cancer therapy in future research.

Mesenchymal stem cell-derived exosomes potentially offer advantages for many therapeutic applications. Among these advantages are immunological tolerance, being effective in cancer treatment, and being simpler to obtain than other cell types. Obtaining exosomes is usually accomplished by growing cells in a culture medium, and then separating the exosomes by a process called ultracentrifugation.[56] This process is quite technical and time-consuming. It has been shown that exosomes obtained from MSCs can be obtained more easily than those obtained from other cells. This is because MSCs produce large amounts of exosomes. Therefore, obtaining exosomes from mesenchymal stem cells is a faster and much easier method than obtaining them from other cell types.[57] In addition, the isolation of MSCs is easy since it has the advantage that MSCs are found in many tissues and can be easily isolated. However, MSCs-derived exosomes also have disadvantages such as standardization difficulties, production costs, inability to determine the effective dose, and not knowing the long-term side effects. For these reasons, the use of MSC-derived exosomes in clinical practice is currently limited, and further research is needed.

MSC-derived exosomes in drug delivery

Liposomes have been the most used and promising particles in the concept of drug delivery applications for a long time. Since they have several advantages as therapeutic agents many others drug delivery vehicles have mimicked their functions and biological aspects.[58] Extracellular vesicles can be also called nature-derived liposomes and have the potentials that overcome some of the drawbacks of synthetic liposomes such as toxicity. Exosomes have been the most studied and clearly defined vesicles that are convenient for the drug delivery vehicle and increase the effects of therapeutic agents. Ideal drug delivery systems should have some properties, such as providing an effective release mechanism, non-fluctuation in the plasma membrane, and long circulation in the blood.[59] Exosomes can satisfy these demands with a variety of features. Since exosomes have some protein and genetic

materials like DNA and RNA, it is possible that such biological compounds can be loaded into them. Due to their presence in several body fluids like urine and blood, exosomes can be well tolerated in the body. Thus, exosomes have much longer circulation and half-life besides increasing efficiency compared to other drug delivery vehicles.[60] Additionally, exosomes can pass the plasma membrane and release the therapeutic agent to the target or recipient cell following their nature.

Jakubec et al.[61] studied the lipid composition of plasma-derived exosome-like vesicles and their ability to cross the blood-brain barrier. The researchers demonstrated that exosome-like vesicles derived from plasma have a high content of lysophospholipid and much richer protein composition, such as transferrin and apolipoprotein E. Furthermore, in the *in vitro* blood-brain barrier (BBB) model, it was observed that the membrane permeability of these exosome-like vesicles increased without influencing neuronal cells. These results suggest that plasma-derived exosome-like vesicles can cross the BBB and be used as a potential carrier for the treatment of neurodegenerative diseases. Crossing the BBB has made exosomes a very significant agent among drug-release vehicles. Exosomes are also suitable vehicles for membrane modifications to direct them to the target cell.

Drug delivery with MSCs-derived exosomes can be also performed to reduce damage to the muscle, bone system, and nerves. Cheng et al.[62] investigated the potential of MSCs to inhibit intervertebral disc degeneration by exosome-mediated miR-21 transfer. Exosomes from MSCs contain miR-21, and miR-21 is thought to inhibit apoptosis in nucleus pulposus (NP) cells. In this study, NP cells were exposed to cytotoxicity, and then cell apoptosis was investigated after delivery of the exosome-miR-21 complex to NP cells. After the transfer of the exosome-miR-21 complex to NP cells, a significant reduction in cell apoptosis was observed. These results suggest that the exosome-miR-21 complex may be a potential therapeutic agent in inhibiting intervertebral disc degeneration. The results of the study show that MSCs can be used in the treatment of degenerative diseases through exosome-mediated miRNA transfer. However, more research is needed, particularly clinical trials evaluating the impact of this approach on humans. Another study performed by Jeyaraman et al.[63] explores the potential use of MSC-derived exosomes for the treatment of knee osteoarthritis. In this study, the effect of MSC-derived exosomes in mice with knee osteoarthritis models was investigated. The results of the study showed that MSC-derived exosomes reduced inflammation and increased cell proliferation in knee cartilage cells. Exosomes also promoted chondrocyte differentiation in cartilage cells.

In the study of Vakhshiteh et al.,[64] it was investigated that exosomes obtained from MSCs overexpressing miR-34-a inhibited cancer growth *in vitro*. These exosomes have been shown to transport miR-34-a to tumor cells, inhibiting the growth and proliferation of tumor cells. This study proposes the use of exosomes derived from miR-34-a overexpressing MSCs as a new approach in cancer therapy. Exosomes can be used as drug carriers and could potentially be effective in cancer therapy by targeting the expression of genes in cancer cells. However, more research is required on the efficacy and safety of exosomes.

Despite the various advantages of MSC-derived exosomes, there are also disadvantages to their use in drug delivery systems. These include limited cargo capacity due to low loading capacity, low targeting efficiency, and regularity challenges due to heterogeneity, destabilization, and tendency to accumulate in organs of the mononuclear phagocyte system such as the liver and spleen.[65] The heterogeneity of exosomes can be heterogeneous in size, and surface charge, cargo content, without being secreted by different or the same cell type. This makes it difficult to obtain consistent results with the use of exosomes during drug delivery, so improvement and optimization studies are required. To eliminate these disadvantages, mimicking exosome-like synthetic structures or using exosomes and these structures as hybrids provides new therapeutic advantages.[66,67]

Applications of MSC-derived Exosomes in Regenerative Medicine

Mesenchymal stem cell-derived exosomes are EVs that have played an important role in regenerative medicine in recent years. The

MSC-derived exosomes can be used in many regenerative medicine applications since they contain factors that promote cell regeneration. These factors can reduce inflammation due to tissue damage or injury, increase cell proliferation, and promote tissue remodeling. In particular, exosomes can be used effectively in the treatment of damaged tissue or organs. Among these applications, their use in many areas, such as cartilage damage, muscle injury, cardiac damage, and nerve damage is being investigated.[68-70]

One of the promising studies in this area was performed by Jiang et al.[71] The study examines the potential effects of MSC-derived exosomes in the treatment of osteoarthritis (OA). MSC-derived exosomes aim to attenuate cartilage damage in chondrocytes by regulating glutamine metabolism, which plays a significant role in the pathogenesis of OA. In the study, the effects of MSC-derived exosomes in mouse models of OA were investigated. In *in vitro* experiments, MSC-derived exosomes increased glutamine synthesis while decreasing glutamine consumption by chondrocytes. In addition, MSC-derived exosomes were observed to increase the proliferation of chondrocytes and reduce apoptosis. *In vivo* studies have shown that injection of MSC-derived exosomes reduces cartilage damage and improves joint function in mice. It was concluded that MSC-derived exosomes might be a potential treatment option in the treatment of OA by regulating glutamine metabolism in chondrocytes.

Exosomes can also be used for skin wounds or severe damage. Diabetes mellitus is a complex pathogenetic chronic disease that can cause chronic skin injuries, and skin damage that takes a long time to heal.[72] In this context, the use of MSC-derived exosomes obtained from the umbilical cord for chronic diabetic wound healing and entire skin regeneration was investigated in the study by Yang et al.[73] It also evaluated the effect of combining MSC-derived exosomes with the pluronic F127 hydrogel. According to the outcomes of the study, combining MSC-derived exosomes with hydrogel provided a significant improvement in the treatment of chronic diabetic wounds. Histological analysis demonstrated that the combination of MSC-derived exosomes and hydrogel increases wound surface neovascularization stimulates granulation tissue formation, and promotes re-epithelialization. It was stated that these effects depend on many factors. MSC-derived exosomes, when examined throughout the study, increased the release of vascular endothelial growth factor and transforming growth factor-beta-1, which are vital factors for wound healing. The increased release of these molecules helped increase cell proliferation and angiogenesis.

In a study by Chew et al.,[74] the effect of using exosomes derived from MSCs to increase the function of periodontal ligament cells (PDLCs) and promote periodontal regeneration was investigated. The study consists of two parts, *in vitro* and *in vivo*. First, PDLCs were treated with MSC-derived exosomes, and different assays were performed. In the second part, it was investigated whether MSC-derived exosomes enhance the functions of PDLCs *in vivo*. For this purpose, MSC-derived exosomes and control groups were applied to the defect area in periodontal defect models in mice. As a result of the analyzes performed after four weeks, it was determined that MSC-derived exosomes promote periodontal regeneration and prevent gingival loss. In the study, it has been indicated that the collagen sponge used together with MSC-derived exosomes increases the functions of periodontal ligament cells and promotes periodontal regeneration. It is shown that the effect of MSC-derived exosomes on periodontal regeneration may be related to increased cell proliferation and differentiation by activating AKT and extracellular signal-regulated kinase signaling pathways.

Applications of MSC-derived exosomes in cancer treatment

Exosomes from a MSC have recently come to the fore as an interesting alternative in cancer treatment. Mesenchymal stem cell-derived exosomes are potentially useful as a new cellular therapy method in cancer therapy. Since these exosomes are small vesicles with lipid bilayer membranes secreted by MSCs and contain various growth factors, cytokines, proteins, and nucleic acids that are responsible for the therapeutic properties of MSCs. Exosomes can modulate the immune response to inhibit the growth and metastasis of cancer cells and exert pro-apoptotic effects on cancer cells.[75] They may

also be effective against cancer cells that fail to respond to chemotherapy. The study by Biswas et al.,[76] highlights the potential of MSC-derived exosomes in cancer therapy. This study investigates how exosomes secreted from MSCs induce the formation of immunosuppressive M2-polarized macrophages in breast cancer. In the study, the effects of MSC-derived exosomes on human peripheral blood mononuclear cells (PBMCs) to which they were exposed at different doses were investigated. The PBMCs exposed to exosomes have been shown to subsequently differentiate into M1 (pro-inflammatory) or M2 (immunosuppressive) macrophages. Mesenchymal stem cell-derived exosomes have been found to increase the formation of M2-polarized macrophages and inhibit M1-polarized macrophages. The study also provides evidence that MSC-derived exosomes enhance the immunosuppressive properties of M2-polarized macrophages and suppress the pro-inflammatory responses of T cells. As a result, it was observed that tumor growth and metastasis were reduced in mice treated with exosomes. In the study conducted by He et al.,[77] it was investigated how exosomes secreted by hUC-MSC are effective in slowing the progression of esophageal squamous cell carcinoma (ESCC) cells. In the study, *in vitro* studies were performed using exosomes from ESCC cells and hUC-MSCs. First, hUC-MSC-derived exosomes have been shown to inhibit enabled homolog (ENAH) protein expression in ESCC cells. As ENAH is a protein known to facilitate the invasion of tumor cells, these results demonstrate the potential effect of exosomes on tumor progression. It has been shown that miRNA-375 inhibits ENAH gene expression in ESCC cells and reduces the migration and invasion of tumor cells. These results can be seen as a promising avenue for the use of hUC-MSC-derived exosomes in cancer therapy.

Applications of MSC-derived exosomes in cardiovascular and neurodegenerative diseases

The potentially important role of MSC-derived exosomes in the treatment of cardiovascular diseases has been the subject of recent research. Cardiovascular diseases are a group of diseases that affect the functions of the heart and blood vessels. Current treatments for these diseases are limited, and additional treatment options are being explored. Research shows that MSC-derived exosomes have anti-inflammatory and regenerative effects in cardiovascular diseases.[78] The importance of MSC-derived exosomes in the treatment of cardiovascular diseases is due to their anti-inflammatory and regenerative properties. Pan et al.[79] investigated the usage of miR-146a-modified MSC-derived exosomes as a potential treatment modality to reduce myocardial damage after acute myocardial infarction (AMI). In the study, AD-MSCs were modified with miR-146-a. Next, exosomes from miR-146a-modified AD-MSCs were used in the mouse model of AMI. These mice were treated to reduce myocardial damage. The results of the study showed that miR-146-a-modified AD-MSC-derived exosomes reduced myocardial damage after AMI. Exosomes have been found to reduce inflammation in the heart muscle and help reconstruct damaged tissue. It has also been found to help prevent myocardial damage by reducing the level of a protein called early growth response factor 1.

Neurodegenerative diseases are a group of diseases characterized by damage to the nervous system over time. Diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis are examples of neurodegenerative diseases. Current treatments for these diseases are limited, and additional treatment options are being explored. Mesenchymal stem cell-derived exosomes could potentially play a significant role in the treatment of neurodegenerative diseases as well as cardiovascular diseases.[80] These exosomes are small vesicles that play a role in communication between cells, and they modulate cell functions thanks to the proteins, nucleic acids, and lipids they contain. Its anti-inflammatory effects are critical in slowing the progression of diseases by reducing inflammation, similar to cardiovascular diseases. In addition, these exosomes may contribute to the preservation of neuronal functions and neuronal regeneration processes.[81]

The study performed by Li et al.[82] investigated the properties of MSC-derived exosomes that could potentially be used in the treatment of

Parkinson's disease. In the study, a microRNA molecule called miR-188-3p was transfected into AD-MSCs. This transfection process caused an increase in miR-188-3p levels in exosomes produced by MSCs. Exosomes from these modified AD-MSCs were then tested in a mouse model of Parkinson's disease. The results of the study showed that miR-188-3p modified AD-MSC-derived exosomes are effective in developing the motor functions of Parkinson's disease. Exosomes have been found to help protect and regenerate neurons in mice, reduce inflammation, and support the healthy functioning of cells by reducing oxidative stress.

FUTURE PERSPECTIVE IN MSC-DERIVED EXOSOME RESEARCH

Exosomes have garnered significant attention in recent times due to their crucial role in cellular communication. Some types of exosomes, such as MSC-derived exosomes, can promote cellular repair and regeneration by controlling cell-to-cell communication and transferring biological materials to target cells. Therefore, exosomes have emerged as a potential treatment option for various diseases. Exosomes can be used as a standalone therapy or in combination with other therapeutic technologies, such as gene editing, drug delivery, and imaging technologies.[83-86] For example, therapeutic nucleic acids like miRNA or small interfering RNA can be loaded into exosomes and delivered to specific cells, providing targeted treatment and reducing side effects, particularly in cancer treatment.[87] However, using exosomes as therapy comes with challenges. For instance, the production and isolation process can be challenging and may need to be directed toward a specific target cell.

Although exosomes have been studied extensively in recent years, research on their therapeutic use is still in its early stages, and further research is required. Also, additional research is required on the structure, production, characterization, and loading of exosomes. Clinical trials and information on the efficacy and safety of exosome-based therapies are essential. Despite being a promising tool for treating various diseases, exosomes' use as therapeutic agents has some limitations, including non-standardized protocols, isolation, and purification techniques.[88] However, the development of exosome-mimicking particles has been gaining momentum due to the natural exosomes' mechanism, origin, and functions.[89] With the further examination of protocols and scale-up processes, breakthrough research can be achieved to treat different diseases. Additionally, exosome-mimicking nanoparticles can be utilized alone or combined with natural exosomes as therapeutic agents in healthcare.

In conclusion, exosomes are small structures that are enclosed by membranes and are secreted by cells. They perform various biological functions, including communication between cells, signaling, and transportation of substances between cells. Exosomes have gained significant attention in recent years due to their potential therapeutic applications, but their biogenesis, composition, and functions are not yet fully understood. However, exosomes that are isolated from specific sources, like MSC-derived exosomes, have been identified as a promising option for cellular therapies. Mesenchymal stem cell-derived exosomes promote cell differentiation, reduce inflammation, promote tissue regeneration, and suppress the immune system. As a result, they are used for therapeutic purposes in various areas, including cardiovascular diseases, neurodegenerative diseases, cancer, and drug delivery systems. Nonetheless, additional research is necessary to expand and standardize clinical practice. Ethical and safety issues regarding the use of exosomes also need to be addressed. Nevertheless, current research suggests that exosomes could be a more effective and safer treatment alternative in the near future.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea, concept and writing the article, literature reviewing: Ö.S.; Supervising, references and fundings: O.E.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: A review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. Int J Nanomedicine 2020;15:6917-34. doi: 10.2147/IJN.S264498.
2. Zeng Y, Qiu Y, Jiang W, Shen J, Yao X, He X, et al. Biological features of extracellular vesicles and challenges. Front Cell Dev Biol 2022;10:816698. doi: 10.3389/fcell.2022.816698.
3. Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: From biogenesis to uptake and intracellular signalling. Cell Commun Signal 2021;19:47. doi: 10.1186/s12964-021-00730-1.
4. Mathieu M, Martin-Jaular L, Lavieu G, Théry C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol 2019;21:9-17. doi: 10.1038/s41556-018-0250-9.
5. Bebelman MP, Smit MJ, Pegtel DM, Baglio SR. Biogenesis and function of extracellular vesicles in cancer. Pharmacol Ther 2018;188:1-11. doi: 10.1016/j.pharmthera.2018.02.013.
6. Shah R, Patel T, Freedman JE. Circulating extracellular vesicles in human disease. N Engl J Med 2018;379:958-66. doi: 10.1056/NEJMra1704286.
7. Janouskova O, Herma R, Semeradtova A, Poustka D, Liegertova M, Hana Auer M, et al. Conventional and nonconventional sources of exosomes-isolation methods and influence on their downstream biomedical application. Front Mol Biosci 2022;9:846650. doi: 10.3389/fmolb.2022.846650.
8. Zhang L, Yu D. Exosomes in cancer development, metastasis, and immunity. Biochim Biophys Acta Rev Cancer 2019;1871:455-68. doi: 10.1016/j.bbcan.2019.04.004.
9. Ha DH, Kim HK, Lee J, Kwon HH, Park GH, Yang SH, et al. Mesenchymal stem/stromal cell-derived exosomes for immunomodulatory therapeutics and skin regeneration. Cells 2020;9:1157. doi: 10.3390/cells9051157.
10. Kugeratski FG, Kalluri R. Exosomes as mediators of immune regulation and immunotherapy in cancer. FEBS J 2021;288:10-35. doi: 10.1111/febs.15558.
11. Guo M, Hao Y, Feng Y, Li H, Mao Y, Dong Q, et al. Microglial exosomes in neurodegenerative disease. Front Mol Neurosci 2021;14:630808. doi: 10.3389/fnmol.2021.630808.
12. Jiang C, Zhang N, Hu X, Wang H. Tumor-associated exosomes promote lung cancer metastasis through multiple mechanisms. Mol Cancer 2021;20:117. doi: 10.1186/s12943-021-01411-w.
13. Trams EG, Lauter CJ, Salem N Jr, Heine U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. Biochim Biophys Acta 1981;645:63-70. doi: 10.1016/0005-2736(81)90512-5.
14. Johnstone RM, Bianchini A, Teng K. Reticulocyte maturation and exosome release: Transferrin receptor containing exosomes shows multiple plasma membrane functions. Blood 1989;74:1844-51.
15. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. 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 2018;7:1535750. doi: 10.1080/20013078.2018.1535750.
16. Jiang L, Dong H, Cao H, Ji X, Luan S, Liu J. Exosomes in pathogenesis, diagnosis, and treatment of Alzheimer's disease. Med Sci Monit 2019;25:3329-35. doi: 10.12659/MSM.914027.
17. Tan Y, Luo X, Lv W, Hu W, Zhao C, Xiong M, et al. Tumor-derived exosomal components: The multifaceted roles and mechanisms in breast cancer metastasis. Cell Death Dis 2021;12:547. doi: 10.1038/s41419-021-03825-2.
18. Ju Y, Bai H, Ren L, Zhang L. The role of exosome and the ESCRT pathway on enveloped virus infection. Int J Mol Sci 2021;22:9060. doi: 10.3390/ijms22169060.
19. Waqas MY, Javid MA, Nazir MM, Niaz N, Nisar MF, Manzoor Z, et al. Extracellular vesicles and exosome: Insight from physiological regulatory perspectives. J Physiol Biochem 2022;78:573-80. doi: 10.1007/s13105-022-00877-6.
20. Chen J, Li P, Zhang T, Xu Z, Huang X, Wang R, et al. Review on strategies and technologies for exosome isolation and purification. Front Bioeng Biotechnol 2022;9:811971. doi: 10.3389/fbioe.2021.811971.
21. Li X, Corbett AL, Taatizadeh E, Tasnim N, Little JP, Garnis C, et al. Challenges and opportunities in exosome research-Perspectives from biology, engineering, and cancer therapy. APL Bioeng 2019;3:011503. doi: 10.1063/1.5087122.
22. Han QF, Li WJ, Hu KS, Gao J, Zhai WL, Yang JH, et al. Exosome biogenesis: Machinery, regulation, and therapeutic implications in cancer. Mol Cancer 2022;21:207. doi: 10.1186/s12943-022-01671-0.
23. Pegtel DM, Gould SJ. Exosomes. Annu Rev Biochem 2019;88:487-514. doi: 10.1146/annurev-biochem-013118-111902.
24. Yue B, Yang H, Wang J, Ru W, Wu J, Huang Y, et al. Exosome biogenesis, secretion and function of exosomal miRNAs in skeletal muscle myogenesis. Cell Prolif 2020;53:e12857. doi: 10.1111/cpr.12857.
25. Li SP, Lin ZX, Jiang XY, Yu XY. Exosomal cargo-loading and synthetic exosome-mimics as potential therapeutic tools. Acta Pharmacol Sin 2018;39:542-51. doi: 10.1038/aps.2017.178.
26. Hoang DM, Pham PT, Bach TQ, Ngo ATL, Nguyen QT, Phan TTK, et al. Stem cell-based therapy for human diseases. Signal Transduct Target Ther 2022;7:272. doi: 10.1038/s41392-022-01134-4.
27. Berebichez-Fridman R, Montero-Olvera PR. Sources and clinical applications of mesenchymal

stem cells: State-of-the-art review. Sultan Qaboos Univ Med J 2018;18:e264-77. doi: 10.18295/squmj.2018.18.03.002.
28. Cofano F, Boido M, Monticelli M, Zenga F, Ducati A, Vercelli A, et al. Mesenchymal stem cells for spinal cord injury: Current options, limitations, and future of cell therapy. Int J Mol Sci 2019;20:2698. doi: 10.3390/ijms20112698.
29. Zhang B, Yeo RWY, Lai RC, Sim EWK, Chin KC, Lim SK. Mesenchymal stromal cell exosome-enhanced regulatory T-cell production through an antigen-presenting cell-mediated pathway. Cytotherapy 2018;20:687-96. doi: 10.1016/j.jcyt.2018.02.372.
30. Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Van Badiavas E. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. Stem Cells Dev 2015;24:1635-47. doi: 10.1089/scd.2014.0316.
31. Harrell CR, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Mesenchymal stem cell-derived exosomes and other extracellular vesicles as new remedies in the therapy of inflammatory diseases. Cells 2019;8:1605. doi: 10.3390/cells8121605.
32. Shen Z, Huang W, Liu J, Tian J, Wang S, Rui K. Effects of mesenchymal stem cell-derived exosomes on autoimmune diseases. Front Immunol 2021;12:749192. doi: 10.3389/fimmu.2021.749192.
33. An Y, Lin S, Tan X, Zhu S, Nie F, Zhen Y, et al. Exosomes from adipose-derived stem cells and application to skin wound healing. Cell Prolif 2021;54:e12993. doi: 10.1111/cpr.12993.
34. Guo M, Yin Z, Chen F, Lei P. Mesenchymal stem cell-derived exosome: A promising alternative in the therapy of Alzheimer's disease. Alzheimers Res Ther 2020;12:109. doi: 10.1186/s13195-020-00670-x.
35. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Nat Rev Nephrol 2018;14:493-507. doi: 10.1038/s41581-018-0023-5.
36. Nasser MI, Masood M, Adlat S, Gang D, Zhu S, Li G, et al. Mesenchymal stem cell-derived exosome microRNA as therapy for cardiac ischemic injury. Biomed Pharmacother 2021;143:112118. doi: 10.1016/j.biopha.2021.112118.
37. Abbaszadeh H, Ghorbani F, Derakhshani M, Movassaghpour A, Yousefi M. Human umbilical cord mesenchymal stem cell-derived extracellular vesicles: A novel therapeutic paradigm. J Cell Physiol 2020;235:706-17. doi: 10.1002/jcp.29004.
38. Xunian Z, Kalluri R. Biology and therapeutic potential of mesenchymal stem cell-derived exosomes. Cancer Sci 2020;111:3100-10. doi: 10.1111/cas.14563.
39. Chen BY, Sung CW, Chen C, Cheng CM, Lin DP, Huang CT, et al. Advances in exosomes technology. Clin Chim Acta 2019;493:14-9. doi: 10.1016/j.cca.2019.02.021.
40. Llorente A, Skotland T, Sylvänne T, Kauhanen D, Róg T, Orłowski A, et al. Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. Biochim Biophys Acta 2013;1831:1302-9. doi: 10.1016/j.bbalip.2013.04.011.
41. Popowski K, Lutz H, Hu S, George A, Dinh PU, Cheng K. Exosome therapeutics for lung regenerative medicine. J Extracell Vesicles 2020;9:1785161. doi: 10.1080/20013078.2020.1785161.
42. Yakubovich EI, Polischouk AG, Evtushenko VI. Principles and Problems of Exosome Isolation from Biological Fluids. Biochem (Mosc) Suppl Ser A Membr Cell Biol 2022;16:115-126. doi: 10.1134/S1990747822030096.
43. Kimiz-Gebologlu I, Oncel SS. Exosomes: Large-scale production, isolation, drug loading efficiency, and biodistribution and uptake. J Control Release 2022;347:533-43. doi: 10.1016/j.jconrel.2022.05.027.
44. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: Is size exclusion chromatography the best option? Int J Mol Sci 2020;21:6466. doi: 10.3390/ijms21186466.
45. Tiwari S, Kumar V, Randhawa S, Verma SK. Preparation and characterization of extracellular vesicles. Am J Reprod Immunol 2021;85:e13367. doi: 10.1111/aji.13367.
46. Kurian TK, Banik S, Gopal D, Chakrabarti S, Mazumder N. Elucidating methods for isolation and quantification of exosomes: A review. Mol Biotechnol 2021;63:249-66. doi: 10.1007/s12033-021-00300-3.
47. Kluszczyńska K, Czernek L, Cypryk W, Pęczek Ł, Düchler M. Methods for the determination of the purity of exosomes. Curr Pharm Des 2019;25:4464-85. doi: 10.2174/1381612825666191206162712.
48. Liu J, Jiang F, Jiang Y, Wang Y, Li Z, Shi X, et al. Roles of exosomes in ocular diseases. Int J Nanomedicine 2020;15:10519-38. doi: 10.2147/IJN.S277190.
49. Sharma S, Sharma U. Exosomes in cardiovascular diseases: A blessing or a sin for the mankind. Mol Cell Biochem 2022;477:833-47. doi: 10.1007/s11010-021-04328-6.
50. Shan SK, Lin X, Li F, Xu F, Zhong JY, Guo B, et al. Exosomes and bone disease. Curr Pharm Des 2019;25:4536-49. doi: 10.2174/1381612825666191127114054.
51. Sun SJ, Wei R, Li F, Liao SY, Tse HF. Mesenchymal stromal cell-derived exosomes in cardiac regeneration and repair. Stem Cell Reports 2021;16:1662-73. doi: 10.1016/j.stemcr.2021.05.003.
52. Pinnell JR, Cui M, Tieu K. Exosomes in Parkinson disease. J Neurochem 2021;157:413-28. doi: 10.1111/jnc.15288.
53. Szwedowicz U, Łapińska Z, Gajewska-Naryniecka A, Choromańska A. Exosomes and other extracellular vesicles with high therapeutic potential: Their applications in oncology, neurology, and

dermatology. Molecules 2022;27:1303. doi: 10.3390/molecules27041303.

54. Min W, Wu Y, Fang Y, Hong B, Dai D, Zhou Y, et al. Bone marrow mesenchymal stem cells-derived exosomal microRNA-124-3p attenuates hypoxic-ischemic brain damage through depressing tumor necrosis factor receptor associated factor 6 in newborn rats. Bioengineered 2022;13:3194-206. doi: 10.1080/21655979.2021.2016094.
55. Lou G, Chen L, Xia C, Wang W, Qi J, Li A, et al. MiR-199a-modified exosomes from adipose tissue-derived mesenchymal stem cells improve hepatocellular carcinoma chemosensitivity through mTOR pathway. J Exp Clin Cancer Res 2020;39:4. doi: 10.1186/s13046-019-1512-5.
56. Lai JJ, Chau ZL, Chen SY, Hill JJ, Korpany KV, Liang NW, et al. Exosome processing and characterization approaches for research and technology development. Adv Sci (Weinh) 2022;9:e2103222. doi: 10.1002/advs.202103222.
57. Staubach S, Bauer FN, Tertel T, Börger V, Stambouli O, Salzig D, et al. Scaled preparation of extracellular vesicles from conditioned media. Adv Drug Deliv Rev 2021;177:113940. doi: 10.1016/j.addr.2021.113940.
58. Liu H, Deng S, Han L, Ren Y, Gu J, He L, et al. Mesenchymal stem cells, exosomes and exosome-mimics as smart drug carriers for targeted cancer therapy. Colloids Surf B Biointerfaces 2022;209:112163. doi: 10.1016/j.colsurfb.2021.112163.
59. Jain KK. An overview of drug delivery systems. Methods Mol Biol 2020;2059:1-54. doi: 10.1007/978-1-4939-9798-5_1.
60. Meng W, He C, Hao Y, Wang L, Li L, Zhu G. Prospects and challenges of extracellular vesicle-based drug delivery system: Considering cell source. Drug Deliv 2020;27:585-98. doi: 10.1080/10717544.2020.1748758.
61. Jakubec M, Maple-Grødem J, Akbari S, Nesse S, Halskau Ø, Mork-Jansson AE. Plasma-derived exosome-like vesicles are enriched in lyso-phospholipids and pass the blood-brain barrier. PLoS One 2020;15:e0232442. doi: 10.1371/journal.pone.0232442.
62. Cheng X, Zhang G, Zhang L, Hu Y, Zhang K, Sun X, et al. Mesenchymal stem cells deliver exogenous miR-21 via exosomes to inhibit nucleus pulposus cell apoptosis and reduce intervertebral disc degeneration. J Cell Mol Med 2018;22:261-76. doi: 10.1111/jcmm.13316.
63. Jeyaraman M, Muthu S, Gulati A, Jeyaraman N, G S P, Jain R. Mesenchymal stem cell-derived exosomes: A potential therapeutic avenue in knee osteoarthritis. Cartilage 2021;13(1_suppl):1572S-85S. doi: 10.1177/1947603520962567.
64. Vakhshiteh F, Rahmani S, Ostad SN, Madjd Z, Dinarvand R, Atyabi F. Exosomes derived from miR-34a-overexpressing mesenchymal stem cells inhibit in vitro tumor growth: A new approach for drug delivery. Life Sci 2021;266:118871. doi: 10.1016/j.lfs.2020.118871.
65. Xi XM, Xia SJ, Lu R. Drug loading techniques for exosome-based drug delivery systems. Pharmazie 2021;76:61-7. doi: 10.1691/ph.2021.0128.
66. Lu M, Huang Y. Bioinspired exosome-like therapeutics and delivery nanoplatforms. Biomaterials 2020;242:119925. doi: 10.1016/j.biomaterials.2020.119925.
67. Shin MJ, Park JY, Lee DH, Khang D. Stem cell mimicking nanoencapsulation for targeting arthritis. Int J Nanomedicine 2021;16:8485-507. doi: 10.2147/IJN.S334298.
68. 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. doi: 10.1016/j.lfs.2020.117447.
69. Hade MD, Suire CN, Suo Z. Mesenchymal stem cell-derived exosomes: Applications in regenerative medicine. Cells 2021;10:1959. doi: 10.3390/cells10081959.
70. Stine SJ, Popowski KD, Su T, Cheng K. Exosome and biomimetic nanoparticle therapies for cardiac regenerative medicine. Curr Stem Cell Res Ther 2020;15:674-84. doi: 10.2174/1574888X15666200309143924.
71. Jiang K, Jiang T, Chen Y, Mao X. Mesenchymal stem cell-derived exosomes modulate chondrocyte glutamine metabolism to alleviate osteoarthritis progression. Mediators Inflamm 2021;2021:2979124. doi: 10.1155/2021/2979124.
72. Li D, Wu N. Mechanism and application of exosomes in the wound healing process in diabetes mellitus. Diabetes Res Clin Pract 2022;187:109882. doi: 10.1016/j.diabres.2022.109882.
73. Yang J, Chen Z, Pan D, Li H, Shen J. Umbilical cord-derived mesenchymal stem cell-derived exosomes combined pluronic F127 hydrogel promote chronic diabetic wound healing and complete skin regeneration. Int J Nanomedicine 2020;15:5911-26. doi: 10.2147/IJN.S249129.
74. Chew JRJ, Chuah SJ, Teo KYW, Zhang S, Lai RC, Fu JH, et al. Mesenchymal stem cell exosomes enhance periodontal ligament cell functions and promote periodontal regeneration. Acta Biomater 2019;89:252-64. doi: 10.1016/j.actbio.2019.03.021.
75. Dai J, Su Y, Zhong S, Cong L, Liu B, Yang J, et al. Exosomes: Key players in cancer and potential therapeutic strategy. Signal Transduct Target Ther 2020;5:145. doi: 10.1038/s41392-020-00261-0.
76. Biswas S, Mandal G, Roy Chowdhury S, Purohit S, Payne KK, Anadon C, et al. Exosomes produced by mesenchymal stem cells drive differentiation of myeloid cells into immunosuppressive M2-polarized macrophages in breast cancer. J Immunol 2019;203:3447-60. doi: 10.4049/jimmunol.1900692.

77. He Z, Li W, Zheng T, Liu D, Zhao S. Human umbilical cord mesenchymal stem cells-derived exosomes deliver microRNA-375 to downregulate ENAH and thus retard esophageal squamous cell carcinoma progression. J Exp Clin Cancer Res 2020;39:140. doi: 10.1186/s13046-020-01631-w.
78. Zheng D, Huo M, Li B, Wang W, Piao H, Wang Y, et al. The role of exosomes and exosomal microRNA in cardiovascular disease. Front Cell Dev Biol 2021;8:616161. doi: 10.3389/fcell.2020.616161.
79. Pan J, Alimujiang M, Chen Q, Shi H, Luo X. Exosomes derived from miR-146a-modified adipose-derived stem cells attenuate acute myocardial infarction-induced myocardial damage via downregulation of early growth response factor 1. J Cell Biochem 2019;120:4433-43. doi: 10.1002/jcb.27731.
80. Xu M, Feng T, Liu B, Qiu F, Xu Y, Zhao Y, et al. Engineered exosomes: Desirable target-tracking characteristics for cerebrovascular and neurodegenerative disease therapies. Theranostics 2021;11:8926-44. doi: 10.7150/thno.62330.
81. Qing L, Chen H, Tang J, Jia X. Exosomes and their microRNA cargo: New players in peripheral nerve regeneration. Neurorehabil Neural Repair 2018;32:765-76. doi: 10.1177/1545968318798955.
82. Li Q, Wang Z, Xing H, Wang Y, Guo Y. Exosomes derived from miR-188-3p-modified adipose-derived mesenchymal stem cells protect Parkinson's disease. Mol Ther Nucleic Acids 2021;23:1334-44. doi: 10.1016/j.omtn.2021.01.022.
83. Salunkhe S, Dheeraj, Basak M, Chitkara D, Mittal A. Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: Strategies and significance. J Control Release 2020;326:599-614. doi: 10.1016/j.jconrel.2020.07.042.
84. Zhang Y, Liu Q, Zhang X, Huang H, Tang S, Chai Y, et al. Recent advances in exosome-mediated nucleic acid delivery for cancer therapy. J Nanobiotechnology 2022;20:279. doi: 10.1186/s12951-022-01472-z.
85. Duan L , Xu L , Xu X , Qin Z , Zhou X , Xiao Y et al . Exosome-mediated delivery of gene vectors for gene therapy. Nanoscale 2021;13:1387-97. doi: 10.1039/d0nr07622h.
86. Alghuthaymi MA, Ahmad A, Khan Z, Khan SH, Ahmed FK, Faiz S, et al. Exosome/liposome-like nanoparticles: New carriers for CRISPR genome editing in plants. Int J Mol Sci 2021;22:7456. doi: 10.3390/ijms22147456.
87. Zhong H, Lu J, Jing S, Xi J, Yan C, Song J, et al. Low-dose rituximab lowers serum Exosomal miR-150-5p in AChR-positive refractory myasthenia gravis patients. J Neuroimmunol 2020;348:577383. doi: 10.1016/j.jneuroim.2020.577383.
88. López de Las Hazas MC, Gil-Zamorano J, Cofán M, Mantilla-Escalante DC, Garcia-Ruiz A, Del Pozo-Acebo L, et al. One-year dietary supplementation with walnuts modifies exosomal miRNA in elderly subjects. Eur J Nutr 2021;60:1999-2011. doi: 10.1007/s00394-020-02390-2.
89. Shojaei S, Hashemi SM, Ghanbarian H, Sharifi K, Salehi M, Mohammadi-Yeganeh S. Delivery of miR-381-3p mimic by mesenchymal stem cell-derived exosomes inhibits triple negative breast cancer aggressiveness; an in vitro study. Stem Cell Rev Rep 2021;17:1027-38. doi: 10.1007/s12015-020-10089-4.