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Chapter

Stem Cell Therapy and Its Products Such as Exosomes: Modern Regenerative Medicine Approach

Leila Dehghani, Amir Hossein Kheirkhah, Arsalan Jalili, Arman Saadati Partan, Habib Nikukar and Fatemeh Sadeghian-Nodoushan

Abstract

Regenerative Medicine is a developing and multidisciplinary field of science that uses tissue engineering, biology, and cell or cell-free therapy to regenerate cells, tissues, and organs to restore their impaired or lost function. Regenerative medicine uses a new element linked to stem cells, which call exosomes, introduces it to the healthcare market. Exosomes are present in almost all body fluids, such as synovial fluid and blood. Exosomes and microvesicles are very efficient mediators of cell-to-cell communication by transferring their specific cargo to recipient cells. Furthermore, the modification of extracellular vesicles is possible that can become an excellent choice for drug delivery systems and vaccines. Isolation of exosomes for their use as therapeutic, research, or diagnostic agents for a specific type of disease is of particular importance. Five techniques have been used to isolate exosomes from different sources, including ultracentrifugation-based, size-based, immunoassay, exosome sedimentation, and microfluidic techniques. The use of exosomes in medicine has many applications, including in Bone and cartilage, dental, immune system, liver, kidney, skeletal muscle, nervous, heart systems, skin and wound, microbial and infectious, and also in cancers. This chapter focuses on stem cells, especially exosomes, as novel approaches in disease treatment and regenerative medicine.

Keywords: stem cell therapy, exosome, regenerative medicine, disease treatment, organ

1. Introduction

Regenerative medicine is a developing and multidisciplinary field of science that uses tissue engineering, biology, surgery, and cell or cell-free therapy to repair and regenerate cells, tissues, and organs to restore their impaired or lost function [18]. Cell-based treatment methods, especially stem cell therapy have been recognized for many years as the main methods in regenerative medicine for their characteristics, such as easy isolation, self-renewal, multidirectional differentiation, immunomodulatory function, and stimulation of tissue regeneration. During the last decade, it was discovered that most of the therapeutic effect of Stem cells is due to different paracrine factors such.
as exosomes. So consequently, cell-free treatment was introduced as a novel approach in regenerative medicine. Although exosomes do not have cell therapy-associated complications such as tumor formation, transplant rejection in the host, and the formation of ectopic tissue, stem cells can differentiate into different types of tissues, which is their main advantage over paracrine factors. This section focuses on stem cells, especially exosomes, as novel approaches in disease treatment and regenerative medicine.

2. Stem cells in clinic, advantages, limitations

Stem cells (SCs) have been extensively known for their reparative actions. There is enormous global anticipation for stem cell-based therapies that are safe and effective. Numerous pre-clinical studies represent encouraging results on the therapeutic potential of different stem cell types, such as tissue-derived stem cells.

SCs are classified into two broad categories according to their differentiation capacity and tissue of origin. Based on stem cell hierarchy, SCs are classified into totipotent, pluripotent, multipotent, or unipotent cells, depending on their cluster of differentiation [1].

If we want to compare adult stem cells, embryonic stem cells, and induced pluripotent stem cells, the positive points of adult stem cells are the ability of transdifferentiation and reprogramming of these cells which is possible but is not well studied, being less likely to be rejected if used in transplants, and successful results have already been demonstrated in various clinical applications. On the other hand, there are some concerns about them such as limitations on the differentiation ability of ASCs which is still uncertain; they are currently thought to be multi or unipotent, being not able to grow for long periods of time in culture, usually, a very small number in each tissue making them difficult to find and purify. There is no technology available to generate large quantities of stem cells in culture, and no major ethical concerns have been raised [2].

Embryonic stem cells possess remarkable properties, including their ability to maintain and grow in culture for extended periods of one year or more. Well-established protocols for their culture maintenance are available, making them a promising research subject. With their pluripotency, they can generate a wide range of cell types, making them an important tool for understanding the process of development. By further studying embryonic stem cells, we can gain a deeper understanding of developmental processes. Also, there are some limitations, such as the inefficient process to generate ESC lines, being unsure whether they would be rejected if used in transplants, therapies using ESC avenues are mainly new, and much more research and testing are needed. Also if they are used directly from the ESC undifferentiated culture prep for tissue transplants, they can cause tumors (teratomas) or cancer development, and finally, the ethical concerns as the embryo is destroyed to acquire the inner cell mass, and the risks for female donors [3].

Also, positive points about induced pluripotent stem cells are that abundant somatic cells of the donor can be used for therapeutic approaches, concerns about histocompatibility mismatch are avoided, they are beneficial for drug development and developmental studies, information learned from the “reprogramming” process may be transferable for in vivo therapies to reprogram damaged or diseased cells/tissues.

Furthermore, there are some limitations such as methods for reproducibility and maintenance, differentiated tissues are not specific, viruses are currently used to introduce embryonic genes and have been shown to cause cancers in mouse studies.
Moreover, as an ethical concern, it should be noted that iPS cells can become embryos if exposed to the right conditions [4].

Although stem cell transplantation has good efficacy, weak immunogenic potential, and high multi-potential differentiation, there are some concerns about that, such as safety considerations in terms of tumorigenicity and transmission of infection, tight regulations, short shelf life, and high cost associated with strict production, transport, and storage conditions. To overcome these challenges, CM's induction of SCs in their native niche to stimulate the regeneration process is a promising cell-free approach [5]. Despite the advantages of this method, such as Immuno-compatibility, improved safety compared with stem cell transplantation, and feasibility of mass production, some limitations should be considered, such as limited therapeutic efficacy due to low concentration of paracrine factors, difficulty in obtaining the CM with a consistent composition, short half-lives of paracrine factors, and requiring frequent administrations with large doses [6]. Stromal Vascular Fraction (SVF) treatment efficacy likely depends upon several patient and treatment-specific characteristics, including the severity and cause of hair loss, treatment frequency, preparation methods, and adjunctive therapies [7]. Literature reviews propose that all kinds of cell types will have the therapeutic application with the potency of regenerative therapy. Different types of cells include many diagnostic and therapy factors. Differential potency of SC such a neurogenesis, synaptogenesis, vasculogenesis, myogenesis, oligodendrogenesis, axonal connectivity, myelin formation, etc.

Although the exact mechanisms by which SCs perform are still unidentified, recent documents have proposed they might be associated with their contents, such as exosomes [8]. Extracellular vesicles (EV) are lipid bilayer-enclosed and small (40–1000 nm) vesicles secreted into biological fluids. EVs are highly heterogeneous in the context of contents, size, and membrane composition, depending on the source of origin. So far, three main categories of EVs have been identified, including exosomes, apoptotic bodies, and cellular microparticles/microvesicles/ectosomes [8]. EVs have been identified as vital components in intercellular communication and information transfer to other cells, affecting both the recipient and parent cells' physiological and pathological functions. Also, the roles of EVs in cancer and autoimmune disease have been suggested in some research [9].

3. Exosomes: properties and applications

Extracellular vesicles (EVs) are small lipid particles secreted from all human cell types, both healthy and malignant. They can be released either directly from the plasma membrane or upon fusion among multivesicular bodies (MVBs) and the plasma membrane. Based on their size, origin, and cargo heterogeneity (i.e., DNA, proteins, various types of RNAs), EVs have been classified into several groups, such as exosomes, microvesicles, apoptotic bodies, and other vesicle types [10].

Two scientists named Pan and Johnston first defined intercellular communication by exosomes in 1983. They discovered that during the maturation of sheep reticulocytes into erythrocytes, transferrin receptors were enclosed in nanovesicles of endosomal origin. During these years, scientists considered the term exosome for these nanovesicles, which are between 30 and 150 nm [11].

Exosomes are present in almost all body fluids, such as synovial fluid and blood. Exosomes and microvesicles are very efficient mediators of cell-to-cell communication by transferring their specific cargo to recipient cells. For example, exosomes are
involved in the delivery of genetic materials causing epigenetic modifications in the target cells, antigen transfer to dendritic cells (DCs) for cross-presentation to T cells, extracellular matrix remodeling, and several signaling pathways [12, 13].

The most crucial feature of EVS, including exosomes, is to be loaded as delivery systems and vaccines because it can be easily loaded with different molecules, such as drugs, antibodies, miRNAs, and siRNAs, especially in anti-tumor treatments, resulting in more specific and efficient systems compared to the carried molecules alone. Furthermore, the modification of EVs is possible that can become an excellent choice for drug delivery systems and vaccines [14].

Exosomes enter the cell through pinocytosis, endocytosis, or direct fusion with the plasma membrane. Today, stem cells are used significantly in regenerative medicine treatment protocols due to their ability and capacity to differentiate into various cell lines. It has also been proven that the ability to heal and regenerate stem cells is due to exosomes secreted from them, which act in paracrine [15, 16]. Similar to cancer, exosomes act as a double-edged sword due to their ability to carry and deliver molecules to target cells in infectious diseases. Exosomes play a crucial role in the pathogenesis of infection but also trigger immune responses to confer protection against pathogens [17]. In general, the advantages of using exosomes in regenerative medicine include the following:

1. The risks associated with treatment with exosomes derived from different cells are relatively lower compared to other cell therapy methods in which the cells themselves are used, which created a project in the field of cell therapy called Cell-free therapy, which reduces the risks of rejection of transplanted cells due to the reaction of the host’s immune system against the transplant.

2. Due to the possibility of direct communication of exosomes with the target cells and the ability to target, they are used as a preferred method over other methods, which have less toxicity and faster cleaning in the body.

3. In vivo preclinical studies have shown that targeting exosomes to specific cells can reduce transported molecules’ concentration and save materials and costs.

4. Structure and biogenesis

The biogenesis of exosomes starts with endocytosis, and the cargo enters the primary endosome membrane by budding. Endocytosis can be dependent or independent of clathrin protein. The early endosome enters the late endosome phase, which has a spherical shape and is located close to the nucleus [18]. The budding of the cargo into the lumen of the endosome causes the formation of intraluminal vesicles (ILVs) with sizes of 30–150 nm, called Multivesicle bodies (MVBs). These multivesicular bodies (MVBs) may fuse with the cell membrane and release their intraluminal vesicles (ILVs), which are exosomes, outside the cell, or they may fuse with the lysosomal membrane to degrade their contents [19]. The membrane components of exosomes that have been identified so far include: lipid rafts containing sphingomyelin, cholesterol, ceramide, phosphatidylserine, and more than 4000 exosomal proteins [20].

Common proteins in all exosomes include transfer proteins such as annexin, Rab GTPase, proteins related to the biogenesis of exosomes such as Alix, TSG101, actin, myosin, and cofilin, as well as tetraspanins such as CD9, CD63, CD81, CD82, CD151, and MHC classes one and two [21, 22]. Sometimes, on the membrane of exosomes, there
are glycoproteins related to targeting lysosomes called Lamp1 or Lamp2, as well as integrins and heat shock proteins such as HSP90 and HSP70. Usually, integrins and tetraspanins play the roles of adhesion and targeting [23, 24].

5. Separation methods

Isolation of exosomes for their therapeutic use, research, or diagnostic agents for a specific type of disease is of particular importance. With the rapid progress of science and technology, techniques for isolating exosomes in a high-quality and high-purity form have been expanded in large quantities. Each technique uses a particular feature of exosomes, such as size, shape, or surface proteins, for their separation. Five techniques have been used to isolate exosomes from different sources, including ultracentrifugation-based, size-based, immunoassay, exosome sedimentation, and microfluidic techniques. For the investigation of the quality of isolated exosomes, several optical and non-optical techniques have been developed to check the size, shape, and quantity of chemical components [25, 26].

Ultracentrifuge: There are usually two types of ultracentrifuge: analytical and preliminary. Analytical ultracentrifugation is used to research the physicochemical properties of particles and molecular interactions of polymeric materials [27]. The preliminary ultracentrifuge is used as the gold standard for the isolation of exosomes because its use is simple and does not require particular expertise. Of course, it is fast and cheap. Separation of exosomes by the type of differential usually includes a series of centrifugation cycles with different centrifugal forces, the duration of which is different from other components based on density and size [28]. A purification step is performed at the beginning of the separation of exosomes from human plasma and serum to get rid of large biological particles. Of course, protease inhibitors are added to the sample to prevent the destruction of exosome membrane proteins [29, 30]. There are two types of density gradient ultracentrifuge: isopycnic and moving zone. The use of density gradient ultracentrifugation to separate extracellular vesicles such as exosomes has received much attention. In the density gradient ultracentrifuge, different densities of the substance are created in the tube, which usually decreases from the bottom to the top, so the exosomes with different densities are placed in a different part of the tubes based on the force exerted on them during the centrifugation [31, 32]. In moving zone ultracentrifuge, samples containing exosomes are placed in a narrow area above the density gradient of the environment, which has a lower density than any of the substances dissolved in the sample, unlike isopycnic ultracentrifugation, which separates only based on density [33, 34].

Based on size: Ultrafiltration is one of the most popular methods of separating exosomes based on size [35]. Methods such as western blotting or electron microscopic methods are used to confirm the successful isolation of exosomes. For cell-free samples such as urine, serum, spinal fluid, and cell fluid culture media, kits based on filtration separation have been developed [27, 36]. Sequential filtration is performed to separate exosomes from the solution on the cell culture medium. Initially, filtration with 100 nm filters is used to separate floating cells, and large cell debris, but components with a size larger than 100 nm but flexible are possible [37]. In this method, in the chromatography column, the stationary phase is a porous substance and is used to separate particles and macromolecules that are smaller in size than exosomes. These substances enter the pores, and when washing the column, the exosomes are separated earlier, which are finally separated by western blotting of the isolated exosomes [38].
**Immunoadfinity capture:** For example, the Enzyme-linked immunosorbent assay or ELISA method is usually used to isolate exosomes in body fluids such as serum or urine. The results of absorption values indicate surface biomarkers produced on the membrane of exosomes. In another method, the surface characteristics of exosomes are evaluated by immunoprecipitation methods based on microplates by ultracentrifugation. This method’s more accurate results are obtained with a smaller sample, which shows its superiority over the ultracentrifugation method [28]. It also depends on the quality of the exosomes and the environment in which they are located [35, 39]. Examples of these diagnostic kits are used to isolate exosomes from the plasma of acute myeloid leukemia (AML) patients that contain abundant amounts of CD34 on their membrane, and separation is done by magnetic beads coated with the target antibody of this marker [28, 40]. To increase the capacity of immunoadfinity capture, the mass spectrometry method is used along with the immunoassay method. For exosome isolation, antibodies on highly porous silica micropipettes that are integrally immobilized are used to isolate CD9-containing exosomes. Another example demonstrating the combination of immunoadfinity trapping with other exosome isolation methods is a magnetically activated cell sorter that uses epithelial cell adhesion molecules to purify isolated tumor exosomes. It uses plasma samples of lung cancer using the method (SEC) [41, 42].

**Based on sedimentation:** Exosomes can be precipitated in biological fluids by changing the solutions containing exosomes or by creating a particular type of dispersion in these solutions. For this purpose, special polymers like polyethylene glycol (PEG) can be used [27]. Typically, the incubation of samples with precipitating substances such as PEG, which have a molecular weight of up to 8000 D, for one night at 4°C causes exosomes to precipitate at a low speed by centrifugation or filtration. This method is an easy separation method that does not require special tools [43]. Several exosome precipitation kits have been commercially developed and available that isolate exosomes from body fluids such as plasma, serum, urine, and spinal fluid. The urinary exosomes isolated with these kits have been proven to be quantitatively higher than in ultracentrifugation [44]. The main weakness of the polymers’ exosome deposition method is co-precipitation with other non-exosome components, such as proteins and other substances [28].

**Separation based on microfluidics:** Rapid advances in microfluidic technology have led to the development and manufacture of devices for the fast and high-yield separation of exosomes; applying such devices saves the use of materials, reagents, and time. An example of such a device is an acoustic nano-filter that uses ultrasound waves to separate the constituents of a sample based on size and density. Larger particles experience more wave pressure and move faster toward the pressure nodes embedded in the device [45]. To take advantage of the size difference between exosomes and other extracellular vesicles, Wang and colleagues have tailored porous nanowire structures on the micropillars of a microfluidic device, which preferentially transport exosomes between 40 nm and 400 nm in diameter. Proteins and other cellular components are filtered out, and the entrapped exosomes are isolated by dissolving the porous silicon nanowires in PBS buffer [46]. To increase the specificity and introduction of exosomes, Chen and his colleagues tried for the first time to integrate the immunoadfinity trapping technique with the microfluidic chip, which is similar to the immunoadsorption methods of exosome isolation based on the specific interaction between exosome membrane proteins and their proven antibodies on a chip. Based on this method, commercial Exochips have been designed, as shown in Table 1, to isolate exosomes. These exochips are immunochips with anti-CD63 function present on the membrane of most exosomes, and a special fluorescent dye called carbocyanine (Dio) stain exosomes are also used in these special chips [47].
6. Application of exosomes in regenerative medicine

The use of exosomes in Medicine has many applications, including in drug delivery to transfer a specific drug or therapeutic molecule, as well as in regenerative medicine and cell therapy, because nanovesicles are made of cell membranes, which are effective due to their genuineness. They have high and typical safety, and also their presence in all body fluids makes them reliable candidates for diagnosing methods (Figure 1).

The role of exosomes in diagnosing and treating various diseases lies in their function as carriers of intracellular communication signals. Recent exosome investigations

<table>
<thead>
<tr>
<th>Methods</th>
<th>Specification</th>
<th>Advantage and disadvantage</th>
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<tbody>
<tr>
<td>Ultracentrifuge</td>
<td>Using a very strong centrifugal force of up to 1,000,000 G [30]</td>
<td>Advantages: Accessible and easy to use Disadvantages: Differential ultracentrifugation is typically linked to contamination and exosome loss because some exosomes in heterogeneous mixtures overlap with other particles in terms of size.</td>
</tr>
<tr>
<td>Based on size</td>
<td>Ultrafiltration Size exclusion chromatography (SEC) [35]</td>
<td>Advantages: works faster than ultracentrifugation and does not require special equipment or expertise. Disadvantages: it is usually repeated two more times [36]</td>
</tr>
<tr>
<td>Immunoaffinity capture</td>
<td>Exosomes have been used for immune targeting based on antibody affinity to an antigen or receptor affinity to a ligand with the goal of isolation. The first characteristic and special feature of the membrane of exosomes is a protein called CD63, which is abundantly expressed on the membrane of human exosomes [28].</td>
<td>Advantages: yields better RNAs than ultracentrifugation. Immune trapping is preferable to ultracentrifugation for isolating colon cancer exosomes [28, 40].</td>
</tr>
<tr>
<td>Sedimentation</td>
<td>Exosomes can precipitate in biological fluids by altering the solutions that contain them or by introducing a specific kind of dispersion into them. Specialized polymers like polyethylene glycol (PEG) can be utilized for this purpose [27].</td>
<td>Advantage: It does not call any specialized equipment. The co-precipitation of other non-exosome components, such as proteins and other substances, is the mechanism of exosome deposition of the polymers’ main drawback [43].</td>
</tr>
<tr>
<td>Microfluidics</td>
<td>An acoustic nano-filter separates sample constituents by size and density using ultrasonic waves. Larger particles feel higher wave pressure and migrate faster to device pressure nodes [45].</td>
<td>Advantage: can reduce the use of materials, reagents, and time. Disadvantage: expensive to build</td>
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Table 1. Summary of exosomes separation methods.
focused on exosomes derived from humans and plants. Based on registered clinical trials (https://clinicaltrials.gov/), exosomes are used in different issues as biomarkers for cancer diagnosis like, lung, breast, colorectal, thyroid, exosome-therapy, drug delivery, and vaccines. In the case of exosome therapy, 35 studies have been registered which 22 clinical trials used MSCs-derived exosomes and most of them have been used in infectious diseases. Although some stem cell therapies have been approved for blood and immune diseases, but there is no approval for exosome therapy and more clinical trials are needed, yet.

7. Bone and cartilage tissue

Clinical studies on laboratory animals have shown the therapeutic effect of mesenchymal stem cells (MSCs) in healing cartilage injuries. The reason for these therapeutic effects is the secretion of various exosomes by these cells. It has been found that intra-articular injection of exosomes derived from mesenchymal stem cells improved osteoporotic defects in model rats. Most articular cartilages have a limited capacity to heal after injury. As challenging surgeries should be done to replace the joint, there is always a fear of rejection of the graft in this treatment [48, 49]. Recently, mesenchymal stem cells have been used to treat these defects. However, using mature mesenchymal stem cells as multipotent stem cells may not be able to optimally repair these damages because, usually, with the increasing age of the donor cells, the ability of these cells to self-regenerate and multiply and differentiate decreases dramatically [50].

Another theory suggests using pluripotent stem cells as permanent sources in cell therapy. Although these cells can differentiate into various cell lines clinically, they may cause tumors and teratoma, and they cannot be considered a reliable treatment source. So an alternative method is to use exosomes derived from these types of cells [51].

In a study by Zang et al., osteoporotic defects were created in two groups of model mice, one as a sample and the other as a control of the same size. For 8 weeks, 100 μL
of exosomes derived from mesenchymal stem cells were injected intra-articularly into the PBS control group and the sample group (the environmental and nutritional conditions of both groups were the same). To observe the progress and effectiveness of the treatment, tissue evaluation was performed. It was observed that four cartilage defects were completely healed within 6 weeks, and hyaline and collagen type 2 were created entirely. In one case, tissue repair was done by fibrosis [48]. In the field of bone regeneration, most of the attention is on exosomes derived from mesenchymal stem cells due to their ability to influence and interact with the bone microenvironment at different levels of regeneration.

At different levels of regeneration, cells such as osteoblasts, osteoclasts, osteocytes, chondrocytes, and endothelial cells are associated with different repair mechanisms [52]. Exosomes derived from MSCs can generally distinguish osteoblasts by transmitting various miRNA cargoes. Exosomes produced by stimulated cells can connect to the extracellular matrix and induce the distinction of osteoblasts through regional interaction with them. In addition, considering the importance of the interaction between osteoclasts and osteoblasts in bone homeostasis, specific targeting molecules to inhibit or induce osteogenesis, such as miR-214-3p, which is secreted from osteoclasts, are of great interest [48, 53]. Another study identified the pathways by which miRNAs in exosomes regulate osteoblastic differentiation.

WNT and PI3K/ AKT pathways directly affect the induction of bone formation. One of the essential miRNAs in this field is miR-27A-3p, which affects different signaling pathways in bone, such as TGFβ, BMP, and WNT, which influence proliferation genes involved in bone distinction, such as STAB2, DLX2, OSX, and Runx2 [52, 54]. According to various research, the bone regeneration capacity of exosomes derived from MSCs depends on the type of tissue from which they are isolated. For example, exosomes derived from MSCs isolated from human adipose tissue can increase the speed of healing and regeneration of bone defects [55].

8. Dental tissue

Recently, studies have been conducted on miR133b derived from certain exosomes of dental dentin cells, which regulate apoptosis in tooth development. This miRNA induces apoptosis in the primary mesenchyme of the dental tissue's upper part and causes the dental tissue's proper formation in the laboratory environment. Such studies highlight exosomes' critical role in signaling growth, differentiation, and regeneration of oral, facial, and cranial tissue [56]. Exosomes derived from adipose tissue stem cells have tremendous healing effects in treating oral and dental diseases. They have opened a clear horizon for dental treatments without the need for surgery. Exosomes may be isolated from mesenchymal stem cells of dental tissues, including dental pulp stem cells (DPSCs). These are multipotent cells that can directly contribute to the regeneration of dental pulp, bone, muscle, nerve, blood vessels, and even the liver [57, 58]. DPSCS-derived exosomes play an important role in regenerative medicine. Research has confirmed that these exosomes in primary cell culture and animal models have a modulatory role and support the immune system and anti-apoptotic activity, similar to MSC-derived. They have the unique ability to regenerate dental pulps. Periodontal ligament cells (PDLCs), known as old sources of multipotent stem cells, are other cells in tooth regeneration. One of these cells' unique features is maintaining the ability of self-regeneration when transplanted [59]. Although extensive research has not been done on the effects of exosomes derived from dental ligament cells, there...
are shreds of evidence about the modulating properties of these cells. In addition, it has been found that exposure of PDLCs to lipopolysaccharide (LPS) produces exosomes that can induce polarity in proinflammatory macrophages [60, 61].

9. Modulation of the immune system

The ability of exosomes to modulate the immune system, and increase or inhibit inflammation, has introduced them as an attractive choice as therapeutic agents. Exosomes can transport different antigens, load on MHC class I and II complexes, and stimulate immune response through epitope presentation by cell-presented antigens [62, 63]. Exosomes derived from dendritic cells (DC) loaded with viral antigens can activate TCD8+ cells. Exosomes secreted from cells infected by bacterial and viral antigens can stimulate the release of macrophages and determine the activity of T cells. Similarly, after being made inside dendritic cells, cancer-specific epitopes can stimulate the activity of cytotoxic T cells against cancer cell antigens [64]. Exosomes derived from regulatory T cells (Tregs) play the role of modulating and sometimes suppressing the immune system. TCD4+, CD25+, and FoxP3+ cells can activate TCRs or receptors on T cells. Exosomes produced by this type of T cells are quantitatively more than other T cells. Treg-derived exosomes can reduce the release of inflammatory cytokines such as IL2 and IFNγ [65]. The suppressive nature of Treg exosomes has been attributed to CD73 ectoenzymes, and the loss of CD73 in Treg exosomes reverses its natural suppressive property. It has been found that the contents of exosomes that move between Treg cells and T effector cells (Teffs) contain miRNAs such as Let-7d, Let-7b, and miR155, which indicate the modulating and inhibitory function of these exosomes [65, 66]. It has been reported that using exosomes derived from cancer cells as a vaccine for chronic myeloid leukemia (CML) patients increases the power of cytotoxic T cells against CML cells. In addition, exosomes derived from mesenchymal stem cells present cancer epitopes on their membrane, which can stimulate the activity of antibody secretion by B cells and Th1 memory cells [67]. Exosomes can be used to transfer molecules that modulate the immune system, such as vitamin D derivatives, which play a role in regulating the immune system in osteoporosis, or as vectors for gene transfer of anti-inflammatory molecules to reduce the damage caused by osteoporosis. Immunotherapy based on exosomes has numerous advantages over cellular immunotherapy because its production is of higher quality, a safer method, and they are more stable and less toxic [68, 69].

10. Liver tissue

The special features of mesenchymal stem cells, such as multipotency and self-renewal, have been used as promising tools for treating liver diseases. According to the figure, exosomes derived from these cells can regenerate various tissues, including the liver, in damaged models [70]. Several studies have been conducted on the therapeutic effects of MSC-derived exosomes in mice models of liver fibrosis. Carbon tetrachloride (CCL4) was used to cause this damage. It was determined that exosomes derived from MSCs isolated from umbilical cord blood have improved liver fibrosis by inhibiting the epithelial-mesenchymal transition of hepatocytes and increasing collagen production [71, 72]. It has been found that exosomes significantly restore the activity of the liver aspartate aminotransferase enzyme and inhibit the smad/TGFβ
signaling pathway by inactivating phosphorylation and increasing the production of type 1 and 3 collagen [73]. Another study showed that hepatic mesenchymal stem cells could release exosomes containing miR-125b, which are transported between these cells and target cells, such as stellate cells that respond to the Hedgehog (Hh) signaling pathway and heal the fibrosis caused by CCL4 damage in mouse models by inhibiting Hh pathway signaling which preventing SMO protein expression [74]. It has been reported that exosomes derived from adipose-derived MSCs (AD-MSC) contain miR-122, which affects hepatocytes and regulates the expression of specific genes such as P4HA1 and IGF1R, which are effective in collagen production and increase the speed of liver fibrosis treatment [75]. Exosomes derived from AD-MSC can significantly reduce the level of alanine and aspartate aminotransferase and concanavalin A, as well as the serum level of pro-inflammatory cytokines such as TNFα, INFγ, IL6, IL8, and also reduces IL1β, which causes severe liver inflammation.

Exosomes derived from MSCs can also improve the acute liver damage caused by acetaminophen or H₂O₂ by affecting the genes of anti-apoptotic proteins such as BCL-XL and transcription activators such as STAT3 and increasing their expression [70, 76].

11. Renal tissue

Exosomes secreted by bone marrow mesenchymal stem cells can enhance the growth of cisplatin-damaged proximal tubule epithelial cells by horizontal transfer of IGF-1 receptor mRNA. It has also been shown that exosomes derived from human umbilical cord blood mesenchymal stem cells can improve acute kidney injury by inhibiting kidney oxidative stress and apoptosis, increasing kidney epithelial cells’ growth [77, 78]. A scientist named Borges discovered that by placing renal tubule epithelial cells in hypoxia condition, these cells release TGF-β1 mRNA-rich exosomes into the culture medium, which can activate fibroblasts to initiate a fibrotic remodeling response [79]. Burger investigated the therapeutic potential of colony-forming cells derived from umbilical cord blood in acute kidney ischemic injury models, which showed the therapeutic abilities of these cells in treating this type of injury due to miR-486-containing exosomes. 5p is derived from these cells, which can repair this damage by targeting the PTEN gene [80]. Research has also been done on the therapeutic abilities of exosomes derived from stem cells isolated from the urine; which has shown that the damage caused by streptozotocin-induced renal damage models by weekly injection of urine-derived stem cell (USCs) exosomes can inhibit apoptosis and increase survival and vessel regeneration [81].

12. Skeletal muscle tissue

Recently, the use of mesenchymal stem cell secretomes, especially exosomes derived from mesenchymal stem cells (MSCs), for skeletal muscle regeneration has been researched. In vivo studies have shown that exosomes derived from MSCs can increase the speed of muscle regeneration by increasing angiogenesis and reducing muscle fibrosis. Concerning skeletal muscle injuries, researchers have discovered miRNAs with anti-apoptotic activity, such as miR-21, and myogenic activities, such as miR-1, miR-133, miR-206, and miR-494, which were able to reduce these types of injuries in mouse models [82, 83]. Choi and colleagues found that exosomes derived from human skeletal myoblasts (hSKMs) during myotube differentiation could...
induce myogenesis response in hASCs. Experiments on skeletal muscle injury model mice confirm that using hSKMs-derived exosomes can accelerate skeletal muscle regeneration by reducing the collagen deposition rate and increasing myofibrils’ regeneration in injured muscles. According to studies, exosomes derived from MSCs regenerate skeletal muscles by strengthening myogenesis and angiogenesis; at least part of these effects are caused by miRNAs such as miR-494 [82, 84].

13. Nervous system

Exosomes have also been investigated to improve regenerative medicine’s central and peripheral nerve systems. They can cross the blood-brain barrier as moderators of inflammatory responses and regeneration of nerve damage. Nervous system injuries are very debilitating for patients and often cause severe skeletal muscle disorders, and the management and recovery of these injuries are complicated and unresolved. Peripheral nervous system damage causes inflammation, loss of neuron function, and destruction, resulting in cell death. Today, we know that exosomes derived from MSCs support nerve growth by stimulating the secretion of growth factors needed to support and stimulate Schwann cells, which play an essential role in myelin production [85, 86]. Exosomes derived from MSCs can significantly induce repair of the nervous system by miR-133b, which is modified with lentiviral expression vectors and determine the overexpression or silencing of miR-133b and thus cause the regeneration of neurons [87]. Recently, it has been found that in debilitating diseases such as Parkinson’s and Alzheimer’s, neurons release exosomes containing α synuclein and β amyloid, respectively. These exosomes can play a role in the nucleation and physical release of these aggregated proteins that cause these diseases [88]. In the research, it has been proven that exosomes can be used as biomarkers of brain damage, for example, exosomes containing miR-9 and miR-124 isolated from blood as biomarkers are used to diagnose acute ischemic stroke (AIS) and also evaluate the amount or degree of damage caused by this ischemia [89]. The effects of exosomes in the regeneration of neurons and the nervous system have also been proven, for example, exosomes derived from oligodendrocytes stimulated with glutamate can increase neurons’ survival in hypoxia conditions without glucose. Exosomes derived from bone marrow tissue stem cells can significantly increase the survival of retinal ganglion cells (RGCs) and the regeneration of their axons [90–92]. Usually, after nerve damage, Schwann cells differentiate and grow and direct axons to their target tissue. It has been found that the exosomes derived from these cells inhibit the activity of RhoA, a GTPase that can cause axons to lengthen and repair them [93, 94].

14. Heart muscle tissue

The protective effects of exosomes in myocardial ischemia re-injury models are being investigated. Scientists have shown that exosomes isolated from cardiosphere-derived cells (CDCs), when injected into mice model of ischemia, can inhibit apoptosis and induce the growth of heart cells. It has been found that these beneficial effects are due to the richness of these exosomes in miR-146a [95]. During another study, it was determined that exosomes secreted from bone marrow mesenchymal stem cells stimulate the formation of umbilical cord vein endothelial cell tubes and inhibit the production of T cells in vitro. In addition, the severity reduces infarction [96].
According to other research on exosomes derived from mouse embryonic stem cells, these exosomes can restore the internal function of the heart after myocardial infarction. With further research, the researchers found that this is due to miR-294 in these exosomes, which are transferred to cardiac progenitor cells [97, 98]. Another research group in China investigated the protective effects of mesenchymal stem cells derived from human umbilical cord blood on acute myocardial infarction (AMI) animal models and noticed the effects of these exosomes in protecting myocardial cells. Apoptosis and increased angiogenesis in the damaged area. They found that these effects are related to the modulation of BCL-2 pro-apoptotic protein family gene expression [99]. Certain exosomes in the heart’s pericardial fluid improve the survival, growth, and communication of endothelial cells in the culture medium. It restores the angiogenic capacity of endothelial cells, and even these exosomes improve blood flow and angiogenesis after ischemic injuries in model mice. Further research has shown that these exosomes contain miR let-7b to carry out this process [100].

15. Skin

Angiogenesis is essential in various physiological processes, including wound healing and skin tissue regeneration. Scientists found that exosomes secreted by mesenchymal stem cells derived from adipose tissue can significantly stimulate the angiogenesis of endothelial cells in vitro and in vivo. Further research showed that these exosomes transfer miR-125a to endothelial cells, which decreases the expression of inhibitory proteins Delta-like 4 (DLL4) that inhibit angiogenesis [101, 102]. Burns is one of the most common skin injuries that significantly intensify inflammatory reactions by increasing the level of factors such as TNFα, IL-1β, and decreasing the level of IL-10. The scientists found that using exosomes derived from umbilical cord blood stem cells successfully reduced the inflammatory reactions caused by burns, and further research revealed that this effect is due to the presence of miR-181c in these exosomes, which reduces pain and severe inflammation caused by burns by reducing TLR4 signaling [103]. It has been determined that exosomes derived from umbilical cord blood endothelial progenitor cells can heal diabetic wounds in rat models. Microarray analysis has shown that exosomes significantly increase the expression of some genes. They change the ERK1/2 signaling pathway, which is very important in the healing and regeneration of these wounds. Studies by Guo and colleagues have shown that platelet-rich plasma-derived exosomes can effectively induce the proliferation and migration of endothelial cells and fibroblasts to increase angiogenesis and regeneration and repair severe skin wounds. Exosomes can also control skin regeneration bipolarily. They can also prevent scarring caused by burn healing and collagen deposition through the induction of phosphorylation that inhibits the WNT/β-catenin YAP pathway [84, 104].

16. Cancer

In the mid-2000s, the first results from clinical trials on exosomes as a treatment for cancer were published. Exosomes derived from dendritic cells (DEXs) are potential targets for cancer therapeutic strategy. DEXs can directly catalyze the transfer of peptide-MHC complexes from their membrane surface to the T cell membrane surface (cross-dressing). Moreover, DEXs can indirectly stimulate T cell responses via
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cross-dressing with dendritic cells or via exosome uptake and processing, following the peptide-MHC complex presentation to T cells. DEXs can also induce activation and proliferation of NK cells by establishing interaction of the NKG2D ligand on DEXs with NKG2D receptors on the NK cell membrane [105]. In 2005, two phase I clinical trials using DEX vaccines were performed. The first trial reported the use of DEXs loaded with HLA-restricted melanoma-associated antigen (MAGE) peptides, which were infused into patients with HLA A2+ non-small cell lung cancer (NSCLC); the second trial reported the use of DEXs derived from DCs pulsed with MAGE and inoculated them to conduct immunization of melanoma patients [106]. Exosomes exhibit features for application as adjuvant carriers, such as optimal size, biocompatibility, stability in the systemic circulation, and target-specific delivery. Recently, an exosome-based adjuvant delivery system was developed using genetically modified murine melanoma B16BL6 cells. The exosomes derived from these cells containing CpG DNA were injected three times at 3-day intervals and successfully induced immunostimulatory signals in mice 7 days after the last immunization [107, 108]. In a recent study by Shi et al., a vaccine with exosomes derived from IFN-γ-modified RM-1 prostate cancer cells under a vaccination regimen of four injections (on days 0, 4, 8, and 12) decreased the number of Tregs. It reduced the metastatic tumor rate in C57BL male mice with lung metastasis [109]. Recently, a non-randomized phase I/II clinical trial showed promising results with a vaccine designed using exosomes derived from DCs pulsed with SART1, a biomarker of esophagus squamous cell carcinoma. Pulsed DCs obtained from patients could generate exosomes that were well tolerated and induced antigen-specific CTLs in seven patients. One patient in this study remained stable for 20 months after DEXs therapy, although he developed lung metastasis after the stable period. The other six patients had progressive disease and died up to 10 months post-vaccination. These findings indicate that developing personalized exosome-based immunotherapy is feasible, although challenging [110]. A phase I clinical trial reported using exosomes derived from ascites (AEXs) in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) as immunotherapy for colorectal cancer. Injection of AEXs for colorectal cancer was safe and well tolerated by all patients during the four weekly doses administered [111].

17. Applied exosomes on scaffolds

The short tissue retention of exosomes after in vivo implantation is still a significant challenge in clinical applications. Scaffold encapsulation of exosomes can enable continuous delivery in the injured environment, thereby improving the therapeutic effect. Researchers have developed several methods to deliver exosomes to the post-infarct environment sustainably. For example, exosomes isolated from cardiomyocyte-derived induced pluripotent stem cells encapsulated in hydrogel patches were directly delivered to the hearts of infarcted rats [112]. The exosome patches demonstrated prolonged exosome release and promoted recovery of the ejection fraction, prevented cardiomyocyte hypertrophy, alleviated the ischemic injury, and promoted heart recovery. Another study loaded endothelial progenitor cell-derived exosomes into a shear-thinning gel to achieve precise administration and sustained delivery [113]. In a rat model of myocardial infarction, the exosome hydrogels enhanced angiogenesis and myocardial hemodynamics around the infarct. The cell-free scaffold material improved the effects of exosome-mediated myocardial therapy. In another study, exosomes isolated from human umbilical cord-derived
MSCs were encapsulated in functional peptide hydrogels to increase their stability and provide sustained release. The exosome hydrogels protected cardiomyocytes from oxidative stress induced by \( \mathrm{H}_2\mathrm{O}_2 \), which improved cardiac function in a rat myocardial infarction model. These studies provide practical and effective methods for exosome-laden scaffolds in myocardial regeneration [114]. Exosome-laden scaffolds are most widely used for skin repair.

Several findings indicate that combining bioactive scaffold materials with the controlled release of exosomes heals skin wounds. For example, exosomes isolated from human umbilical cord-derived MSCs encapsulated in polyvinylalcohol (PVA)/Alg nanohydrogels were used to heal diabetic wounds. The PVA/Alg nanohydrogel promoted cell proliferation, migration, angiogenesis, enhanced the efficacy of exosomes, and accelerated the healing of diabetic wounds. In another study, exosomes were loaded in a novel injectable bioactive hydrogel called FHE. Exosomes isolated from adipose-derived MSCs were loaded into FHE hydrogel through electrostatic interactions with poly-\( \varepsilon \)-L-lysine. The exosome hydrogel promoted angiogenesis, cell proliferation, and granulation tissue formation at the wound site and accelerated the healing of diabetic wounds and skin regeneration [115, 116]. In another study, methylcellulose-chitosan hydrogels loaded with exosomes isolated from placenta-derived MSCs were shown to heal diabetic wounds and form new tissues similar to natural skin. Similarly, chitosan/silk hydrogels with swelling and moisturizing capabilities loaded with exosomes isolated from gingival-derived MSCs promoted collagen epithelial regeneration and angiogenesis and accelerated the healing of diabetic skin defects [117, 118]. Chitosan scaffolds have also been shown to provide controlled release of exosomes isolated from synovium-derived MSCs, which accelerated wound healing by increasing the formation of granulation tissue and angiogenesis [118, 119]. Modified exosomes also have the potential to stimulate bone regeneration. For example, miR-375 was enriched in exosomes by overexpression in parental cells. The exosomes were loaded into a hydrogel and injected into a rat skull defect model. The exosomes were continuously released into the wound, which enhanced bone regeneration [120]. Liu et al. developed a photoinduced imine functional group cross-linking hydrogel glue to generate a decellularized tissue patch for cartilage regeneration. The patch retained stem cell-derived exosomes in the cartilage for a long time. In addition, the exosome-laden scaffold integrated with the natural cartilage matrix induced cell migration in the cartilage defect and promoted the repair and regeneration of articular cartilage [121]. Another study constructed a cell-free bone tissue engineering system by combining poly (lactic-co-glycolic acid) (PLGA)/polydopamine (pDA) scaffolds and exosomes isolated from human adipose-derived stem cells. The exosomes were slowly and continuously released from the scaffold, which promoted the migration of MSCs and significantly enhanced bone regeneration [112].

18. Exosome for microbial diseases

MSCs express various types of anti-microbial peptides and proteins (AMPs). Some of them are known for anti-bacterial properties, such as cathelicidin LL-37, \( \beta \)-defensin-2 (BD-2), hepcidin, and Lipocalin-2 (Lcn2) [122]. It is suggested in recent studies that MSCs can improve bacterial infection in preclinical models by AMPs. Therefore, MSCs can enhance the innate immune response against bacteria. It is suggested in recent studies that MSCs can improve bacterial infection in preclinical models by AMPs. Therefore, MSCs can enhance the innate immune response against bacteria [123].
In a study by Yagi et al. in 2020, the anti-microbial activity of human Adipose-derived MSCs (AD-MSCs) on *Staphylococcus aureus* was assessed. The findings showed that human AD-MSCs conditioned medium significantly prevented the growth of *S. aureus*. The results also showed the critical anti-microbial activity of cathelicidin LL-37 in AD-MSCs [124]. A previous study also showed that the anti-microbial activity of BM-MSCs against the growth of Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*S. aureus*) bacteria was mediated by LL-37 [125]. On the other hand, human umbilical cord blood-derived MSCs attenuated acute lung injury through *E. coli* infection in mice. The results demonstrated that MSCs secreted BD-2 through the TLR-4 signaling pathway and mediated the anti-microbial effects [126].

Moreover, menstrual-derived MSCs secreting hepcidin in synergy with antibiotics in sepsis were responsible for bacterial clearance. MSCs also secret different growth factors, such as the Keratinocyte growth factor (KGF), to exert anti-bacterial activity. In the research performed by Lee et al. on an *E. coli* infection model in an *ex vivo* perfused human lung, BM-MSCs improved alveolar fluid bacterial clearance and mitigated inflammation [127, 128]. The metabolomics and immunomodulatory effects of MSCs-MVs are performed by enhancing the intracellular ATP levels in injured alveolar epithelial cells and reducing the secretion of inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) in human monocytes. It should be considered that MSC-MVs expressed Cyclooxygenase2 (COX2) mRNA. COX2 is the crucial enzyme in prostaglandin E2 (PGE2) synthesis that is a critical factor for transforming the polarization of M1 into M2 macrophages. As articles suggest, the enhancement in PGE2 secretion by monocytes following the transfer of COX2 mRNA from MSC-MVs to these cells caused the phenotype switch from M1 to M2. MSC-MVs, by direct transfer of KGF or indirectly by activating monocytes, mitigated lung inflammation, cytokine permeability, bacterial growth, and improved survival. This therapeutic effect of MVs was abrogated by KFG neutralizing antibody, proposing a possible mechanism for the anti-bacterial effect of MSC-MVs [129, 130]. As the anti-bacterial effect of KGF in MSCs was previously reported [128], these studies supported the hypothesis that MVs can partly conserve the anti-microbial effects of parent cells, by using growth factors, including KGF. Cell-Exo can overcome the shortage of stem cells to treat microbial and other infectious diseases and provide a new generation in medical science from cellular to acellular therapy.

Both intact and engineered exosomes have been applied, and their efficacy on various infectious diseases has been assessed in preclinical studies and limited clinical trials. Although exosomes perform part of their antimicrobial activity by directly transferring mRNA, miRNA, and protein cargos, their beneficial effects are mainly indirectly applied through reprogramming immune cells and activating innate and adaptive immune responses.
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References


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[38] D-k K, Nishida H, An SY, Shetty AK, Bartosh TJ, Prockop DJ. Chromatographically isolated CD63+ CD81+ extracellular vesicles from mesenchymal stromal cells rescue cognitive impairments after TBI. Proceedings of the National Academy of Sciences. 2016;113(1):170-175


[64] Bhatnagar S, Schorey JS. Exosomes released from infected macrophages contain Mycobacterium avium glycopeptidolipids and are proinflammatory. The Journal of Biological Chemistry. 2007;282(35):25779-25789


MiR-9 and MiR-124 in the serum exosomes of acute ischemic stroke patients. PLoS One. 2016;11(9):e0163645


[95] Ibrahim AG-E, Cheng K, Marbán E. Exosomes as critical agents of cardiac regeneration triggered by cell therapy. Stem Cell Reports. 2014;2(5):606-619


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