



The Potential Use of Mesenchymal Stem Cells and Their Derived Exosomes for Orthopedic Diseases Treatment

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Abstract

Musculoskeletal disorders (MSDs) are conditions that can affect muscles, bones, and joints. These disorders are very painful and severely limit patients' mobility and are more common in the elderly. MSCs are multipotent stem cells isolated from embryonic (such as the umbilical cord) and mature sources (such as adipose tissue and bone marrow). These cells can differentiate into various cells such as osteoblasts, adipocytes, chondrocytes, NP-like cells, Etc. Due to MSC characteristics such as immunomodulatory properties, ability to migrate to the site of injury, recruitment of cells involved in repair, production of growth factors, and large amount production of extracellular vesicles, these cells have been used in many regenerative-related medicine studies. Also, MSCs produce different types of EVs, such as exosomes, to the extracellular environment. Exosomes reflect MSCs' characteristics and do not have cell therapy-associated problems because they are cell-free. These vesicles carry proteins, nucleic acids, and lipids to the host cell and change their function. This review focuses on MSCs and MSCs exosomes' role in repairing dense connective tissues such as tendons, cartilage, invertebrate disc, bone fracture, and osteoporosis treatment.

Keywords MSCs · Exosomes · MSDs · Orthopedic disease · Regenerative medicine

Abbreviations

MSDs	Musculoskeletal disorders
MSCs	Mesenchymal stem cells
DLS	Dynamic light scattering
SEM	Surface electron microscopy
TEM	Transient electron microscopy
NSAIDs	Nonsteroidal anti-inflammatory
IDO	Indolamine 2,3-dioxygenase

GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
PGK	Phosphoglycerate kinase
PGM	Phosphoglucomutase
ENO	Enolase
TLR	Toll-like receptors
IL-1	Interleukin-1
TNF	Tumor Necrosis Factor
BMP	Bone morphogenic protein
HIF	Hypoxic induced factor
VEGF	Vascular endothelial growth factor
COL1	Collagen Type 1
MAPK	Mitogen-activated protein kinase
LncRNA	Long non-coding RNA
TGF-β	Transforming growth factor-beta
NF-kB	Nuclear factor kappa-B
NP	Nucleus pulposus
ECM	Extracellular matrix
IDD	Intervertebral disc disease
MMPs	Matrix metalloproteinase
HSPs	Heat shock protein
PTEN	Phosphatase and tensin homolog
AGEs	Advanced glycation end products

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ER	Endoplasmic reticulum
NLRP	Nucleotide-binding oligomerization domain, Leucine-rich Repeat and Pyrin domain containing

Introduction

Orthopedic diseases are the most common cause of disability worldwide and include muscles, bones, nerves, joints, ligaments, tendons, and other connective tissue diseases. In general, damage to these tissues is due to their wounding or degeneration. The prevalence of these diseases increases with age, and The prognosis in these patients is usually poor [1]. People with musculoskeletal diseases (MSDs) have pain in different parts of the body with similar characteristics and prognosis and may respond to similar treatments [2]. Among the various treatments, we can mention non-pharmacological therapies, complementary therapies, pharmacological therapies, surgery, cell therapy, and combination therapy. These injuries are associated with a lot of pain, and there is usually no definitive cure for them, and the available treatments are usually used to reduce the pain. These include the use of corticosteroids, simple painkillers, and nonsteroidal anti-inflammatory drugs (NSAIDs) [3]. However, the effectiveness of these treatments is unclear and inconsistent. Nowadays, Cell therapy is one of the methods used in regenerative medicine, such as musculoskeletal diseases.

MSCs are used in the regeneration of different tissues due to their characteristics [4]. Self-renewability and the ability to differentiate into various cells are two characteristics of stem cells, and also they can be seen in MSCs [5]. These cells proliferate in vitro as plastic-adherent heterogeneous cells and have a fibroblast-like morphology. MSCs form colonies in vitro cultures and can be differentiated into bone, cartilage, and fat cells. However, researchers are trying to find and use all the potential of MSCs to create safe and alternative treatments. Therapeutic amplification of circulatory MSC, genomic manipulation of MSCs, combination therapy of mesenchymal stem cells with drugs, and use of their exosomes are examples of these efforts [6].

The use of MSCs derived exosomes has also been expanded to reduce cell therapy's side effects. Exosomes are lipid bilayer nanoparticles that, because they have a membrane similar to the body's cells, easily fuse with them and transfer their contents into target cells.

This article will discuss studies of MSCs and their exosomes role in bone diseases treatment such as osteoporosis and bone fractures and various types of degenerative diseases of dense connective tissue treatment, including cartilage, tendon, ligament, and Intervertebral disc.

Mesenchymal Stem Cell

MSCs, like other stem cells, are undifferentiated and can divide during self-renewal and differentiate into different cell types and tissues [7]. The sources of these cells are divided into three categories according to the place of collection. 1) Prenatal-derived sources such as placenta, umbilical cord, and umbilical cord blood 2) Embryonic sources such as amniotic fluid and embryonic tissue 3) Adult sources such as bone marrow, adipose tissue, muscle, and peripheral blood. The secretion of soluble factors from MSCs is another way that plays a role in the homeostasis of various tissues [8]. Different tissues derived MSCs' positive and negative markers are listed in Table 1.

Under standard conditions, MSCs have the ability to adhere to plastic [9]. They have also been shown to differentiate into fatty adipose cells, osteoblasts, chondrocytes, tenocytes, skeletal myocytes, heart muscle cells, smooth muscle cells, and even neurons [10, 11]. Since the beginning of studies on stem cells, special attention has been paid to MSCs from various sources. Therefore, these cells have become pioneers in the field of stem cell therapy studies.

Mesenchymal Stem Cell and the Immune System

Interestingly, MSCs exhibit anti-inflammatory properties when exposed to high levels of pro-inflammatory cytokines but enhance inflammatory responses when exposed to low levels of interferon-gamma and tumor necrosis factor-alpha (TNF- α) [12, 13]. The physiological properties of MSCs make them commonly used for tissue regeneration, transplant rejection, autoimmune diseases, and sepsis. Due to easy access, isolation, culture, and storage, they have become one of the main cell therapy options. Other functions of MSCs include migration to damaged tissues, increased angiogenesis, prevention of fibrosis, neuroprotective effects, suppression of the immune system, and suppression of hematopoietic stem cell apoptosis has a role in their therapeutic application [14].

These cells have been used for the treatment of skeletal diseases, multiple sclerosis, liver cirrhosis, rheumatoid arthritis, Alzheimer's, transplant rejection, graft versus host disease, inflammatory bowel disease, type 1 diabetes, asthma, and allergic diseases, etc. [15]. Table 2 shows some clinical trials in the field of MSCs in musculoskeletal diseases therapy (Table 2).

Although many studies have used MSCs as a treatment for various tissue disorders, problems such as tumorigenesis, fibrosis, pro-inflammatory conditions in the treated cell tissue, cell rejection and injection toxicity, functional

Table 1 Various tissues MSCs positive and negative surface markers

MSC tissue source	Positive markers	Negative markers
Adipose tissue	CD105, CD73, CD36, CD90, CD44, CD29, CD151, CD49d [149, 150]	CD45, CD34, CD14, CD11b, CD19, HLA-DR, CD34, CD38, CD31, CD106 [150–152]
Bone marrow	CD29, CD31, CD44, CD49a, CD49b, CD49c, CD49d, CD49e, CD51, CD54, CD58, CD61, CD71, CD73, CD90, CD102, CD104, CD105, CD106, CD120a, CD120b, CD121a, CD124, CD146, CD166, CD221, CD271, SSEA-4, STRO-1, Sca-1 [153–155]	CD11a, CD11b, CD13, CD14, CD19, CD34, CD45, CD133, CD31, CD86, HLA-DR [153]
Umbilical cord blood	CD29, CD44, CD73, CD90, CD105, CD166 [156, 157]	CD14, CD31, CD34, CD45, CD106, HLA-DR [156]
Synovial fluid	CD9, CD10, CD13, CD44, CD54, CD55, CD90, CD105, CD166, D7-FIB, CD49a, CD147, CD73, CD140a, CD49 [158, 159]	CD14, CD45, CD34, CD117, CD62e, CD20, CD113, HLA-DR, CD68, CD31, ALP, CD62, HLA-DR [158, 159]
Dental pulp	CD29, CD44, CD105, CD146, CD117 and STRO-1, SSEA-4, CD146, CD73, CD44, CD10, CD123 [160, 161]	HLA-DR, CD106, CD34, CD7, CD31 [160]
Wharton jelly	CD90, CD105, CD73, CD29, CD44 [9, 162, 163]	CD3, CD34, CD45, CD14, CD19, HLA-DR [163]
Amnion	CD73, CD29, CD49f, Oct4, Nanog, Sox2, SSEA-3, SSEA-4, Rex1 [164]	CD14, CD20, CD34, CD45 [165]

erosion, and the restricted ability of MSCs to differentiate are the barriers that can greatly overwhelm the therapeutic efficacy of these cells. MSCs, affect the immune system cells function and reduces inflammation and modulates injury site microenvironment. Mesenchymal stem cells produce the IL-1B receptor, which inhibits B cell differentiation by

trapping interleukin 1, stimulates macrophage type 2 responses, and increases their differentiation into M2 [16]. Also, they produces IL-10 and stimulates the expression of MHC class II, CD45, and CD11 on monocytes, reduces T cell responses and increases their differentiation into Treg [17], and suppress DC migration and maturation.

Table 2 Clinical trials that use mesenchymal stem cells to treat in various diseases

Study Title	Information provided by (Responsible Party):	ClinicalTrials.gov Identifier
1 Mesenchymal Stem Cells Transplantation in Newly Diagnosed Type-1 Diabetes Patients (MSCTXT1DM)	Tehran University of Medical Sciences Iranian Stem-Cell Council	NCT04078308
2 Mesenchymal Stem Cells for Progressive Multiple Sclerosis Sweden	Ellen Iacobaeus, Karolinska Institutet	NCT03778333
3 Allogenic Bone Marrow Mesenchymal Stem Cells Infusion in Patients With Steroid-refractory GVHD	National Institute of Blood and Marrow Transplant (NIBMT), Pakistan	NCT02824653
4 Bone Regeneration With Mesenchymal Stem Cells	Alejandro Gonzalez-Ojeda, Instituto Mexicano del Seguro Social	NCT02755922
5 Mesenchymal Stem Cells for the Treatment of Rectovaginal Fistulas in Participants With Crohn's Disease (RVF)	Amy Lightner, The Cleveland Clinic	NCT04519697
6 Mesenchymal Stem Cell Transplantation for Acute-on-chronic Liver Failure	Han Ying, Xijing Hospital of Digestive Diseases	NCT03668171
7 Mesenchymal Stem Cell for Acute Respiratory Distress Syndrome Due for COVID-19	Martín Iglesias, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran	NCT04416139
8 Clinical Trial of Umbilical Cord Mesenchymal Stem Cell Transfusion in Decompensated Liver Cirrhosis	Shandong Qilu Stem Cells Engineering Co., Ltd	NCT03529136
9 Mesenchymal Stem Cells for Multiple Sclerosis	Ellen Iacobaeus, Karolinska Institutet	NCT01730547
10 Use of Mesenchymal Stem Cells in Inflammatory Bowel Disease	Hanan Jafar, University of Jordan	NCT03299413
11 Allogeneic Human Mesenchymal Stem Cells for Alzheimer's Disease	Stemmedica Cell Technologies, Inc	NCT02833792
12 Allogeneic Human Cells (hMSC) Via Intravenous Delivery in Patients With Mild Asthma (ASTEC)	Marilyn Glassberg, University of Miami	NCT03137199

GVHD Graft Versus Host Disease, RVF Rectovaginal Fistulas, hMSC Human Mesenchymal Stem cell

Mesenchymal stem cells continuously secrete indolamine 2,3-dioxygenase (IDO). Sequential reduction of tryptophan by IDO inhibits allogeneic T cell responses, stimulates IL-4 secretion in Th2 cells, and reduces IFN- γ production by Th1 cells [18]. These cells also inhibit T-cell responses by secreting PD-L1 and induce apoptosis and non-response in these cells. Besides, mesenchymal stem cells inhibit NK cells' response by secreting factors such as prostaglandin E2, TGF-B1, NO, and IL-6 [12, 19].

Mesenchymal Stem Cell-derived Exosomes

Due to the mentioned limits, in recent years, scientists have turned their attention to the indirect use of MSCs, which are based on extracellular vesicles (EVs) derived from these cells [10]. Apoptotic body, microvesicles (MVs), and exosomes are three types of EVs and divided based on their size, content, and formation [20]. Apoptotic bodies are 50–4000 nm in size and are typically produced in the last stage of apoptosis from apoptotic cells. These EVs are heterogeneous and contain membrane contents (such as phosphatidylserine), nuclear material, and cellular organelles [21]. Unlike apoptotic bodies, microvesicles are shedding directly from the healthy cells membrane. Like the apoptotic body, these EVs have a heterogeneous morphology and their size range from 100 to 1000 nm. Microvesicles can affect the expression of genes by transmitting mi-RNA to neighboring cells. Besides, because MVs release from the cell is not dependent on endocytosis, they lack endocytosis-related proteins [22]. Exosomes by 30–120 nm size are the smallest EVs and are produced during late endosome membrane inward invagination and the multiple vesicular bodies (MVBs) formation [23]. After forming exosomes inside MVBs, they are secreted to the extracellular environment through endocytosis by MVBs membrane fusing with cell membrane [24]. Today, exosomes are divided into three groups based on size: large exosomes (exo-L size is between 90 to 120 nm), small exosomes (exo-S size is between 60–80 nm), and exomers which are 35 nm in size [25].

MSCs produce large amounts of exosomes compared to other cells, which makes them clinically viable for exosome separation and therapy [26]. Exosomes have common markers that seen in them and can be referred to as tetraspanins (CD63, CD9, CD81, CD82), fusion involved proteins (flotillins, CD9, annexin, GTPases), adhesion molecules, gap junction related proteins (Connexins-43) [27], heat shock proteins (HSC70 and HSC90), MHC-1, MHC-2, membrane transporters (GTPases), Rab proteins [28], lysosomal proteins (Lamp2b) and proteins involved in multivesicular body biogenesis (Alix and TSG101) [29, 30].

Exosomes are separated in a variety of ways, such as ultracentrifugation, density gradient centrifugation,

pegylation-based methods, and the use of kits. After characterizing the exosome through various methods such as dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), ELISA, it can be used for therapeutic applications.

Exosomes' uses have not the risk of genetic instability and immunosuppression following allogeneic administration in the *in vivo* models. Studies show that exosome therapy can be considered a new strategy to overcome stem cell therapy deficiencies [31].

Exosomes are one of the paracrine cellular communication vesicles. They can carry cytokines, chemokines, growth factors, different enzymes, different signaling molecules, miRNAs, lipids, and transcription factors by a lipid bilayer membrane. Based on studies, cargos present in MSC exosomes include enzymes involved in the synthesis of ATP (glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglucomutase (PGM), enolase (ENO) [32], angiogenesis stimulating enzymes (VEGF, inducer extracellular matrix metalloproteinase (EMMPRIN) and MMP-9) [33], Various transcription factors (transcription factor with Octamer 4 (Oct-4), HoxB4 and Rex-1) [31], tumor growth inhibitory miRs (miR-23b, miR-214, miR-451, miR-223, miR-31, miR-24, miR-125b and miR-122) [34] and inflammation regulating miRs (miR-155 and miR-146) [35]. Thus, bilayer lipids protect nucleic acids and proteins from degradation in the extracellular environment, enabling efficient transport. Table 3 shows some new studies that used MSCs derived-exosomes to repair and regenerate tissue in orthopedic diseases (Table 3).

Exosomes are smaller and less complex than their parent cells and also have less protein in the membrane. Therefore, they are easier to separate and store and have lower immunogenicity than cell therapy [36]. Furthermore, exosomes systemically are less likely to be trapped in the lungs or liver. Exosomes can transmit information through various ways, such as juxtacrine and solution signaling [37]. Exosomes have advantages over the use of their source cells: 1) Their use prevents the transfer of cells that may contain immunogenic molecules and even mutated or damaged DNA. 2) Exosomes are Nano-sized, and They can enter any organ and move easily inside it, while the cells are larger and cannot migrate to the site of injury through capillaries. 3) Exosomes, due to the presence of homing molecules on Their surface, can migrate to different parts of the body. 4) Because exosomes are native to the body, their surface has biochemical properties similar to their derived cells, enabling them to prevent phagocytosis, fusion with cell membranes, and lysosomal fusion [38].

Due to the mentioned features, MSCs-derived exosomes have become one of the dynamic fields in regenerative

Table 3 New studies used mesenchymal stem cells derived-exosomes to repair and regenerate tissue in orthopedic diseases

number	Study name	MSC source	Affected tissue	Study type	Exosome dose	reference
1	Bone marrow mesenchymal stem cell-derived exosomes promote tendon regeneration by facilitating the proliferation and migration of endogenous tendon stem/progenitor cells	Bone marrow	tendon	In vivo and in vitro	5 μ L of exos (4 μ g/ μ L)	[166]
2	TGF- β 1-containing exosomes derived from bone marrow mesenchymal stem cells promote proliferation, migration, and fibrotic activity in rotator cuff tenocytes	Bone marrow	tendon	In vitro	20 μ g/mL	[167]
3	Mesenchymal stem cell-derived exosomes ameliorate intervertebral disc degeneration via anti-oxidant and anti-inflammatory effects	bone marrow	intervertebral disc	In vivo	100 μ g/ml	[132]
4	Hypoxic mesenchymal stem cell-derived exosomes promote bone fracture healing by the transfer of miR-126	umbilical cord	bone	in vitro and in vivo	100 μ g/mL	[74]
6	MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity	E1-MYC 16.3 human embryonic stem cell derived MSC line	cartilage	In vitro	10 μ g/ml	[115]
7	Sphingosine-1-phosphate mediates the therapeutic effects of bone marrow mesenchymal stem cell-derived microvesicles on articular cartilage defect	bone marrow	cartilage	in vitro and in vivo	100 μ g/mL	[168]
8	Human bone mesenchymal stem cells-derived exosomes overexpressing microRNA-26a-5p alleviate osteoarthritis via down-regulation of PTGS2	umbilical cord	cartilage	In vitro and in vivo	unclear	[169]
9	Exosomes derived from miRNA-210 overexpressing bone marrow mesenchymal stem cells protect lipopolysaccharide-induced chondrocytes injury via the NF- κ B pathway	Bone marrow	ligament	In vitro	unclear	[170]
10	Intra-Articular Injections of Mesenchymal Stem Cell Exosomes and Hyaluronic Acid Improve Structural and Mechanical Properties of Repaired Cartilage in a Rabbit Model	E1-MYC 16.3 human embryonic stem cell-derived MSC line	ligament	In vivo	200 μ g/mL	[171]

TGF- β Transforming growth factor-B, *MSC* Mesenchymal stem cell, *PTGS2* Prostaglandinendoperoxide synthase 2, *NF- κ B* Nuclear factor kappa-B

medicine. Tissue destruction in many orthopedic diseases is one of the most important causes of loss of function. If we repair these tissues, their function will be mostly reversible. MSC-derived exosomes' therapeutic effect in heart, kidney, lung, skin, brain, liver, autoimmune, and musculoskeletal diseases has been demonstrated [39]. These exosomes can be used to treat various diseases such as type 1 diabetes, macular degeneration, chronic kidney disease, ischemic stroke [40], Alzheimer's [41], multiple sclerosis [42], sepsis, hepatitis [43], Chronic liver disease [36], skin disease [44] and musculoskeletal disease.

MSCs-derived exosomes have been shown to have therapeutic effects by modulating the immune system, stimulating cell proliferation, promoting angiogenesis, preventing apoptosis, and suppressing oxidative stress [5]. These exosomes help to homeostasis maintenance and cell repair by providing and transporting active enzymes to restore normal cell activity [45]. Proteomic analyses of MSCs exosomes show the presence of more than 200 immunomodulatory molecules in them [46]. Also, these exosomes increase proliferation in the target cell and prevent apoptosis by activating the Ras/Raf/MEK/ERK and PTEN/PI3K/AKT/mTOR signaling pathways [47].

Apart from the therapeutic ability, MSC-derived exosomes have the ability to migrate to lesion sites. It is also possible to modify the surface molecules of exosomes to migrate more and better to the site of injury [48]. This feature of exosomes makes them a good vehicle and a transport system that can deliver drugs directly to the site of disease [49]. Systemic exosome injections, such as intravenous, intraperitoneal, or subcutaneous, resulting in the rapid clearance of exosomes from the bloodstream and their accumulation in the liver, spleen, lungs, and gastrointestinal tract [50, 51]. Also, as a significant point, regardless of the injection route, most of the systemically injected exosomes are rapidly taken up by macrophages in the reticuloendothelial system and eliminate from the body [52]. Therefore, the biological distribution of exosomes after systemic administration can be divided into two stages. 1) Rapid distribution in liver, spleen, and lungs approximately 30 min after administration. 2) Removal of exosomes by hepatic and renal processing 1 to 6 h after administration [53]. The half-life of exosomes is administered topically (Such as the skin surface and ocular surface) is shorter due to their cleansing by fluids (sweat and tears) and exposure to external factors [54]. Figure 1 shows

the main mechanisms involved in the therapeutic applications of MSCs and their exosomes (Fig. 1), and Table 4 describes some mechanisms of MSC and MSC-derived exosomes involved in regenerative medicine.

MSCs and their Exosomes Role in Bone Regeneration

Bone regeneration is a complex process, and many cells, such as osteoblasts, osteoclasts, endothelial cells, chondrocytes, and MSCs, play a role in this process. Bone regeneration may occur in two ways: 1) intramembranous ossification that creates flat bones, and 2) endochondral ossification creates long bones [55]. Intramembranous osteogenesis performed by MSCs-derived osteoblasts direct calcification of bone. But endochondral ossification has a complex step that is regulated by chondrocytes [8]. Bone regeneration does not always happen wholly and correctly. Therefore, to increase the efficiency of bone repair, various treatments have been used. One of these treatments is the use of MSCs, which have been highly attentive for about two decades. Different

The main mechanism of MSCs and their exosome in treatment of orthopedic diseases

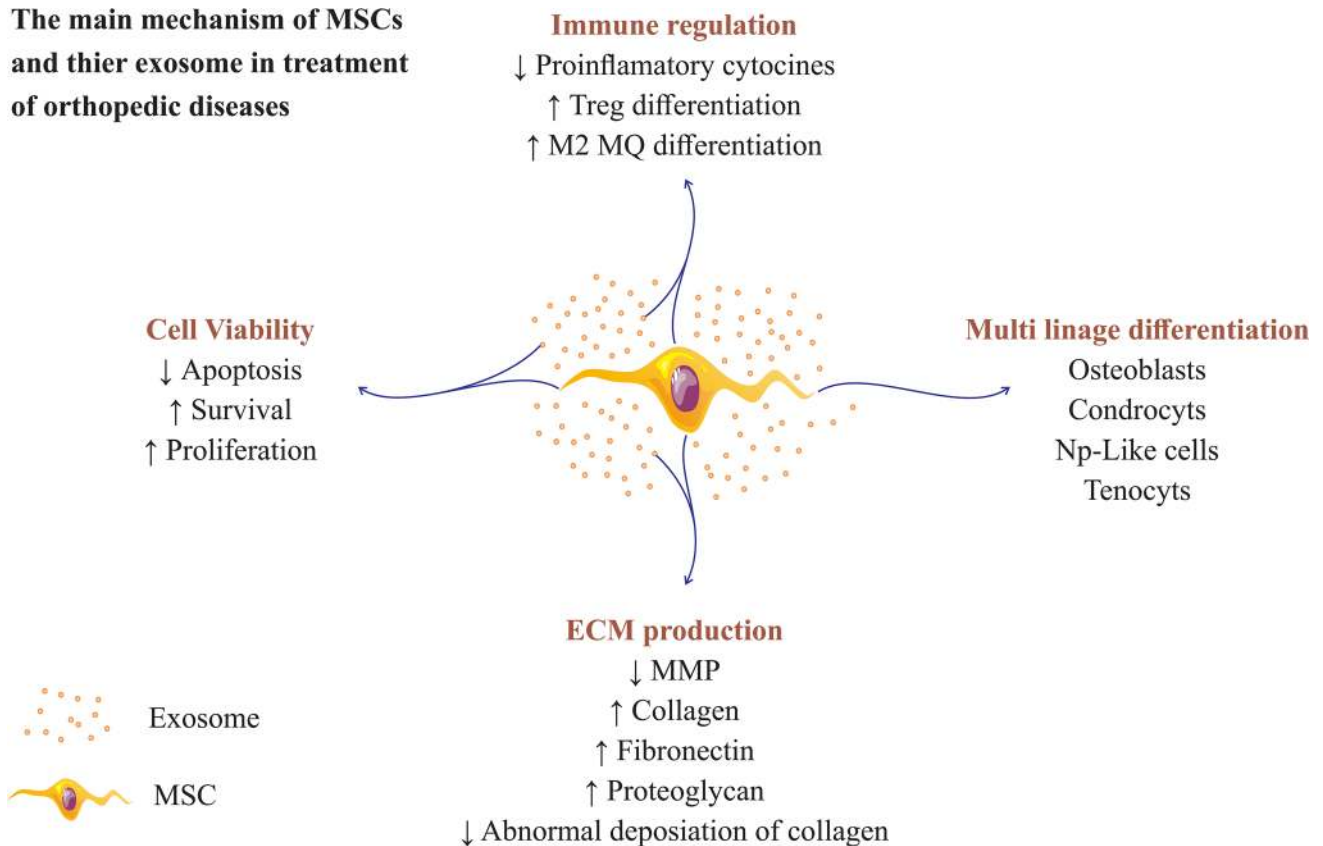


Fig. 1 Mesenchymal stem cells and their exosome use various mechanisms to repair tissue in orthopedic diseases. So MSCs and MSCs-derived exosomes can be increased extracellular matrix production,

increased cell viability, immunomodulatory properties, and multiplication differentiation

Table 4 Mechanisms of MSC and MSC-derived exosomes involved in regenerative medicine abbreviation

disorders	MSC related mechanisms	MSC derived exosome related mechanisms
bone regeneration and Osteoporosis improvement	<ol style="list-style-type: none"> 1. increased monocyte differentiation to M2 macrophage 2. decreased production of proinflammatory cytokines such as IL-6, IL-1, and TNF-α 3. Migration to bone injury site by specific chemokine receptors 4. Stimulation of osteoblastic differentiation 5. Increase angiogenesis at the site of injury 6. Creating a regenerating microenvironment exosomes 	<ol style="list-style-type: none"> 1. carry osteogenic related mi-RNAs such as miR-196a, miR-27a, and miR-206 2. increasing the recruitment of MSCs to the fracture site by stimulation of MCP-1, MCP-3, and SDF-1 production 3. increasing mineral deposition in osteoblasts 4. increasing angiogenesis by stimulating VEGF expression and suppress SPRED1 suppression 5. increasing the differentiation of hMSCs into osteoblasts by stimulating COL1, ALP expression 6. increase MSC osteoblastic differentiation by activating the BMP-2/Smad1/RUNX2 signaling pathway 7. increasing survival, proliferation, and differentiation MAPK signaling pathway in osteoblasts 8. inhibit apoptosis in BM-MSCs by miR-1263 / Mob1 / Hippo signaling pathway
cartilage repair	<ol style="list-style-type: none"> 1. producing ECM components such as collagen, fibronectin, proteoglycans, and glycosaminoglycans 2. prevent chondrocyte apoptosis 3. modulate the immune microenvironment of the injury site 	<ol style="list-style-type: none"> 1. create a regenerative environment for tissue repair 2. stimulation of cell proliferation through adenosine catalysis, enzymes ERK1 / 2 and AKT 3. increased extracellular matrix synthesis 4. increase chondrocytes mitochondrial function by transferring inactive glycolytic enzymes to them 5. Inhibition the function of NF-κB transcription factor 6. transmit anti-inflammatory factors
IDD	<ol style="list-style-type: none"> 1. stimulate proliferation in the NPCs 2. differentiate to NP-like cells 3. prevents abnormal deposition and aggregation of type 1 collagen by MMP12 and HSP47 production 4. increase angiogenesis and pain-inducing nerve fibers growth 5. exert immunomodulatory effects on NP cells 	<ol style="list-style-type: none"> 1. modulate the inflammatory environment 2. increase proliferation in NPCs 3. increase ECM production in NPCs 4. decrease NPCs apoptosis via suppression PTEN expression (by mir-21 transmission) 5. reducing the stress of the NPC 6. reduce AGEs related endoplasmic reticulum stress in NPCs
tendon	<ol style="list-style-type: none"> 1. immunomodulatory effect 2. differentiate to tenocytes 3. stimulate proliferation and differentiation in CD146 + progenitor cells by the FAK/ERK1/2 signaling pathway 	<ol style="list-style-type: none"> 1. increase the tendon stem cell migration 2. increase the anti-inflammatory macrophages migration to the site of injury 3. increase the expression of COL1a1 and COL3a1

ECM extracellular matrix, *TNF- α* tumor necrosis factor alpha, *NF- κ B* Nuclear factor kappa-B, *IL* interleukine, *MCP* monocyte chemotactic protein, *SDF* Stromal cell-derived factor, *RUNX2* Runt-related transcription factor 2, *BMP* bone morphogenic protein

in vivo and in vitro studies have shown the effectiveness of these cells in increasing bone regeneration. These cells' therapeutic effectiveness depends on various factors such as differentiation stage, injection method, and injection dose. These cells increase the regeneration of the bone in several ways [56]. 1) Decreased inflammation and immunomodulatory properties such as increased macrophage differentiation to M2 phenotype and decreased production of proinflammatory cytokines [57] 2) Migration to bone injury site by specific chemokine receptors [58] 3) Stimulation of osteoblastic differentiation of injury site cells [59] 4) Increased angiogenesis [60] 5) Creating a regenerating microenvironment [59]. Also, nowadays, MSCs are used to transmit genes to increase bone regeneration. The selected genes can be transferred to MSCs using viral vectors such as adenoviruses or non-viral vectors such as liposomes [61]. For example, it has been

reported that Ad-BMP2-MS-C grafting in rabbits is associated with new bone formation [62].

Various stimulatory factors such as bone morphogenic protein 2 (BMP-2) [63], BMP-7 [64], Wnt11 (that increase the osteogenic potential of BMP-9) [65], nanohydroxyapatite [10], and strontium [66] stimulate MSCs differentiation into osteoblasts.

MSC exosomes play an important role in bone regeneration by stimulating osteogenesis and angiogenesis while interacting with various cells, such as Bone marrow-derived MSCs (BM-MSCs) and endothelial cells [67]. BM-MSCs exosomes contain up-regulated levels of three osteogenic-related miRNAs, miR-196a, miR-27a, and miR-206, of which miR-196a is the most important exogenous osteogenic regulator [68, 69].

Successful bone regeneration is closely related to successful angiogenesis, and if angiogenesis is defective,

bone regeneration will fail. MSCs exosomes are captured by endothelial cells, which activate the Hypoxia-inducible factors (HIF-1 α) and produce VEGF [70]. This pathway leads to the activation of eNOS and, eventually, NO production, which, regulating ERK1/2 and activating the protein kinases ERK and c-Jun, independent of VEGF/VEGF-R2 signaling, leads to increased angiogenesis. Also, the HIF-1 α increases angiogenesis by activating the PI3K/Akt signaling path [71]. However, it is essential to consider that the microenvironment properties affect the function of exosomes produced by MSCs. For example, it has been shown that the pre-angiogenesis and stimulatory features of osteoblastic differentiation by exosomes produced from bone marrow MSCs are impaired in cases with type 1 diabetes [72].

Statistical analysis shows that during the differentiation of MSCs into osteoblasts, nine miRNAs, including let-7a, miR-199b, miR-218, miR-148a, miR-135b, miR-203, miR-219, miR-299-5p and miR-302b, increase in MSCs exosomes, while expression of four miRNAs, including miR-221, miR-155, miR-885-5p, miR-181a, and miR-320c decreases. Interestingly, all enriched miRNAs play a role in the activity and function of osteoblasts [73].

Numerous studies have demonstrated the effectiveness of MSCs exosomes in healing bone fractures. To ensure bone fracture healing, there must be three basic conditions: proper angiogenesis, proper ossification, and stabilization of the two fractures together. These exosomes improve bone fracture by increasing the migration of MSCs to the fracture site, increasing mineral deposition, as well as increasing angiogenesis, and increasing the differentiation of MSCs into osteoblasts by miR-21. MSC exosomes stimulate the expression of genes associated with osteoblastic differentiation and angiogenesis such as COL1, ALP, and VEGF [74] and contain bone repair-related cytokines such as monocyte chemoattractant protein-1 (MCP-1), monocyte chemoattractant protein-3 (MCP-3), stromal cell-derived factor-1 (SDF-1) that increases the healing of bone fractures. Given the characteristics of CD9^{-/-} mice, such as reduced bone repair and reduced exosome release from cells, it is hypothesized that defects in fracture healing in these mice compared to wild-type mice may be related to defect in exosome release. Studies have shown that injection of MSC exosomes increases the rate of fracture healing in CD9^{-/-} mice [69].

Nonunion is one of the disorders that can occur after a bone fracture. Using BM-MSCs derived exosomes to Nonunion Prevention has been tested today. In vitro results show that these exosomes increase bone differentiation by activating the BMP-2/Smad1/RUNX2 signaling pathway [75]. Smad proteins bind to RUNX2 to stimulate the differentiation of endothelial cells at the fracture site into osteoblasts and regulate collagen expression in osteoblasts (Fig. 2). These findings suggest that the use of BM-MSCs derived exosomes may be a

promising therapeutic approach in the treatment of bone graft fractures [76].

In a study by Liu et al. and colleagues, both in vitro and in vivo analyses showed that MSCs' exosomes under hypoxic stress improved bone fractures healing more than normal exosomes. Induction of hypoxia leads to more exosome production from MSCs, and these exosomes are better and more captured by other MSCs. Hypoxia also leads to increased expression of HIF-1 α in MSCs, which is a significant factor in the positive regulation of miR-126 expression. Thus, these exosomes contain large amounts of miR-126 and increase angiogenesis in endothelial cells by suppressing the expression of SPRED1 and activating the Ras/Erk signaling pathway. Besides, miR-126 has been shown to play an essential role in promoting angiogenesis during embryonic development by targeting PIK3R2, an inhibitor of angiogenic signals, and cell survival in response to VEGF [74].

MSCs and Their Derived Exosomes Role in Osteoporosis Improvement and Prevention

Osteoporosis is one of the most common age-related skeletal disorders that, in many cases, can lead to bone fractures, and its prevalence is higher in women than men [77, 78]. Hormonal, nutritional, behavioral, and genetic factors can contribute to osteoporosis development and progression [79, 80]. Among them, estrogen and glucocorticoid deficiency are the most common causes of osteoporosis [81]. Disruption of bone metabolic mechanisms, such as osteoclast imbalance of bone digestion and osteoblast formation of new bone, can lead to osteoporosis [79, 82]. In the process of osteoporosis repairing, osteoblasts are very important, and their function is to secrete osteoids to regenerate bone. BM-MSCs can differentiate into osteoblast cells [83, 84]. So MSCs play a role in bone tissue homeostasis due to their osteogenic and adipogenic differentiation abilities under physiological conditions [85]. Maintaining a balance between these differentiation abilities is essential to preventing osteoporosis [86]. Studies show that estrogen at normal concentrations stimulates bone formation and inhibits adipocyte formation, while at high concentrations, it has an adverse effect [87, 88]. Mesenchymal stem cells lose their ability to maintain bone homeostasis due to aging, menopause, and ovariectomy leading to the accumulation of large numbers of fat cells in the bone, loss of bone mass, and eventually osteoporosis [89]. Osteoblasts proliferate, mature, and produce extracellular matrix proteins for bone formation [90]. Several osteoporosis treatments have been suggested, including 1) Antiresorptive drugs including estrogen, bisphosphonates, selective estrogen response modulators (SERMs), and monoclonal antibodies including

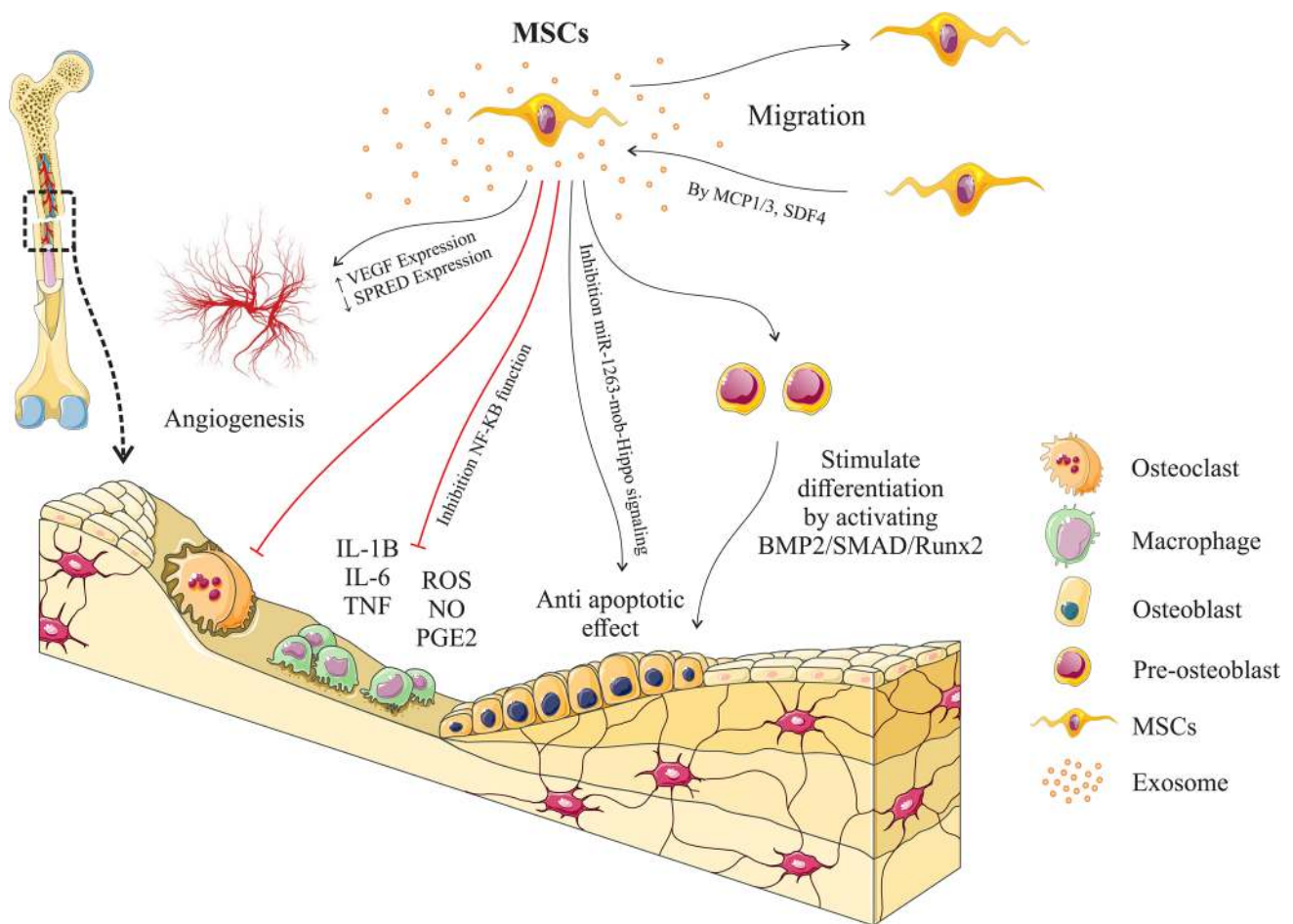


Fig. 2 Different functions of mesenchymal stem cell exosomes in bone fracture healing: These vesicles help bone healing by modulating the inflammatory environment, stimulating the differentiation of osteogenic progenitor cells into osteoblasts, stimulating angiogenesis,

and stimulating the migration of circulating mesenchymal stem cells to the site of injury. Mesenchymal stem cells can also differentiate into other cells to help in the repairing process, leading to faster fracture healing

denosumab. 2) Anabolic drugs, including Teriparatide (recombinant human parathyroid hormone), abaloparatide, and Wnt signaling pathway activators such as romosozumab 3) Combination therapies [91]. All of these treatments have side effects, so efforts to find new therapies are ongoing. Due to the reasons mentioned above, the use of mesenchymal stem cells and their exosomes can be a new treatment for osteoporosis.

A study states that MSCs derived exosomes improve and prevent osteoporosis by increasing survival, proliferation, and differentiation of osteoblast cell line (hFOB 1.19) via the mitogen-activated protein kinase (MAPK) signaling pathway, whose molecular pathways are not currently known [92].

Exosomes of MSCs contain GLUT3 protein and mRNA. This protein is one of the most important osteoblast differentiation markers and maturation, which increases significantly in hFOB1.19 cells treated with exosomes derived from MSCs. Also the level of pro-caspase 3 and 9 increases, and the amount

of broken caspase 3 and 9 decreases in these cells. According to the mentioned cases, these exosomes' use leads to increased proliferation and decreased apoptosis in the cell line related to osteoblast (hFOB1.19) [93].

In a study by Bao-cheng Yang et al., Human umbilical cord mesenchymal stem cell (HUC-MSCs) derived exosomes were used to treat hind limb unloading (HLU)-induced osteoporosis in rats. The miR-1263 in these exosomes can bind directly to 3' UTRs of Mob1 and inhibit Mob1 expression. Inhibition of Mob1 activates YAP, a significant regulator of the Hippo signaling pathway, thus inhibiting HLU-induced apoptosis in BM-MSCs compared with controls. BM-MSCs are precursors of osteoblast lineage and prevent osteoporosis by differentiating into these cells. Therefore, this study states that exosomes derived from HUC-MSCs inhibit apoptosis in BM-MSCs and prevent osteoporosis in rats through the miR-1263 / Mob1 / Hippo signaling pathway [94].

Exosomes derived from BM-MSCs can effectively stimulate osteoblast activity and prevent osteoporosis. A study in this field states that BM-MSCs do this by a lncRNA called MALAT1. MALAT1 binds to miR-34c and inactivates it, which increases the expression of SATB2 in osteoblasts (hFOB1.19). SATB2 is the target gene for miR-34b/c. It is also a scaffold protein that enhances the activity of two transcription factors, Runx2 and ATF4, which play an essential role in strengthening osteogenic differentiation. Therefore, this study suggests that exosomal MALAT1 derived from BM-MSCs may increase osteogenic activity and reduce osteoporosis symptoms [95].

MSCs and Their Derived Exosomes Role in Cartilage Repair and Regeneration

Many studies have confirmed the therapeutic effect of MSC in cartilage repair in animal and clinical studies. Osteoarthritis (OA) is the most common form of chronic joint disease characterized by the destruction of articular cartilage, the destruction of menisci and ligaments, the thickening of subchondral bone, and the formation of osteophytes [96]. There is currently no cure for OA, and most OA treatments are prescribed to manage pain, stiffness, and swelling [97, 98]. Because articular cartilage damage is of particular importance for OA pathology, it is crucial to restoring the articular cartilage's integrity and function in stopping or reversing OA progression. However, repairing and regenerating damaged articular cartilage is challenging because cartilage generally has an inadequate healing capacity and also challenging to treat due to the low level of blood vessels and blood supply. In recent decades, MSCs have been used to repair cartilage tissue because of their ability to differentiate into different tissues, including cartilage tissue [99]. Various strategies, such as co-culture of MSCs with cartilage cells, have increased MSC differentiation into chondrocytes and improved these cells' regenerative properties. For example, concomitant use of MSCs and chondrocyte differentiation-inducing factors, such as TGF- β superfamily activators [100], affects MSCs' proliferation and metabolic activity [101].

In large cartilage lesions, along with surgery, tissue engineering technology is used to recover cartilage tissue. Mesenchymal stem cells delay cartilage degeneration progression and lead to pain relief and improved function in patients with osteoarthritis and rheumatoid arthritis [101]. MSCs based Treatments for cartilage lesions include three groups. 1) MSCs transplantation 2) Injection of MSCs cells with scaffolds, and 3) Injection of MSCs-derived exosomes [102]. These cells improve cartilage function by increasing the production of ECM components such as collagen, fibronectin,

proteoglycans, and glycosaminoglycans [103, 104]. MSCs can prevent chondrocyte apoptosis through the paracrine effect [105].

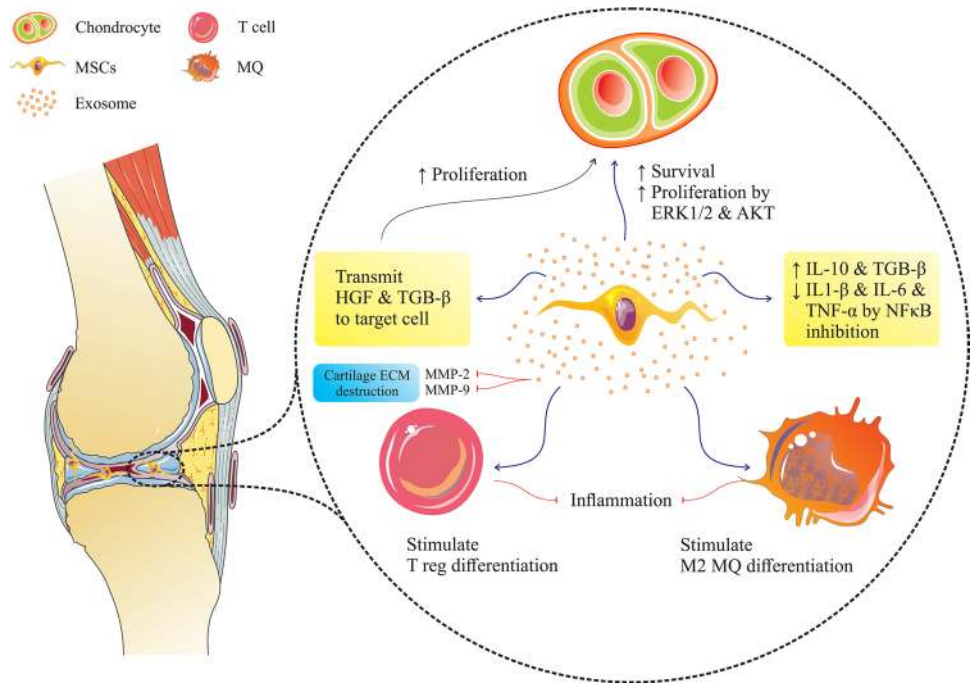
Over time, various studies have demonstrated the therapeutic effects of MSC derived exosomes in treating and recovering cartilage tissue [106]. MSCs exosomes may modulate the immune microenvironment to reduce the proinflammatory cell phenotype and create a regenerative environment for tissue repair [107, 108]. It has also been reported that the therapeutic efficacy of MSC derived exosomes does not depend on the tissue source of MSCs [109]. These exosomes perform therapeutic functions to regenerate cartilage through various mechanisms, including the transmission of biological mediators, stimulation of cell proliferation, increased extracellular matrix synthesis, and immunomodulatory properties [104].

Usually, during the aging process in mitochondria of chondrocytes, the proteins associated with the electron transport chain and ATP production decrease, and mitochondrial function is impaired [110]. In such a scenario, the capacity of the cartilage cells to repair is significantly reduced. MSC exosomes contain inactive glycolytic enzymes such as phosphoglucokinase and pyruvate kinase, ATP-producing enzymes such as adenylate kinase and nucleoside diphosphate kinase [32, 111]. It seems that the transfer of these enzymes by exosomes can compensate for the decreased ATP production in cartilage chondrocytes.

In order to regenerate cartilage tissue, it is vital to restore cell count, tissue structure, and function. MSC exosomes increase proliferation in various cells by phosphorylation through adenosine catalysis enzymes, ERK1/2 and AKT. Also, MSCs exosomes catalysis the adenosine production (activating survival kinases from Mediated by adenosine receptors) [112] from extracellular ATP released from damaged tissues (stimulates immune cells to kill damaged and stimulated cells) [113] with CD73 on their surface [114, 115].

In OA pathology, pro-inflammatory cytokines and matrix metalloproteinases (MMPs) [116] are mainly produced by the synovium and immune cells, such as macrophages, and play a role in the initiation and progression of OA. Therefore, modulation of the pro-inflammatory environment in damaged cartilage or OA is significant in cartilage regeneration. In various studies related to OA treatment with MSCs-derived exosomes [117], the concentrations of IL-1 β and TNF- α , IL-6, MMPs, and NO are significantly reduced (due to the inhibition of NF- κ B) [118] and the secretion of IL-10 and TGF- β increases. These exosomes transmit anti-inflammatory factors such as hepatic growth factor (HGF), Heme oxygenase-1, TGF- β 1, and stimulate the induction of suppressor cells such as Treg [119] and M2 macrophages in the region of inflammation and reduce cartilage damage and stimulate tissue regeneration (Fig. 3).

Fig. 3 The effect of mesenchymal stem cell exosomes on cartilage regeneration: These exosomes help regenerate cartilage by repressing the immune system, stimulating the production of the extracellular matrix, and stimulating the proliferation and survival of chondrocytes through the ERK1/2 and AKT pathway



MSCs and Their Derived Exosomes role in Ameliorate Intervertebral Disc Destruction (IDD) Treatment

The intervertebral disc (IVD) is made up of the inner nucleus pulposus (NP) and the outer annulus fibrosus (AF), which act as the load-bearing and buffering unit of the spine [120]. Intervertebral disc disease (IDD) leads to back and neck pain [121], during which proinflammatory cytokine production and macrophage migration increase and extracellular matrix degradation occur [121, 122]. IDD progression begins and accelerates with NP cell depletion and ECM destruction [123]. One of the important signs of IDD is reducing the number of viable and functional cells and its replacement by a significant number of aging and apoptotic cells. The use of stem cells increases the viability of intervertebral disc cells and prevents disease progression [124]. Today, one of the most exciting treatments for this disease is the use of MSCs. After transplantation, MSCs can differentiate into NP-like cells and stimulate proliferation in NPCs, leading to intervertebral disc regeneration [125, 126]. Many studies have shown that the use of MSCs reduces IDD in many animal models but can not completely regenerate the disc [127]. Fibrosis is one of the leading causes of IDD that can be caused by inflammation or injury and autoimmune disorders.

Interestingly, MSCs transplantation prevents abnormal deposition and aggregation of type 1 collagen in the pulposus nucleus by modulating MMP12 and HSP47 mediators [128]. Mesenchymal stem cells increase angiogenesis and pain-inducing nerve fibers growth by decrease the inflammatory

mediators through immunomodulatory effects on NPCs [129]. Also, MSCs are ameliorating IDD by their ability to differentiate into NPCs [130]. In order to increase the efficacy of MSCs in IDD treatment, these cells are incubated with NPC exosomes before transplantation to stimulate MSCs to differentiate into NP-like cells [124].

MSC exosomes modulate the inflammatory environment [129] and, after affecting NPCs, lead to increased proliferation, decreased apoptosis, and increased extracellular matrix production of these cells [124, 131]. Due to the decrease in the amount of mir-21 in exhausted NPCs and the presence of high levels of mir-21 in MSCs exosomes, the addition of these exosomes to the exhausted NPCs reduces apoptosis and leads to suppression of PTEN expression by mir-21 transmission [126].

Advanced glycation end products (AGEs) produced by the Millard reaction typically accumulate in aging and damaged tissues and are closely related to inflammation, metabolic dysfunction, and endoplasmic reticulum (ER) stress. Since AGEs increase apoptosis in NPC by inducing ER stress and activating the unfolded protein response (UPR), inhibiting ER stress induced by AGEs can be a suitable therapeutic target for IDD treatment. MSCs exosomes inhibit apoptosis in these cells and inhibit disease progression by reducing the stress of the NPC endoplasmic reticulum containing AGEs by activating the AKT and ERK signaling pathways [103]. After the effect of MSCs derived exosomes on the exhausted NPCs, their proliferation was significantly increased compared to the control. The expression of Aggrecan, collagen II, sox-9, and TIMP-1 genes were also increased considerably. IL-1β, iNOS, COX-2, IL-6, MMP13, MMP-1, MMP-3, and

other enzymes involved in extracellular matrix degradation expression was significantly decreased so these cells can form a healthy extracellular matrix [132].

A new study has shown that miR-532-5p levels are decreased in apoptotic NPCs [133], while its amount in TNF- α -stimulated bone marrow mesenchymal stem cell exosomes are very high [134]. TNF- α stimulated BM-MSCs derived exosomes has better effects on NPCs than native BM-MSCs-Exos. miR-532-5p reduces apoptosis, ECM degradation, and fibrosis deposition by targeting RASSF5 (pro-apoptotic gene) 3'-UTR and silencing it [135].

Research has shown that the pathogenesis of IDD is closely related to ROS and oxidative stress [136]. Thus, attenuating mitochondrial dysfunction mediated by oxidative stress can increase the cellular homeostasis of NPCs and protect against IDD. A study by Chen Xia and colleagues shows that the use of H₂O₂ leads to increased ROS and oxidative stress and leads to abnormal mitochondrial structure, increased ECM degrading enzymes, decreased ECM synthesis-related enzymes, increased NLRP3, and apoptosis in NPCs. MSC exosomes contain mitochondrial proteins and

improve NPCs' mitochondrial function by transferring these proteins to them [132]. Studies show that these exosomes can merge with mitochondria membrane after entering the NPCs. Besides, MSCs exosomes contain anti-oxidant proteins such as Trap 1, Gpx 4, and Prdx-1, preventing apoptosis by transporting them to cells containing oxidative stress [137].

MSCs and their Exosomes Role in Tendon Repair

Tendon damage occurs in athletes and physical activity and leads to severe pain. Also, people with multi-morbidity are more vulnerable to tendon damage as aging occurs. Today, MSC is used to repair tendons in many studies [138]. However, the direct use of MSC still faces inevitable challenges, such as operating costs, maintaining cell viability, and storage [139].

Because the Inhibition of exosome production by MSCs reduces their ability in tendon regeneration, the regenerative effect of MSCs may be exerted, at least in part, by exosomes [140]. Evidence suggests that MSCs derived exosomes can inhibit tendon stem cell apoptosis and stimulate their proliferation, migration, and tenogenic differentiation [141].

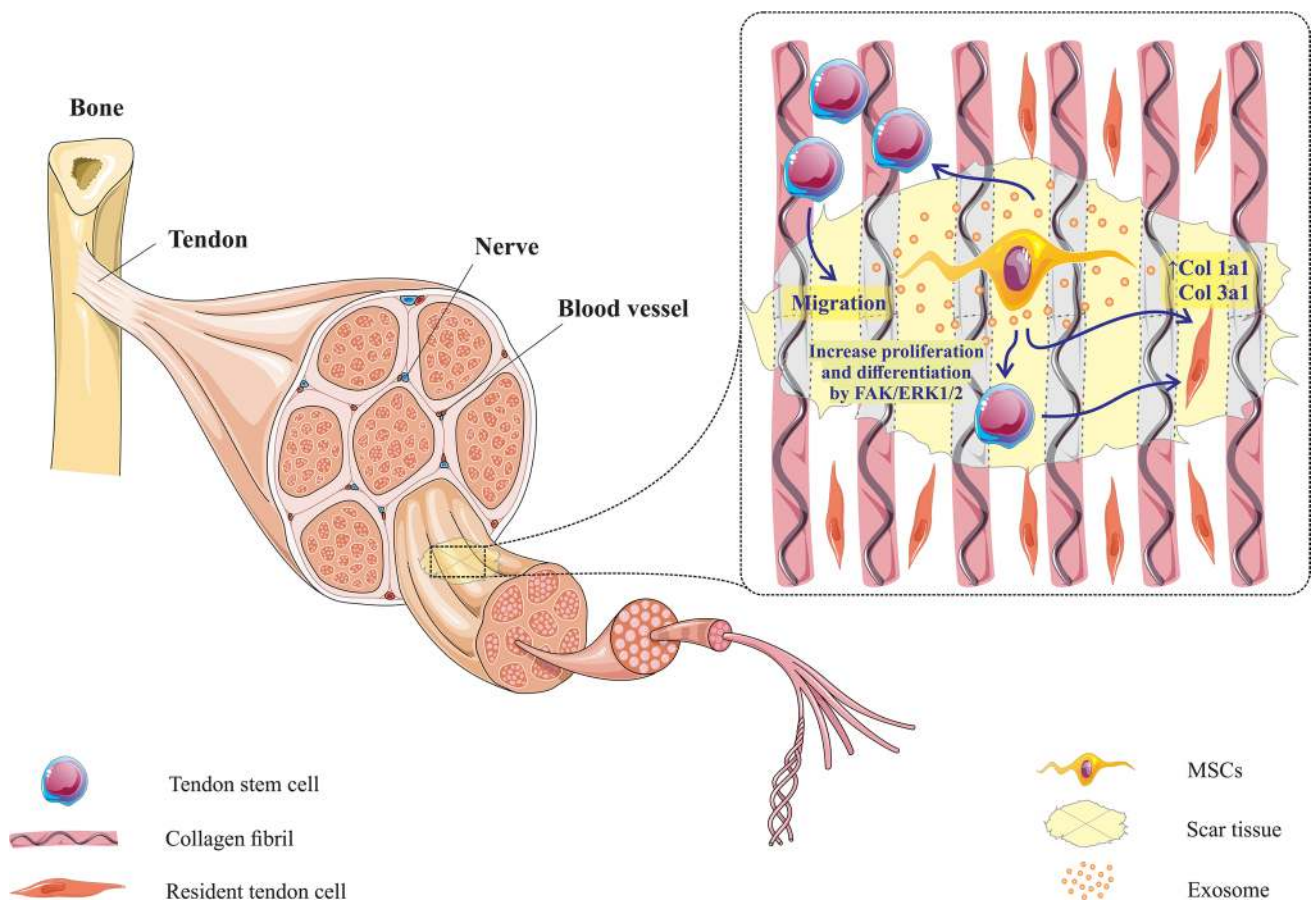


Fig. 4 Mesenchymal stem cell exosomes help regenerate tendon tissue by increasing tenogenesis of tendon stem cells through the SCX and TNmd pathways. Also, these exosomes increase the expression of COL1a1 and COL3a1 in the tendon cells present at the site of injury

Inhibiting inflammation at the site of tendon damage is one of the first steps in tissue repairing. MSCs derived exosomes prevent inflammation by increasing the migration of CD163+ cells (markers of anti-inflammatory macrophages) to the tendon regeneration site. Also, the MSCs derived exosomes can affect macrophages and convert them to the M2 phenotype to participate in anti-inflammatory and regenerative reactions [142]. During this event, IL-10 and IL-4 cytokines' expression increased in macrophages, and the expression of interferon-gamma, IL-1B, and IL-6 cytokines decreased. These macrophages also increase angiogenesis by stimulating endothelial cell migration [143]. Studies show that the use of MSCs exosomes increases the CD146+ cell migration (tendon stem cell maker) to the site of injury [108]. These exosomes also stimulate the tenogenesis of tendon stem cells by stimulating mRNA expression of Scx and Tnmd genes (Fig. 4). These exosomes promote the expression of COL1a1, which encodes type I collagen, and COL3a1, which encodes type III collagen to regenerate the ECM in the injured Achilles tendon [144].

Conclusion

According to the above, mesenchymal stem cells and exosomes produced from them are one of the attractive fields in the treatment of various diseases. These cells and their exosomes can stimulate differentiation in the cells at the injury site and prevent cell apoptosis. Due to their immunomodulatory properties, they modulate inflammatory responses and prevent immune responses mediated disease. Mesenchymal stem cells produce chemotactic factors to recruit other stem cells and immune cells involved in tissue regeneration. Mesenchymal stem cells communicate with the surrounding cells by producing micro-vesicles, such as exosomes, and transmit various information. Although exosomes do not have cell transfer problems, their use is complicated due to their nanosize and are relatively expensive. Also, naive exosomes secreted by mesenchymal stem cells do not have much ability to induce optimal responses [145]. Therefore, today, by manipulating mesenchymal stem cells and their exosomes, increases their therapeutic application. These include loading various drugs into the exosomes for better efficiency. Drug loading in exosomes done through pre-load and post-load methods. In pre-load, by adding the cargos such as drugs, miRNA, and various proteins to mesenchymal stem cells' culture medium, they pick up the cargo and manifest inside the exosomes [146]. However, in the post-load method, after exosome isolation, the cargo is loaded in the exosome by various methods such as incubation, freeze–thaw, sonication, and electroporation [147]. Also, in some cases, by changing the exosomes' surface molecules, their efficiency can be increased for therapeutic purposes

[148]. However, using exosomes to treat various diseases is a new field in research, and its development can be a good platform for various applications.

Also, since there is no definitive treatment for orthopedic diseases, trying to induce tissue regeneration in the damaged tissues can be helpful in these diseases. Based on the above information, it can be concluded that the use of MSCs derived exosomes is one of the desired treatments that can stimulate this regeneration at the injury site.

Authors' Contributions Kosar Malekpour and Marziah Zahar wrote the manuscript, Ali Hazrati and Angelina Olegovna Zekiy edited final version of the manuscript, Jamshid G. Navashenaq and Alexander Markov draw the schematic figure and tables, Leila Roshangar and Majid Ahmadi designed and supervised the study, and correspondence during the paper submission.

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Data Availability The data supporting the conclusions of this article are all online.

Declarations

Competing Interests The authors declare that they have no competing interests.

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