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Review article

Human umbilical cord mesenchymal stem cells derived-exosomes in diseases treatment

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ABSTRACT

Exosomes are extracellular vesicles with the size of 40–100 nm in diameter and a density of 1.13–1.19 g/mL, containing proteins, mRNAs, miRNAs, and DNAs. Exosomes change the recipient cells biochemical features through biomolecules delivery and play a role in cellular communication. These vesicles are produced from body fluids and different cell types like mesenchymal stem cells (MSCs). Evidence suggests that mesenchymal stem cells-derived exosome (MSC-EXO) exhibit functions similar to MSCs with low immunogenicity and no tumorization. MSCs can also be isolated from a variety of sources including human umbilical cord (HUC). Because of the non-invasive collection method, higher proliferation and lower immunogenicity, HUCMSC-EXO has been frequently used in regenerative medicine and various diseases treatment compared to the other MSC-EXO resources. This review aimed to investigate the applications of HUCMSC-EXO in different diseases.

1. Introduction

Stem cells are divided into embryonic and adult stem cells. Mesenchymal stem cells (MSCs) are one of the most important adult stem cells [1]. These cells are characterized by plastic adhesion properties, differentiation ability into osteocytes, chondrocytes, and adipocytes *in vitro*, positive for CD73, CD90, and CD105, and negative for major histocompatibility complex class II (MHC-II), CD11b, CD14, CD31, CD34, and CD45 [2]. MSCs can be isolated from different sources such as umbilical cord, umbilical cord blood, bone marrow (BM), adipose tissue, Wharton's jelly, cervical tissue placentae, skeletal muscle tissue, ammonia fluid, liver tissue, dental pulp, synovial membranes, saphenous veins, lung and dermal tissues and periodontal ligaments [3]. Due to their differentiation ability, *in vitro* expansion and release of trophic materials, along with immunoregulatory properties, MSCs are suitable candidates for tissue repair and treatment of various diseases, such as diabetes, organ transplantation, graft-*versus*-host disease, cardiovascular, inflammatory, liver, lung, kidney, neurological, autoimmune, bone and cartilage diseases as well as [4,5]. Due to the non-invasive isolation method, no ethical concerns, lower immunogenicity, faster self-renewal ability, more stable doubling time and higher proliferation potency, human umbilical cord MSCs (HUCMSC) are preferred candidates for cell-based therapies and regenerative medicine compared to the other sources [6]. However, HUCMSC have relative limitations in the maintenance of biological activity, the quantification of bioactive substances, and the logistics delivery in clinical therapies [7]. Therefore, finding a cell free method with the same output and efficacy seems to be necessary. Exosomes are one of the members of the extracellular vesicles, which have attracted researchers, attention as a novel strategy for cell free-based therapies, due to their numerous biological activities and cellular communications [8,9]. In HUCMSC-EXO studies, all the advantages of HUCMSC, such as therapeutic factors transfer from HUCMSC with low immunogenicity, outstanding self-renewal and immunoregulation characteristics have been reported to be conserved [7]. Additionally, no tumorigenic outcomes have been reported in these studies. Therefore, in this review, the application of HUCMSC-EXO in different diseases will be discussed.

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2. Exosome

The term exosome was first described by Trams et al. in 1981 [10]. Exosomes are released *via* the fusion of multivesicular bodies with plasma membranes into the extracellular environment [11]. Exosomes are spherical particles with two lipids layers containing sphingomyelin, cholesterol, ceramide, phosphatidyl choline and phosphatidyl ethanolamine [12]. Exosomes are 40–100 nm in diameter and a density of 1.13–1.19 g/mL [13]. These vesicles are released from B lymphocytes, cytotoxic T cells, neurons, schwann cells, oligodendrocytes, platelets, intestinal epithelial cells, dendritic cells, and mast cells. Additionally, exosomes have also been found in body fluids like saliva, blood, breast milk, semen, bile, urine, cerebrospinal fluid, ascites fluid, and amniotic fluid [14]. Exosomes can also deliver biomolecules including lipids, carbohydrates, nucleic acids, and proteins from one cell to another, leading to genetic information exchange, host cell reprogramming and cellular communication [9,13]. The main obstacles for exosome use include disadvantages of different isolation and purification methods, exosomal antigen and drug load and cargos delivery to the target cell [15]. There are also challenges in the production of large-scale exosomes, high quality and uniform exosome collection, and optimization of exosome storage conditions [16].

2.1. Exosome composition

Exosomes composition differs based on their sources. The protein and lipid content of exosomes has been measured by various methods, such as fluorescence-activated cell sorting, Western blotting, mass spectrometry and immuno- Electron microscopy. Rabs and Annexin, including Annexin I, II, V, and VI are cytosolic proteins present in exosomes which contribute to the formation of exosome docking, membrane fusion, and the kinetic regulation of cytoskeletal membranes. Additionally, adhesion molecules such as intercellular adhesion molecule-1, CD11a, CD11b, CD11c, CD18, CD9 fat-globule EGF-factor VIII (MFG-E8), CD58, CD146, CD166 have also been identified in exosomes [17,18]. Exosomes also contain heat-shock proteins (Hsp70 and Hsp90), which facilitate peptides loading to the MHC I and II [19]. Participating proteins causing vesicle formation and trafficking include lysobisphosphatidic acid (LBPA)-binding protein Alix, which is present in exosome [20]. Of the proteins found in most exosomes, there are transmembrane transport and integration-related proteins (*e.g.*, Annexin, G protein, Flotillin), heat shock proteins (Hsp70, Hsp90) and tetraspanins (CD82, CD81, CD63, CD9) [21,22]. The lipid content of exosome also varies depending on their production source, and includes double unsaturated phosphatidylcholine, glycoside GM3, phosphatidylserine, double-unsaturated phosphatidylethanolamine, ganglia, cholesterol and sphingomyelin [23]. Exosomes contain noncoding RNAs or fragments, including overlapping RNA transcripts, protein-coding region, structural RNAs, repeats, fragments of transfer RNAs, Y RNAs, short hairpin RNAs, small interfering RNAs (siRNAs), microRNA (miRNA), messenger RNA (mRNA), and DNA [24]. They also contain various miRNAs including miR-1, miR-15, miR-16, miR-17, miR-18, miR-181, and miR-375 [25]. miR-21, miR-100, miR-143, miR-146a, mir-181, and miR-221 have also exclusively been found in HUCMSC-EXO [26]. Moreover, various cytokines such as Tumor Necrosis Factor-α (TNF-α), Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF), Interleukin (IL)-2, IL-6, IL-8, IL-10, IL-15, IL-1β, are expressed in exosomes [27]. Fig. 1 shows the exosome composition in detail.

2.2. Exosome isolation

Four methods are proposed for the exosome isolation and purification as follows:

2.2.1. Exosome isolation by ultracentrifugation

Ultracentrifugation using sucrose density gradients or sucrose cushions is one of the methods suggested for exosome isolation [28]. The advantages of this method are cost-effectiveness and contamination risk with separation reagents, and large samples requirement. Because of successive centrifugation, this method is considered as a time-consuming approach. Also, due to high speed centrifugation, exosomal damage is possible. However, as it is accepted by most researchers, this method, in combination with the sucrose gradient and sucrose cushions yields higher exosome quantity and purity [29,30]. The ultracentrifugation method in combination with sucrose cushions is summarized in Fig. 2.

2.2.2. Size-based exosomes isolation

The size-dependent exosome isolation is consisted of several methods including separation with the size exclusion chromatography (SEC) and ultrafiltration procedures [14]. Faster performance, higher exosome yields, no need for special equipment and good portability, are the main advantages of this approach. However, low exosome purity, clogging and vesicle trap possibility, and the loss of exosomes due to membrane attachment are challenging problems in this method [29,30].

2.2.3. Exosome precipitation

Polyethylene glycols (PEGs) can be used for the precipitation procedure [31]. Then, it can isolate exosome at low speed centrifugation or filtration [14]. The advantages of this method are easy to perform and no need for special equipment [30]. However, low purity and yield and high costs are disadvantages of this procedure [29].

2.2.4. Affinity-based exosomes capture

Theoretically, antibodies against exosomes markers including CD81, CD63, or CD9, as well as a dedicated lectin targeting mannose can be used for exosomes isolation [14]. The advantages of this method are the isolation of specific exosomes and high purity compared to other methods. The disadvantages of this method include high cost, low yields and the use of cell-free samples [30].

2.3. Exosome characterization and identification

A variety of microscopes such as transmission electron microscopy (TEM) [32], scanning electron microscopy (SEM) [33], and atomic force microscopy (AFM) have recently been used to measure the size and morphology of the exosomes. TEM is the most frequently used instrument in studies. Furthermore, exosomal proteins measurement using the Bradford method [34] bicinchoninic acid assay (BCA) and enzyme linked immunosorbent assay (ELISA) has been considered in different studies [35]. Nanoparticle tracking analysis (NTA), immunoaffinity based capture method (IAC), dynamic light scattering (DLS), surface plasma resonance (SPR) have also been used to measure the size distribution and particle concentration of the samples [36]. In most studies, the NTA method is the preferred approach. Flow cytometry and western blotting analysis are also used to identify exosomes surface markers [37]. Most studies have also been identified exosome surface markers including CD9, CD63, and CD81 using western blotting. Table 1 represents the characteristics of human umbilical cord mesenchymal stem cells-derived exosome (HUCMSC-EXO) in studies.

3. Human umbilical cord mesenchymal stem cells derived-exosome

MSCs have been isolated from different sources. One of these sources is human umbilical cord [38]. Due to the non-invasive isolation method, no ethical concerns, and the low immunogenicity, this source of MSCs has attracted the researchers' attention [6]. However, reports have indicated that the injection of MSCs may cause tumor spreading. Additionally, MSCs differentiation, potentially has led to tissue ossification or calcification in animal models [39,40]. Evidence suggests that

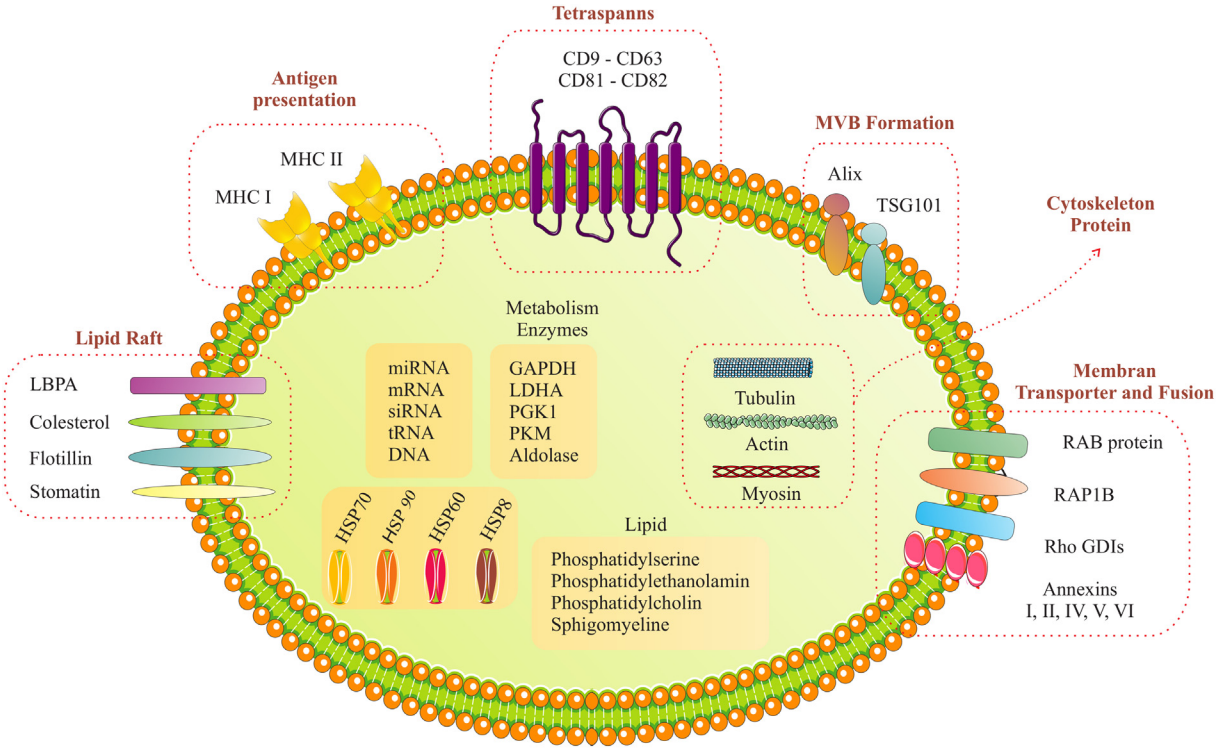

Fig. 1. Exosome composition. Exosomes are spheroidal shaped and two-layer lipid particles containing various types of proteins, lipids, DNAs, non-coding RNAs, miRNAs, and mRNAs which cause genetic information exchange and reprogramming of the recipient cells. Cluster of Differentiation (CD), Major Histocompatibility Complex (MHC), Lysobisphosphatidic Acid (LBPA), Rho GDP Dissociation Inhibitor (Rho GDI), RAS Related Protein 1B (RAP1B), RAS Related Protein (RAB), Multivesicular Body (MVB), Tumor Susceptibility Gene 101 (TSG101), microRNA (miRNA), Messenger RNA (mRNA), Small interfering RNA (siRNA), transfer RNA (tRNA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Lactate Dehydrogenase A (LDHA), Phosphoglycerate Kinase 1 (PGK1), Pyruvate Kinase Muscle (PKM), Heat Shock Protein (HSP).

exosomes have the same functionality as MSCs, with no concerns about MSCs injection complications [41]. The HUCMSC-EXO are extracellular vesicles of 40–100 nm in size. HUCMSC-EXO expresses CD9, CD81 markers. It also contains several types of cytokines, containing the highest levels of IL-6 and IL-8. Based on recent data, HUCMSC-EXO has been shown to stimulate cell proliferation and protection against H_2O_2 induced apoptosis [27]. Furthermore, HUCMSC-EXO has no harmful effects on the kidneys and liver. Other evidences, such as systemic anaphylaxis, hemolysis, pyrogen, hematology indexes, vascular and muscle stimulation have introduces HUCMSC-EXO as a safe therapeutic mediator [42].

4. Application of HUCMSC-EXO

4.1. Kidney diseases

Studies have shown that the use of BM-MSC and microvesicles can improve acute kidney injury (AKI) [43,44]. Moreover, HUCMSC-EXO have been shown to have therapeutic effects on cisplatin-induced nephrotoxicity *in vitro* and *in vivo*. Cisplatin is an anticancer drug causing nephrotoxicity *via* oxidative stress-inducing renal tubular epithelial cell apoptosis. However, HUCMSC-EXO treatment has reduced creatinine (Cr) levels, blood urea nitrogen (BUN) proximal kidney tubules necrosis, apoptosis, oxidative stress induced by cisplatin and formation of abundant tubular protein casts *in vivo*. Additionally, *in vitro* treatment by HUCMSC-EXO also has reduced oxidative stress, the number of apoptotic cells, and p38 mitogen-activated protein kinase (p38MAPK) activation pathway followed by an increased caspase 3 expression and cell multiplication in NRK-52E cells. HUCMSC-EXO also promoted cell proliferation through extracellular-signal-regulated kinase (ERK) 1/2 pathway activation. Wang et al. [45] in a study, examined the effects of HUCMSC-EXO pretreatment on cisplatin-induced renal toxicity prevention. The results indicated the significant role of autophagy in tissue damage alleviation. They also showed that HUCMSC-EXO pretreatment causes autophagy activation through decreasing phosphorylated mammalian target of rapamycin (mTOR), as well as increased expression of the autophagy-related genes (ATG5 and ATG7) and the autophagy marker protein (LC3B) in NRK-52E cells. It also reduced apoptosis and inflammatory responses. mTOR is an evolutionarily conserved nutrient-sensing serine/threonine protein kinase. Therefore, HUCMSC-EXO may be considered as a therapeutic strategy for prevention of cisplatin side effects. Jia et al. [46] study also showed that 14-3-3ζ induces autophagy. 14-3-3ζ interacts with ATG16L probably through a phosphorylation-dependent manner. This interaction leads to elevated localization of ATG16L in auto phagosome precursors. The ATG12-ATG5-ATG16 complex is an ubiquitin-like conjugation system that is involved in elongation and expansion steps of autophagosome formation.

4.2. Ocular disease

Idiopathic macular hole (MH) is one of the reasons of visual impairment [47]. Pars plana vitrectomy (PPV) is the main treatment for MH [48]. Intravitreal injection of HUCMSC and HUCMSC-EXOs with air, heavy silicon oil, 20% SF6, or 14% C3F8 tamponade for 7 patients with large and refractory MH after standard PPV combined with internal limiting membrane peeling was performed by Zhang et al. [49] Six MH patients were closed and 1 patient remained in the flat-open state. In 5 patients with MH closure, the best-corrected visual acuity (BCVA) was ameliorated. No alterations in BCVA were observed in one MH closure patient with a 4-year MH history. In one MSC treated patient, a fibrous membrane was observed on the retina. An inflammatory

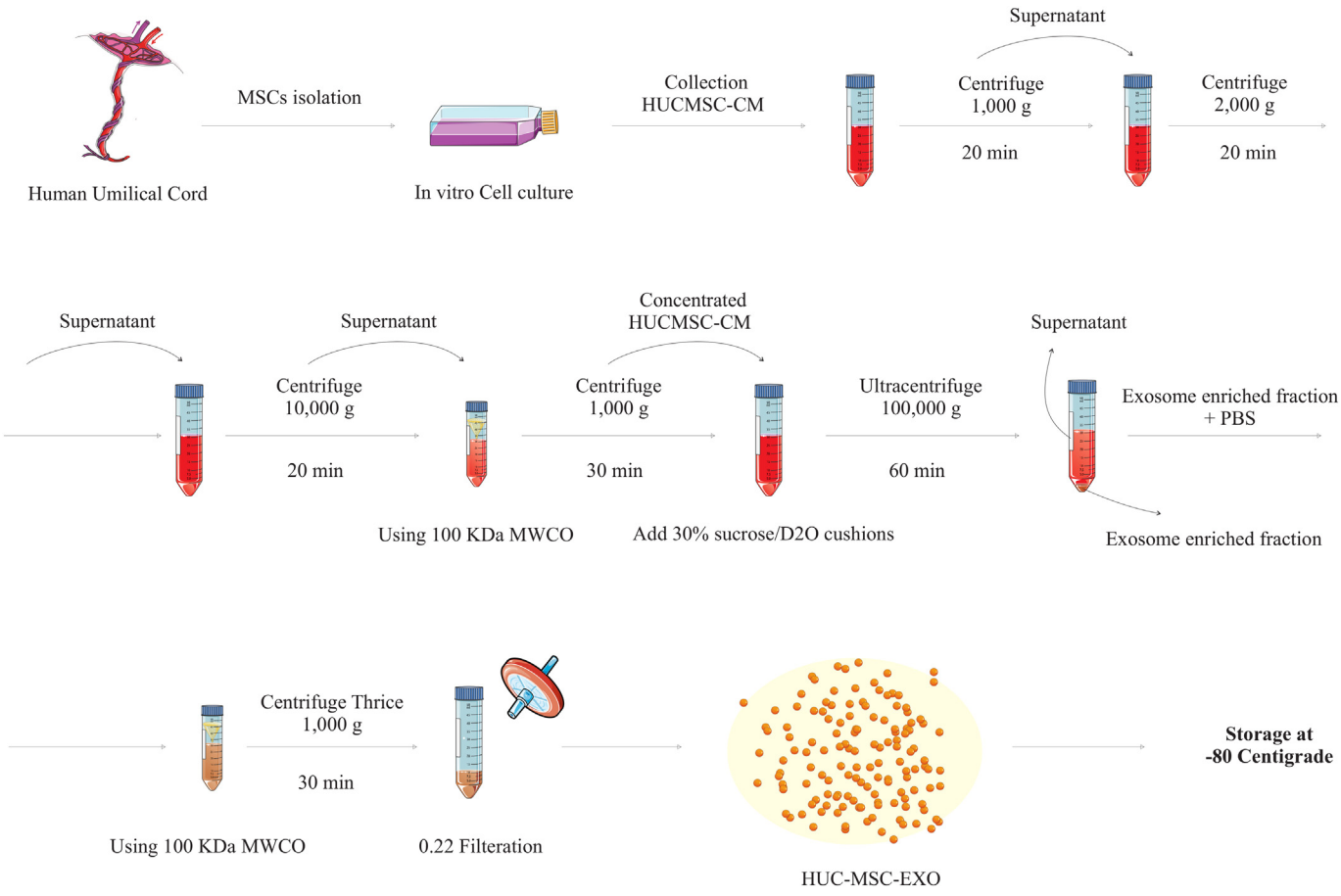

Fig. 2. Exosome isolation by sucrose cushions ultracentrifugation. Ultracentrifugation in combination with sucrose cushions will result in higher yield and purity. However, the time-consuming property of this method is a challenging issue. Mesenchymal Stem Cells (MSCs), Human Umbilical Cord Mesenchymal Stem Cells Condition Medium (HUCMSC-CM), Molecular Weight Cut-Off (MWCO), Deuterium Oxide (D2O), Phosphate-Buffered Saline (PBS).

Table 1
Characterization and identification of human umbilical cord mesenchymal stem cells derived exosome (HUCMSC-EXO).

Morphology		Protein concentration		Size distribution	Surface marker		Refrence
Method	Morphology	Method	Concentration		Western Blotting	Flow Cytometry	
TEM	–	BCA	–	NTA	CD9,CD63	–	[88]
TEM	Saucer	BCA	–	NTA	CD9, CD81	–	[86]
TEM	–	BCA	(2.4 μg/μl)	NTA	CD9, CD63 CD81,TSG101	–	[85]
TEM	–	BCA	–	NTA	CD9,CD63,CD81	–	[78]
TEM	Cup	BCA	(3.98 mg/mL)	NTA	CD9,CD63	–	[89]
TEM	Round	–	–	NTA	HSP70,CD9,CD63	–	[103]
TEM	Round or Oval	BCA	–	–	CD63,CD81	–	[104]
TEM	–	ELIZA	(1.0–1.4 mg/mL)	–	CD63,HSP60	CD81	[80]
SEM	Cup	BCA	–	–	CD9,CD63,CD81	–	[49]
TEM	Spheroid	BCA	–	NTA	CD9,CD63,CD81	–	[77]
TEM	Spheroid	BCA	–	NTA	CD9,CD63,CD81	–	[7]
SEM	Cup	–	–	–	CD63	–	[64]
SEM	Spheroid	BCA	–	–	–	–	[57]
TEM	Spheroid	–	–	NTA	CD9,CD63	CD9, CD63	[37]
TEM	–	BCA	–	–	CD9,CD81	–	[87]
TEM	Spheroid	–	–	–	CD9,CD63,CD81	–	[44]
TEM	Spheroid	–	–	NTA	CD9,CD63,CD81	–	[45]
TEM	–	BCA	–	–	CD9,CD63,CD81	–	[46]
TEM	Spheroid	BCA	–	NTA	CD9,CD63,CD81	–	[97]
TEM	–	BCA	–	NTA	–	–	[98]
TEM	Cup	BCA	–	NTA	CD9,CD81	–	[100]
TEM	Spheroid	BCA	–	–	–	CD9,CD63,CD81	[108]

Abbreviations: TEM: transmission electron microscopy; SEM: scanning electron microscopy; BCA: bicinchoninic acid assay; ELIZA: enzyme linked immunosorbent assay; NTA: nanoparticle tracking analysis; CD: Cluster of Differentiation.

reaction was also reported in a patient who received high doses of MSCs-EXO. This study showed that intravitreal injection of MSC-EXO and MSCs at the end of a regular PPV may improve the anatomic and visual outcomes of refractory MH surgery. The main limitation of this study was the absence of control group. The retinal damages caused by infection and ischemia cause irreversible visual impairment [50]. Currently, there is no neuroprotection therapy for retinal damage. Although, there are several treatments such as stem cell transplantation [51] and treatment with 7,8-dihydroxyflavone [52], antagonist of *N*-methyl-d-aspartic acid receptors [53], Ca^{2+} channel blockers [54], anti-inflammatory agents [55], for retinal cells function improvement, these methods have not been accepted in clinical therapies. Previous studies have shown that intravenous injection of MSCs inhibits apoptosis and inflammatory reactions in retinal laser damages [56]. In a study by Yu et al. [57] the Intravitreal injection of HUCMSC-EXO and MSCs were compared in mouse models. It was observed that HUCMSC-EXO inhibited apoptosis and inflammatory responses just like MSCs. Studies have also shown that retinal damages may release pro-inflammatory mediators such as monocyte chemotactic protein (MCP-1), TNF-α, and intercellular adhesion molecule 1 (ICAM1). In the early stages of damage, increased mRNA expression of these factors is observed. The intravitreal injection of MSC-EXO has also reduced the expression of MCP-1. Age-related macular degeneration (AMD) is the main reason of blindness in people over 65 years [58]. Choroidal neovascularization (CNV) and the presence of the brushed membrane and retinal pigment epithelium (RPE) layer are the main pathological characteristics of AMD. The deposition of RPE metabolite usually causes macular edema and RPE atrophy, retinal hemorrhage and histological destruction due to CNV leading to visual dysfunction in AMD [59–61]. Intravitreal injection of anti-vascular endothelial growth factor (antiVEGF) drugs, photodynamic therapy, or laser photocoagulation, triamcinolone acetonide on CNV membrane are the primary medical treatments regimen of AMD in the CNV early stages [62]. The role of MSC-EXO in VEGF inhibition has also been demonstrated [63]. In He et al. [64] study, the effects of HUCMSC-EXOs on VEGF-A expression was evaluated in light retinal pigment epithelial (RPE) cells and laser induced choroidal neovascularization (CNV) in human and rats, respectively. The HUCMSC-EXOs vital proteins or RNAs reduced the expression of VEGF-A, *in vitro*. Furthermore, HUCMSC-EXO resulted in decreased VEGF-A expression, damage reduction and gradually improvement of the CNV's visual function histological structures, *in vivo*.

4.3. Alzheimer's disease

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders causing cognitive and memory impairment [65]. Amyloid-β (Aβ) peptide induces a neuroinflammatory process in the central nervous system (CNS) In AD, and excessive Aβ accumulation is observed [66,67]. In a study by Ding et al. [7] the effects of HUCMSC-EXO in AβPP/PS1 transgenic AD mouse models and BV2 cells were investigated. The accumulation and the formation of Aβ plaque can activate microglia as the major component of neuroimmuno-regulation [68]. Microglia, similar to macrophages, has two completely different functional activation states [69]. Microglia, in pro-inflammatory activated states, causes neurodegeneration by increasing the pre-inflammatory cytokines such as TNF-α and IL-1β leading to Aβ accumulation and reduces Aβ clearance. Microglia in an alternative state causes neuroprotective action by increasing anti-inflammatory cytokines including TGF-β and IL-10, leading to inflammation improvement and increases Aβ clearance. Additionally, these cytokines cause return tissue homeostasis, alleviates pro-inflammatory immune responses, and wound healing [7]. Autophagy is the physiological degradation of organelles and proteins and can be increased by pro-inflammatory cytokines. Inflammation induces autophagy in the peripheral blood mononuclear cells (PBMCs) [70,71]. HUCMSC-EXO injection has also reduced autophagy in PBMCs as a result of decreased inflammation. Indeed, PBMCs phagocyte more Aβ depositions. Neprilysin (NEP), is a type II integral membrane protein that can modulate the Aβ cleavage. Increased NEP expression has been reported to reduce Aβ deposition.the Insulin-degrading enzyme (IDE) is also another zinc metallopeptidase causing Aβ clearance in the brain. The results of this study also revealed that alternatively activated microglia following HUCMSC-EXO use, increases the NEP expression and IDE [7]. Other studies also have reported that Aβ plaque deposition around the microglia in the brain results in neuronal and synaptic loss, leading to cognitive and personality disorders and memory impairment [72,73]. In this study, injection of HUCMSC-EXO also increased spatial learning and memory improvement in an AD mice model [7].

4.4. Inflammatory bowel disease

Inflammatory bowel disease (IBD) is the gastrointestinal tract non-infectious, relapsing inflammatory illness [74]. IBD is mainly caused by infiltration of inflammatory cells such as macrophages [75]. Presently, existing treatments for IBD are based on anti-inflammatory drugs, immune modifying or immunomodulatory agents, thiopurine agents and anti-TNF monoclonal antibodies, however, clinical outcomes of the mentioned methods are not satisfactory [76]. Mao et al. [77], examined the effects of HUCMSC-EXOs on the treatment of dextran sulfate sodium (DSS)-induced IBD in mice. The results showed that IL-10 expression is increased as an anti-inflammatory cytokine, whereas, pre-inflammatory cytokines expression including IL-6, TNF-α, inducible nitric oxide synthase (iNOS), IL-1β, and IL-7 are decreased. IL-7 also activates mucosal inflammation in IBD. The level of this cytokine is higher in the colon tissue of colitis patients than that of healthy individuals. It also reduces the macrophages infiltration into the colon tissues. It has been reported that HUCMSC-EXOs inhibits IL-7 expression in macrophages and relieves inflammatory responses in IBD patients. Additionally, ubiquitination plays an important role in inflammatory response regulation. Wu et al. [78] used HUC-MSC-EXO in DSS-induced IBD mice model and showed an increased expression of inflammatory cytokines such as IL-1β, TNF-α, IL-6, and NEDD8 activating enzyme E1 subunit 1 (NAE1) in the IBD group. However, in the HUCMSC-EXO treatment group, the expression level of these cytokines was significantly decreased.

4.5. Nerve injury–induced pain

Peripheral nerve injury can induce neuropathic pain [79]. In Shiue et al. [80] study, intrathecal administration of HUCMSC-EXO into the L5/6 spinal nerve ligation (SNL) in a rat pain model, led to exosomes homing in dorsal root ganglion, ipsilateral L5 spinal dorsal horn, and peripheral axons in immunofluorescent assays. HUCMSC-EXO also suppressed the expression of glial fibrillary acidic protein (GFAP), ionized calcium binding adaptor molecule 1 (Iba1), 2′,3′-cyclic-nucleotide 3′-phosphodiesterase (CNPase), c-Fos, and leading to SNL-induced mechanical allodynia and thermal hyperalgesia reversal. Additionally, HUCMSC-EXO decreased and increased neuroinflammatory (IL-1β and TNF-α) and anti-inflammatory cytokines, as well as neurotrophic factors (brain-derived and glial cell line-derived neurotrophic factors) in the ipsilateral L5/6 dorsal root ganglion of nerve-ligated rats, respectively. The homing capability and anti-inflammatory/regenerative properties of HUCMSC-EXO make them a probable candidate for nerve injury-induced pain.

4.6. Spinal cord injury

M1 macrophages secrete inflammatory cytokines such as Interferon-gamma (IFNγ), IL-6, TNF-α, and IL-1β which damage the host cells [81,82]. Indeed M2 macrophages inhibit inflammatory immune responses by the production of IL-4 and IL-10 cytokines which increases the angiogenesis and removes necrotic parts [83,84]. Studies have revealed that HUC-MSC-EXO administration in the treatment of spinal

cord injury (SCI) induces M1 to M2 phenotype polarization and performance improvement. This polarization also reduces inflammatory cytokines such as IFN-γ, IL-6, TNF-α, and macrophage inflammatory protein-1 alpha (MIP-1α) [85].

4.7. Pancreatic ductal adenocarcinoma

miR-145-5p5p, as a tumor suppressor, is downregulated in various types of cancers, including pancreatic ductal adenocarcinoma cells (PDAC), hepatocellular carcinoma, colorectal, gastric, and breast cancers. Ding et al. [86] study revealed that HUCMSC-EXOs-mediated delivery of miR-145-5p to PDAC, inhibits PDAC cell proliferation and invasion, increases arrests cell cycle, apoptosis, and reduces Smad3 expression *in vitro*. Additionally, the *in vivo* result showed a reduced xenograft tumors growth following miR-145-5p overexpression in mice.

4.8. Liver

Liver fibrosis is caused by chronic injuries such as viral hepatitis, alcohol abuse and drugs. Liver transplantation is an ultimate cure for this disease. However, due to the limited number of donors and high mortality rates in patients with liver fibrosis, finding alternative therapies is crucial. HUCMSC-EXO was used to treat carbon tetrachloride (CCl4)-induced mouse liver fibrosis. The results indicated fibroblasts derivation from hepatocytes through epithelial to mesenchymal transition (EMT) and collagen production. Various studies have also shown that after the phosphorylation, Smad3, and Smad2 form a complex with Smad4 and then translocate into the nucleus to regulate the transcription of target genes responsible for EMT, such as collagen type I, snail, and Smad7. Additionally, TGF-B1 activates the Smad 2/3 pathway and causes EMT. HUCMSC-EXO transplantation also caused TGF-B1 downregulation and consequent Smad2 phosphorylation inhibition, as well as EMT reversion, *in vivo*. Treatment of human liver cell line HL7702 with HUCMSC-EXO also resulted in EMT, reversed EMT-associated markers expression, and spindle-shaped cells, *in vitro*. Moreover, HUCMSC-EXO ameliorates serum aspartate aminotransferase (AST) activity, reduces TGF-B1, Smad2 phosphorylation, and collagen type I and III, *in vivo* [87]. Oxidative stress also is increased in chronic liver diseases such as viral hepatitis, alcoholic liver disease, liver fibrosis, and nonalcoholic fatty liver disease. Furthermore, oxidative stress is a major factor in hepatocarcinogenesis progression. However, use of antioxidants is a good therapeutic strategy. HUCMSC-EXO injection into acute and chronic liver injury and CCl4-induced liver tumors showed a reduced acute liver injury and liver fibrosis and restrained the liver tumors growth followed by apoptosis and oxidative stress reduction *in vitro* and *in vivo*. The effect of HUCMSC-EXO was also compared with bifendate, and dimethyl diphenyl bicarboxylate (DDB). DDB is a synthetic intermediate of schisandrin C, which is currently used for hepatitis treatment with minimal side effects. The results showed hepatoprotective effects of HUCMSC-EXO and different antioxidant. Moreover, HUCMSC-EXO exhibited stronger antioxidant activities than that of DDB in suppression of CCl4-induced liver injury and tumor development [37]. On the other hand, the use of HUCMSC-EXOs reduced the NLRP3 inflammasome expression, inflammatory factors and ALT and AST levels *in vitro* and *in vivo* in acute liver failure models [88].

4.9. Acute myocardial ischemia

Myocardial infarction (MI) is one of the death reasons worldwide, causing irreversible myocardial cell loss and cardiac function [89]. The use of stem cells in acute myocardial infarction (AMI) treatment has been reported in clinical studies [90,91]. In a study, Yuanyuan Zhao et al. [89] examined the effects of HUC-MSC-EXO in AMI. They injected HUC-MSC-EXO intravenously into a rat AMI model. AMI rats were created by ligation of the left anterior descending (LAD) coronary artery. Echocardiography findings showed an improved cardiac function after 4 weeks of HUC-MSC-EXO injection. Additionally, reduced cardiac fibrosis was also observed after Masson's trichrome staining.

4.10. Cutaneous wound healing

Previous studies have shown that MSC-EXO plays critical role in tissue repair. However, the role of MSC-EXO has not been investigated in cutaneous wound healing yet. It has been reported that Wnt/βcatenin signaling plays critical role in skin development [92–95] and wound healing [96]. HUCMSC-EXO was used to repair skin in a rat skin burn model. The results showed that HUCMSC-EXO-derived Wnt4 protein activates Wnt/βcatenin signaling in skin cells. This activation also promotes proliferation and cell migration and wounds healing. It has also been shown that heat stress inhibits insulin/protein kinase B (AKT) signaling and induces apoptosis in skin cells. Additionally, HUCMSC-EXO activates AKT signaling and reduces cell apoptosis in the burned skin model [97]. As mentioned, MSC-EXO increases tissue regeneration. However, it is still unclear how MSC-EXO can control stem cell expansion after regeneration responses to prevent overcrowding and dysplasia. In the current study, they examined the ability of HUCMSC-EXO in collagen expression and stem cell expansion regulation in a rat deep second-degree burn model. The results showed that HUCMSC-EXO treatment promotes Wnt/βcatenin signaling self-regulation at the remodeling phase of cutaneous regeneration and prevents collagen deposition and increases cellular expansion in week 4 after transplantation. Under high cell density conditions, 14-3-3ζ which is presented in HUCMSC-EXO, leads to the formation of an inhibitory complex, resulting in YAP (YES-associated protein) phosphorylation in Ser127. Studies have shown that YAP and p-LATS (Large Tumor Suppressor) form a complex, and 14-3-3ζ is necessary for the formation of this complex to activate Hippo pathway. 14-3-3ζ transfer also results in p-LATS accumulation. As a result, high cell density represents a condition to activate the Hippo-YAP signaling and HUCMSC-EXO-mediated 14-3-3ζ delivery ensures a sufficient level of p-LATS to phosphorylate YAP. These findings demonstrate that HUCMSC-EXO functions not only as an “accelerator” of the Wnt/βcatenin signaling to restore damaged skin tissue, but also as a “brake” of the signaling pathways by YAP modulating to orchestrate controlled cutaneous regeneration [98].

4.11. Type 2 diabetes mellitus

The main causes of Type 2 diabetes mellitus (T2DM) are β-cell dysfunction pancreatic β-cell mass loss and peripheral insulin resistance, which increase blood glucose levels. Currently, the main treatments for T2DM are daily injections of insulin and chemical drugs such as thiazolidinedione, metformin, and sulfonylurea [99]. However, these treatments have side effects such as subcutaneous nodule, diarrhea, obesity, and metropia and temporarily reduce the glucose level in blood. Therefore, finding curative methods with fewer side effects is needed. The effects of HUC-MSC-EXO on a T2DM rat model were examined. T2DM in rats was established by streptozotocin (STZ) and high-fat diet (HFD). The liver and skeletal muscles play an important role in maintaining the blood glucose balance. The results showed that HUCMSC-EXO increases glucose uptake in skeletal muscles by increasing the expression and membrane translocation of Glucose transporter type 4 (GLUT4) and glycolysis. HUCMSC-EXO also reduced glycogenolysis as a result of increased storage of glycogen in the liver to maintain hemostasis. Insulin resistance is responsible for the pathogenesis of T2DM. HUCMSC-EXO caused restored tyrosine residues phosphorylation of insulin receptor substrate 1 (IRS-1) and activation of AKT signaling pathway through pro-inflammatory cytokines, such as TNF-α inhibition. This phenomenon promotes insulin sensitivity of tissues. Additionally, HUCMSC-EXO increases insulin levels from pancreatic β-cells by inhibiting STZ-induced β-cell apoptosis [100].

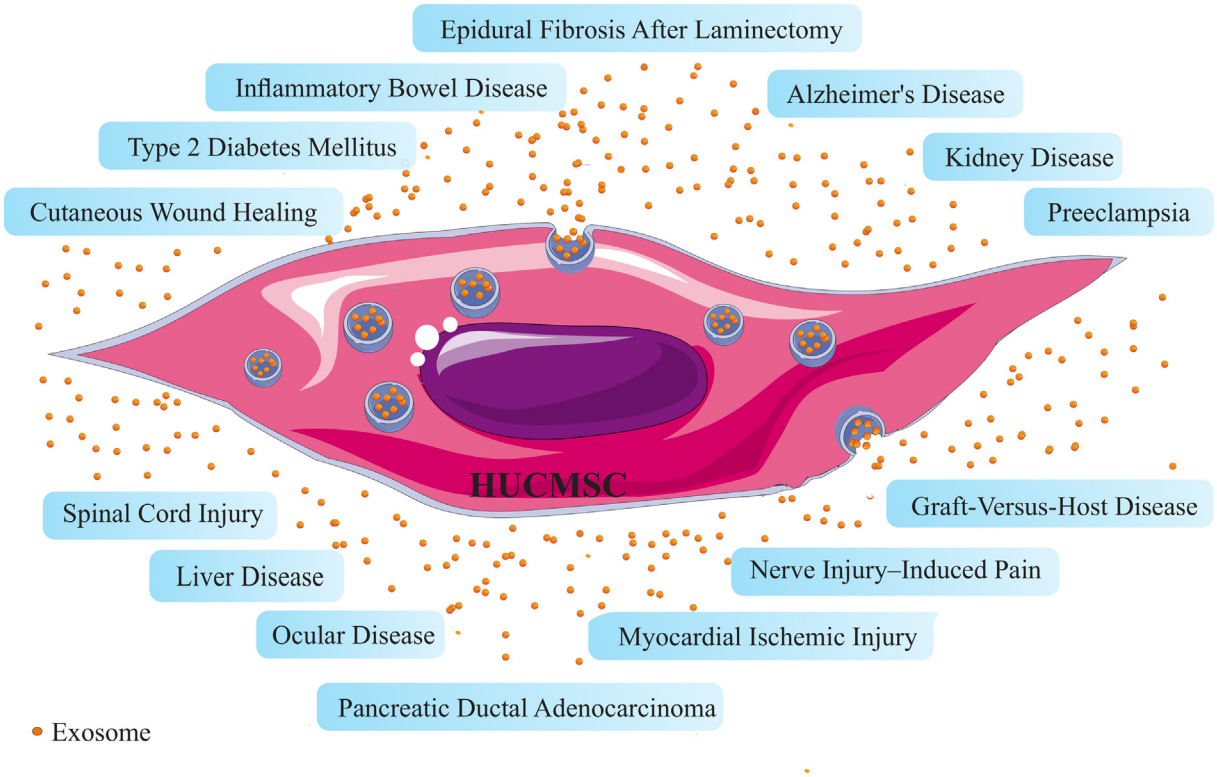

Fig. 3. application of human umbilical cord mesenchymal stem cells-derived exosome (HUCMSC-EXO) in different diseases.

4.12. Epidural fibrosis after laminectomy

Unsuccessful lumbar surgery sometimes may result in failed back surgery syndrome (FBSS). Adhesions and epidural fibrosis are the main reasons of a FBSS [101,102]. Wang et al. [103] constructed biomaterials containing the bacterial cellulose (BC) anti-adhesion membrane, consisted of exosomes. The use of this membrane on epidural fibrosis post-laminectomy in a rabbit model showed a three-dimensional structure formation making it appropriate for supporting the space between the dural mater and surrounding tissues. Furthermore, this membrane was able to inhibit epidural adhesions and epidural fibrosis in failed back surgery syndrome.

4.13. Preeclampsia

Preeclampsia (PE) is known to be associated with hypertension and proteinuria during pregnancy. Xiong et al. [104] in a study, examined the effects of low, medium and high dosages of HUCMSC-EXO in a mice PE model. Data revealed a decreased blood pressure and 24-h urinary protein on 17 and 19 days of pregnancy. On the day 21, increased fetus number, quality, placental quality, micro-vascular density (MVD), VEGF expression and reduced apoptosis, soluble fms-like tyrosine kinase receptor-1 (sFlt1) were also observed. SFlt1 is a PE biomarker which is increased during this disorder. Accordingly, the dose-dependent treatment with HUCMSC-EXO improved the morphology of the placental tissue by cell apoptosis inhibition and promoted angiogenesis in placental tissue.

4.14. Graft-versus-host disease

The only way for specific hematologic malignancies treatment is the allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, allo-HSCT shows side effects such as acute graft-*versus*-host disease (aGVHD). aGVHD is one of the main causes of death in allo-HSCT patients [105–107]. Regenerative and immunoregulatory properties of MSCs make them as a good candidate for cell therapy during allo-HSCT. However, the use of MSCs has recently been restricted due to uncontrollable difference monitoring of MSCs and the increased risk of death from pneumonia after allo-HSCT. Recently, it has been shown that MSC-EXO reduces the clinical manifestations of refractory aGVHD patient. HUCMSC-EV includes exosomes and microvesicles. The effects of HUC-MSC-EVs on aGVHD in a mouse model of allo-HSCT showed that HUCMSC-EV significantly reduces the manifestations of aGVHD and increases the rat's survival. Additionally, the results of *in vivo* and *in vitro* studies have indicated an increased and decreased anti-inflammatory (IL-10) and inflammatory (IFN-γ, IL-2, and TNF-α) cytokines, respectively [108]. applications of HUCMSC-EXO are briefly illustrated in Fig. 3.

5. Conclusion

Nowadays, MSCs are widely used in tissues repair and diseases treatment. Among MSC's different sources, human umbilical mesenchymal stem cells have widely been considered because of easy isolation methods, higher proliferation, faster self-renewal ability, and lower immunogenicity. However, the use of HUCMSC has limitations in the maintenance of biological activity, the quantification of bioactive substances, and the logistics of delivery in clinical therapies. Therefore, finding therapeutic strategies without cell interference is needed. Exosomes are produced from a variety of sources such as HUCMSC and are involved in cellular communication. As a result, exosomes can be replaced instead of cell-based therapies. In this review, applications of HUCMSC-EXO were mentioned in a variety of diseases. However, most of these studies have been performed in the animal models and the use of HUCMSC-EXO in human clinical studies should be further studied.

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Declaration of competing interest

Authors declare no conflict of interests.

Author contributions

Yoda Yaghoubi wrote the manuscript. Majid Zamani and Mehdi Talebi draw the schematic figures and tables. Aliakbar Movassaghpour and Amir Mehdizadeh wrote some parts of the manuscript and edited the final version of the manuscript and submitted the paper. Mehdi Yousefi designed and supervised the study, whole correspondence during the paper submission, handling the revisions and re-submission of revised manuscripts up to the acceptance of the manuscripts.

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