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REVIEW ARTICLE

Current developments and therapeutic potentials of exosomes from induced pluripotent stem cells-derived mesenchymal stem cells

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Abstract: Mesenchymal stem cells (MSCs) are multipotent cells derived from adult human tissues that have the ability to proliferate in vitro and maintain their multipotency, making them attractive cell sources for regenerative medicine. However, MSCs reportedly show limited proliferative capacity with inconsistent therapeutic outcomes due to their heterogeneous nature. On the other hand, induced pluripotent stem cells (iPSC) have emerged as an alternative source for the production of various specialized cell types via their ability to differentiate from all three primary germ layers, leading to applications in regenerative medicine, disease modeling, and drug therapy. Notably, iPSCs can differentiate into MSCs in monolayer, commonly referred to as induced mesenchymal stem cells (iMSCs). These cells show superior therapeutic qualities compared with adult MSCs as the applications of the latter are restricted by passage number and autoimmune rejection when applied in tissue regeneration trials. Furthermore, increasing evidence shows that the therapeutic properties of stem cells are a consequence of the paracrine effects mediated by their secretome such as from exosomes, a type of extracellular vesicle secreted by most cell types. Several studies that investigated the potential of exosomes in regenerative medicine and therapy have revealed promising results. Therefore, this review focuses on the recent findings of exosomes secreted from iMSCs as a potential noncell-based therapy.

Keywords: Exosomes; Extracellular vesicles; Induced mesenchymal stem cells; Mesenchymal stem cells

1. INTRODUCTION

1.1. Mesenchymal stem cells

Stem cells are unspecialized cells capable of self-renewal and differentiation into any cell type. Based on their differentiation potential, stem cells can be categorized into totipotent, pluripotent, multipotent, oligopotent, and unipotent cells.[1] Compared with other stem cell types, mesenchymal stem cells (MSCs) are favored due to their multipotency, immunological compatibility, lower probability to form teratoma, and fewer ethical challenges.[2,3] Other therapeutic advantages of MSCs can be ascribed to their immunomodulatory activities and tissue regeneration and homeostasis maintenance abilities.[4,5]

1.2. Therapeutic drawbacks of MSCs

In the last decade, vast applications of MSC transplantation in several diseases, including stroke, myocardial infraction, and retinal degeneration, have been reported in clinical trials. However, several challenges have arisen when using MSCs in therapy, including the heterogeneity of MSCs due to donor variations, differentiation capacities, and the stability of stemness among MSCs isolated from different sources; the immunocompatibility of MSCs; low viability of the transplanted cells; and the variable expansion possibilities under different culture conditions.[6–14] Despite a large number of clinical trials (Fig. 1) carried out to test the therapeutic potential of MSCs, the therapeutic efficacy remains unclear due to inconsistent clinical outcomes. Hence, a newer approach focusing on the bulk generation of induced MSCs (iMSCs) via induced pluripotent stem cells (iPSCs) may provide a more practical solution for clinical application.

1.3. iPSCs and iMSCs

iPSCs are embryonic stem-like cells derived via the reprogramming of somatic cells through the introduction of pluripotent-associated genes (Oct3/4, Sox2, Klf4, and c-Myc).[15,16] The

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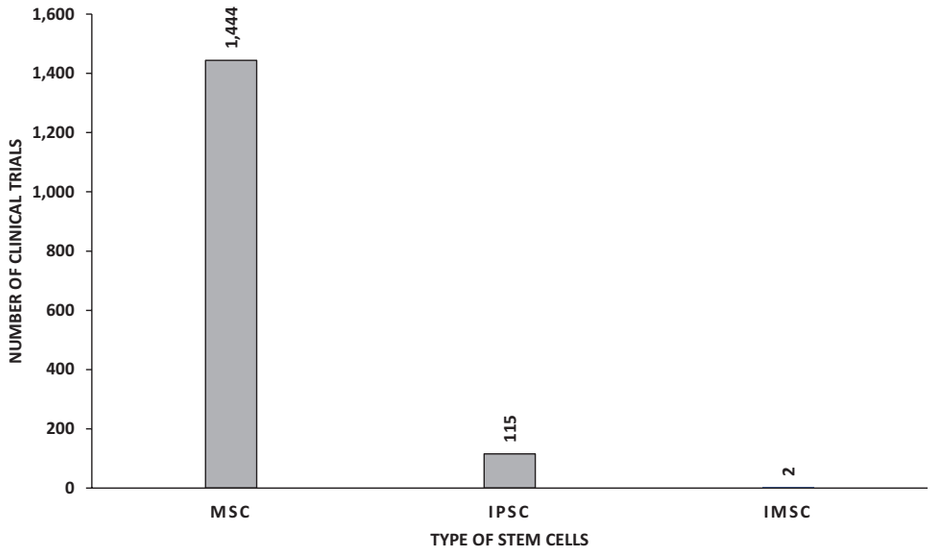

Fig. 1 The number of clinical trials registered for mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), and induced mesenchymal stem cells (iMSCs) as of August 31, 2022 (https://www.clinicaltrials.gov).

pluripotent-associated genes are delivered/transduced into the cells using viral and nonviral vectors via integrative or nonintegrative systems.[17] Vectors in the integrated systems may be further classified into viral integrated vectors (retrovirus, lentivirus, and inducible or excisable retro- or lentivirus) and non-basic fibroblast growth factor (bFGF)-viral integrated vectors (plasmid/linear DNA, or transposons such as piggyBac or sleeping beauty). Likewise, the nonintegrative system can also be subcategorized into nonintegrative viral vectors (adenovirus or Sendai virus) and nonintegrative nonviral vectors (episomal vectors including plasmids or minicircles, RNA, or protein).[17] The generated iPSCs have high self-renewal and proliferation capacities and can differentiate into the three germ layers. These iPSCs resemble embryonic stem cells (ESCs): They exhibit ESC-like morphology, express ESC markers (SSEA3/4, Oct4, TRA-1-81, TRA-1-60, and Nanog), and form teratomas when injected into immunocompromised mice.[17] They are considered immunologically safe in autologous transplantation and bypass ethical concerns, unlike the use of ESCs.[18–20] The regenerative properties of iPSCs may open opportunities for tissue replacement and repair, as observed from 115 clinical trials (https://www.clinicaltrials.gov). Although the use of iPSCs may also be complicated by other challenges such as potential immune rejection, formation of teratoma, lowered reprogramming efficiency due to epigenetic memory, and genetic instability,[21–26] the generation of iMSCs from the differentiation of iPSCs opens up new possibilities in regenerative medicine and cell therapy. Numerous studies have reported the ability of iMSCs to differentiate into various tissues, to control the immune response, to yield various paracrine factors and cytokines, to secrete exosomes, and to have a high proliferation rate. In addition, iMSCs incorporate the advantages of MSCs and iPSCs with no immunogenicity, low senescence, and are patient-specific, thereby eliminating the requirement of immunosuppression in recipients.[27–32]

1.4. iMSC isolation and characterization

The most common iMSC production method is via the reprogramming of somatic cells into iPSCs, followed by the spontaneous differentiation of iPSCs into iMSCs via the deprivation of the culture medium from pluripotent signals.[33] Generally, the differentiation of iPSCs into iMSCs can be categorized into four main approaches: induction by specific growth factors, physiochemical stimulation from biomaterial matrices and small molecule inhibitors, supplementation with platelet lysate, and activation of neural crest cells (NCCs). The generation of iMSCs may be stimulated by the addition of typical MSC growth factors including platelet-derived growth factor alpha polypeptide b (PDGF-AB)/bFGF or medium containing high serum concentrations after mesoderm induction.[33]

Alternatively, the differentiation of iPSCs into iMSCs may occur via physiochemical stimulation using biomaterial matrices that activate the epithelial-to-mesenchymal transition of epithelial cells.[34] Coating materials such as thin, fibrillar type I collagen act as a matrix that mimics the structure of physiological collagen, resulting in the formation of iMSCs.

Another approach for deriving iMSCs is by using platelet lysate as a supplement in a two-step procedure. In the first stage (0–14 days), PSCs are cultured on Matrigel-coated dishes with the addition of platelet lysate (10%), a ROCK inhibitor, and B27 supplement. These additions promote notable secretion of growth factors and avoid cell death after plating. The second stage includes culturing the cells without coating and involves the addition of 10% platelet lysate for the remaining 14 days.[35] iMSCs can also be generated from NCCs via activation of canonical Wnt signaling and suppression of transforming growth factor-β (TGFβ) signaling.[36]

To obtain ideal iMSCs for clinical applications, the establishment of iMSCs under feeder-free and serum-free culture conditions is essential. One such example is the two-step procedure used by Menendez et al[37] to isolate iMSCs. In the first step, iPSCs are dissociated into single cells, followed by culturing these cells for 2 weeks in chemically defined medium to obtain feeder-free, serum-free conditions. The culture is then supplemented with a Wnt signaling activator and Activin/Nodal/TGFβ signaling inhibitor (Fig. 2) to induce differentiation into iMSCs.[37]

The differentiated iPSCs (iMSCs) are characterized by following the International Society for Cell & Gene Therapy (ISCT) developed by Dominici et al.[38] The criteria include: the ability to adhere to plastic; expression of a typical panel of MSC surface markers [CD105(+), CD73(+), CD90(+), CD44(+), and CD73(+), CD11b(−), CD79a(−), CD19(−), CD 34(−), CD45(−), and MHC II]; and the ability to differentiate into adipogenic, chondrogenic, and osteogenic lineages.

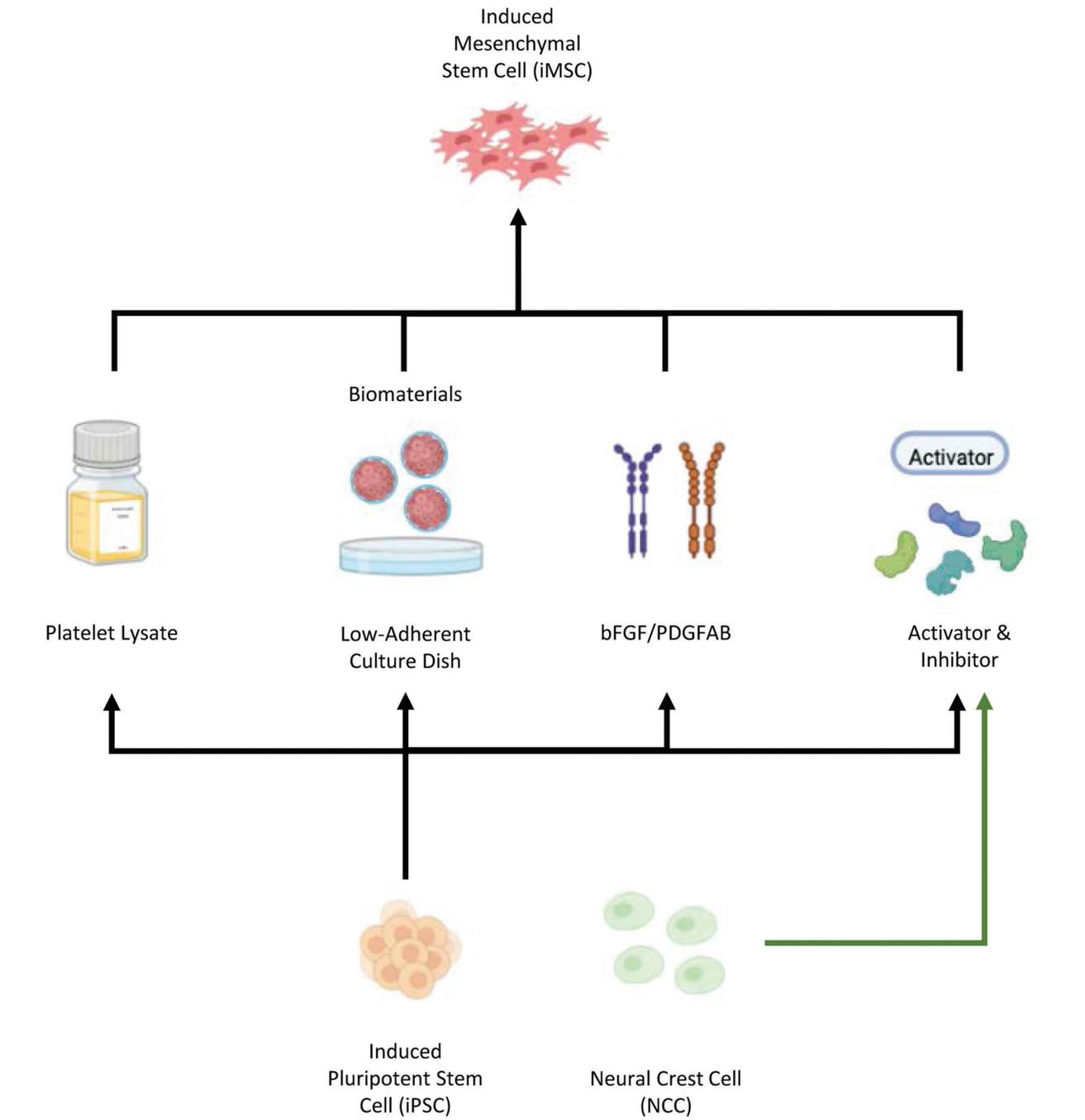

Fig. 2 Methods of induced pluripotent stem cell (iPSC) differentiation to induced mesenchymal stem cells (iMSC).

1.5. Therapeutic potential of iMSCs

Several preclinical studies have involved testing iMSCs derived from different sources on diseases including osteonecrosis, periodontal disease, inflammatory bowel disease, myocardial infraction, and cancer. These studies have revealed promising therapeutic potential (Table 1).[28,39–47]

Clinically, two phase I and II trials have been carried out on graft-versus-host disease and coronavirus disease 2019 (COVID-19). A phase I open-label multicenter dose-escalation study used iMSCs to treat steroid-resistant graft-versus-host disease, with safety, tolerability, and efficacy as the primary endpoints. The administration of iMSCs was well tolerated and safe with no adverse reactions post-iMSC treatment.[48] Another pilot, multicenter, open-label randomized controlled phase I/II trial was performed to determine the role of CYP-001 in adult patients with respiratory failure. The primary objective was to assess respiratory dysfunction via determination of the arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2) ratio between groups (NCT04537351).

Considering early differentiation markers, iMSCs express mesenchymal genes with downregulation of pluripotent-associated genes. However, the gene expression levels of the tri-lineage differentiation have shown that bone marrow–derived MSCs (BM-MSCs) outperform iMSCs, suggesting that iMSCs may represent a discrete cell population different from BM-MSCs.[49] Similarly Kang et al[50] showed that iMSCs retain osteogenic and chondrogenic potential with less adipogenicity compared with their MSC counterparts, suggesting that iMSCs may not necessarily comply with the ISCT criteria and hence may not be adequate to verify the robust derivation of MSCs from iPSCs. These findings imply the need to develop distinguishing criteria for the basic characteristics of iMSCs, instead of following the ISCT criteria that have been implicated in the definition of MSCs.

2. REGENERATIVE FATE/POTENTIAL OF STEM CELLS

The regenerative fate of stem cells occurs due to their differentiation into specialized cells, stimulation of endogenous cells for repair, or producing paracrine factors including growth factors, cytokines, and extracellular vesicles (EVs). The immunological properties of MSCs arise mainly via the paracrine mechanism in which EVs, particularly exosomes, appear to play an important role.[51,52] Hence, it is possible to find anti-inflammatory and immunosuppressive properties of MSCs/iMSCs in MSC/iMSC-derived exosomes. For example, injured cells in damaged tissues may not primarily fulfill the repair mechanism via a direct cellular manner, suggesting that the therapeutic outcome occurs due to paracrine effects mediated via the MSC/iMSC secretomes.[53]

3. GENERAL FEATURES OF EVS

The utility of MSC/iMSC-derived EVs as a noncellular-based therapy is recognized as an alternative approach to overcome the possible risks that MSCs/iMSCs may pose for clinical applications. EVs are natural nanoparticles encapsulated by a lipid bilayer and released in response to the microenvironment. EVs transport signals from their parental cells, including nucleic acids, proteins, lipids, and mitochondria, and cause physiological changes to their target cells.[54,55] EVs are categorized into three major groups based on their origin and size. The first division of EVs is exosomes derived from endosomes: Exosomes are formed by the fusion of multivesicular bodies (MVBs) with the plasma membrane. Second, microvesicles are shed from the plasma membrane. Finally, apoptotic bodies are formed during apoptosis (Table 2).[56,57] Within the EV subpopulation, exosomes are gaining increasing interest for regenerative therapy as well as immunomodulation.[54,55,58]

Table 1

iMSCs in diseases

No.	Disease	MSC source	Aim	Findings	References
1	Osteonecrosis	BM–SC of osteonecrosis of the femoral head	Bone repair	Improved regeneration and infiltration	39
2	Periodontal disease	Tail–tip fibroblast	Inhibit inflammation and bone loss	Controlled chronic inflammatory response	40
3	Periodontal regeneration	Human foreskin	Periodontal regeneration	Improved regeneration	41
4	IBD	Skin fibroblast	Therapeutic effect of different MSC sources in IBD	Reduced inflammation and lesion score	28
5	IBD	Adipose tissue	IBD cellular therapy	Improved survival. Failed to reduce inflammation	42
6	Myocardial infraction	Derma fibroblast	Effects of myocardial transplantation in infarcted myocardium	Increased vascular density, reduced infract size	43
7	Breast cancer	Male blood	Role of iPSC–MSC in cancer therapy	Decrease in invasive potential, homed to cancer	44
8	Breast cancer	Peripheral blood mononuclear cells	Personalized treatment of BRCA1–associated hereditary breast cancer	Pro–angiogenic signature due to the over expression of angiogenic factors (VEGF, PDGF, and ANGPT) and HIF–1α	45
9	Melanoma	Foreskin fibroblast	Role of iMSC–IL12 in melanoma	Induced apoptosis and melanoma growth inhibition	46
10	Multiple cancers	Exfoliated tubular epithelial cells (urine cells)	Role of iMSC–TRAIL in melanoma, liver, breast, and lung cancer	Induction of apoptosis in melanoma, liver, breast, and lung cancers and tumor growth inhibition	47

The table represents the therapeutic potentials of iMSC in several diseases.

BM–SC = bone marrow–mesenchymal stem cells; BRCA1 = breast cancer gene 1; IBD = inflammatory bowel disease; iMSC–IL12 = induced mesenchymal stem cells–Interleukin 12; iMSC–TRAIL = induced mesenchymal stem cells–tumor necrosis factor related apoptosis inducing ligand; iPSC–MSC = induced pluripotent stem cells-derived mesenchymal stem cells.

Table 2

Biological properties of extracellular vesicles

No	Extracellular vesicle	Size, nm	Shape	Biomarkers	Content
1	Exosomes	30–100	Cup shaped	Tetraspanins (CD63, CD9, CD81, CD82, CD49, CD24, and CD53), heat shock proteins (HSP60, HSP70, HSP20, HSP90), flotillins, GTPases (Rab31, Rab11, Rab35, and Rab27), TSG101, ALIX, and ESCRT (0, I, II, and III)	Mitochondrial DNA, mRNA, miRNA, noncoding RNA, and specific proteins
2	Microvesicles	100–1000	Irregular	Integrins (CD61, CD51, and CD41), metalloproteinase, flotillin–2, and selectins	Histones, chemokines, annexin V positivity, and adhesion molecules
3	Apoptotic bodies	500–3000	Heterogeneous	Histones, chemokines, annexin V positivity, and adhesion molecules	Cytoplasm with packed organelles

The table illustrates the size, shape, biomarkers, and content of exosomes, microvesicles, and apoptotic bodies (Adapted from Dilsiz[56] and Xiao et al[57]).

ALIX = ALG–2 interacting protein X; CD24 = cluster of differentiation 24; CD4 = cluster of differentiation 41; CD49 = cluster of differentiation 49; CD51 = cluster of differentiation 51; CD53 = cluster of differentiation 53; CD61 = cluster of differentiation 61; CD63 = cluster of differentiation 63; CD81 = cluster of differentiation 81; CD82 = cluster of differentiation 82; CD9 = cluster of differentiation 9; DNA = deoxyribonucleic acid; ESCRT (0, I, II, & III) = endosomal sorting complexes required for transport (0, I, II, & III); GTPases = guanosine triphosphate binding proteins; HSP20 = heat shock protein 20; HSP60 = heat shock protein 60; HSP70 = heat shock protein 70; HSP90 = heat shock protein 90; miRNA = micro ribonucleic acid; mRNA = messenger ribonucleic acid; Rab11 = RAS-related protein Rab–11; Rab27 = RAS-related protein Rab–27; Rab31 = RAS-related protein Rab–31; Rab35 = RAS-related protein Rab–35; RNA = ribonucleic acid; TSG101 = tumor susceptibility gene 101.

4. EXOSOME CONTENT, BIOGENESIS, SECRETION, UPTAKE, AND BIOLOGICAL FUNCTION

The surface of exosomes comprises unique lipids and proteins such as fusion proteins, platelet-derived growth factor receptor, membrane transport proteins, transmembrane proteins, lysosome-associated protein 2B, sphingomyelin, and cholesterol. Exosomes contain tetraspanins (CD63, CD81, and CD9), heat shock proteins (HSP70 and HSP90), and GTPases. With the aid of these markers, exosomes can be differentiated from other EVs including microvesicles and apoptotic bodies.[59] These proteins are useful as biomarkers for exosomal detection and impact the recipient cells based on their specific functions. Exosomes are also distinguished from other membrane vesicle populations by their characteristic lipid bilayer. The average thickness of the lipid bilayer of an exosome is about 5 nm, resulting in a significant difference between the sinking coefficient of exosomes and other protein aggregates.[60] The accumulation of lipid components like ceramides can also distinguish exosomes from other vesicles that contain cholesterol and lysosomes, which are commonly associated with exosomes.[61] In addition, saccharide chains such as alpha-2,6-sialic acid, polylactosamine, mannose, and *N*-linked glucans are abundant on the outer surface of exosomes.[62]

The biogenesis of exosomes occurs when MVBs bud inward to form intraluminal vesicles that are formed via the endocytosis of the endoplasmic reticulum/Golgi secretory pathway. Next, MVBs fuse with the cellular membrane followed by fusing with either the lysosome for degradation or the plasma membrane to release exosomes into the extracellular space.[57] The released exosomes then enter the cells via fusion to the recipient cell, endocytosis, or receptor–ligand interaction on the recipient cell.[57,63]

Exosomes can be released from several cells such as neurons, intestinal epithelial cells, adipocytes, fibroblast, and tumor cells. Exosomes are also found in several biological fluids including blood, urine, amniotic fluid, saliva, cerebrospinal fluid, and breast milk.[64–68] Previous studies have shown that exosomes can act as cellular trash, expelling excess and/or nonfunctioning cellular components.[69] Several recent studies have revealed that exosomes play an important role in several biological processes including differentiation, apoptosis, antigen presentation, coagulation, cell homeostasis, angiogenesis, intracellular signaling, immunomodulation, and inflammation. These functions are attributed to the ability of exosomes to translocate enzymes, lipids, RNA, and proteins, thus affecting the physiological and pathological processes in various diseases including neurodegenerative diseases, autoimmune diseases, infections, and cancer.[70]

Exosomes may mediate intercellular communication to deliver exosomal content to recipient cells either directly or indirectly to influence physiological and pathological processes. Exosomes can cross the blood–brain barrier, help reduce inflammation, allow multiple intravenous administrations without side effects, and improve neurological and motor functions.[70] Additionally, exosomes play a vital role in the prognosis and diagnosis of a variety of health conditions including neurodegenerative diseases, liver and kidney diseases, numerous heart and lung diseases, and cancers.

The role of MSC-derived exosomes had been tested in several disease models including kidney, neuromuscular, liver, skeletal, skin, cardiovascular, and respiratory diseases, as well as cancer. MSC-derived exosomes promote angiogenesis and neurogenesis, inhibit the expression of proinflammatory cytokines to exert anti-inflammatory effects, and stimulate tissue regeneration by promoting extracellular matrix remodeling.[71–78] Exosomes derived from ESCs and iPSCs also show therapeutic potential similar to MSC-derived exosomes.[79,80]

5. EXOSOME ISOLATION AND VERIFICATION

Exosomes may be isolated using several methods, namely ultracentrifugation, sucrose density- gradient separation, ultrafiltration, size-exclusion chromatography, immunoaffinity capture, microfluidic technique, and polymer-induced exosomal isolation.[56] The isolated exosomes are then verified using nanoparticle tracking analysis, dynamic light scattering, transmission electron microscopy, and detection of exosomal markers (Fig. 3).[81] These

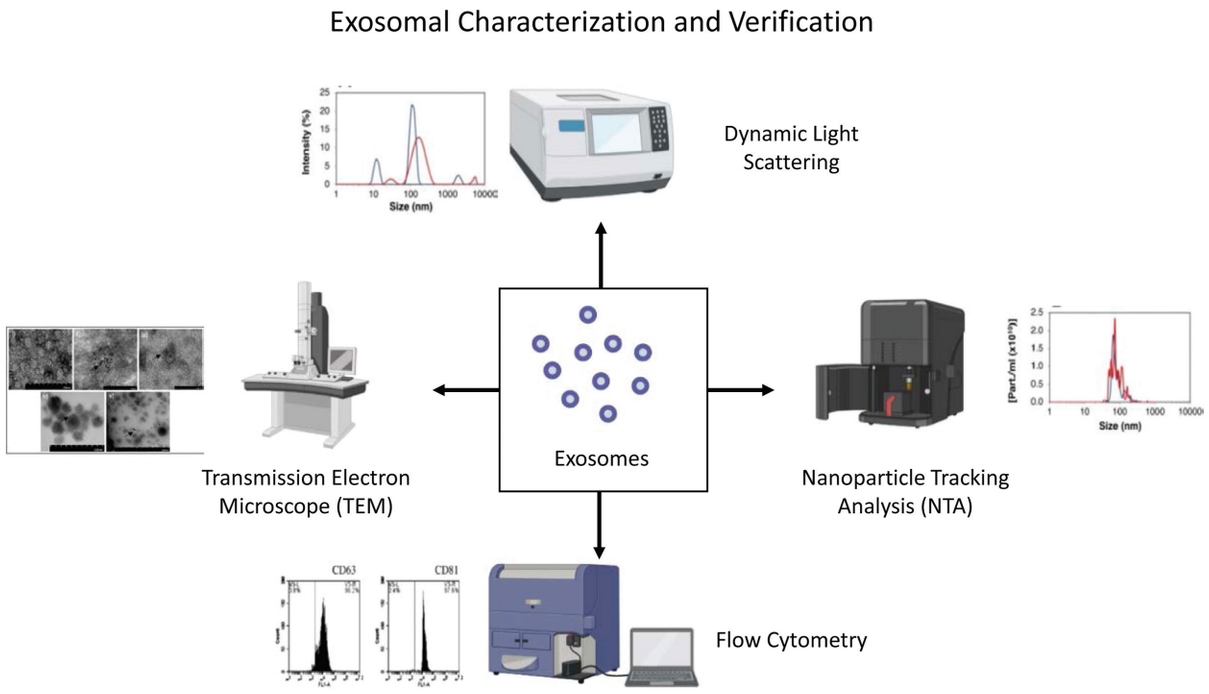

Fig. 3 Exosomal verification and characterization.

techniques are crucial for exosomal characterization because they help researchers to understand the properties and functions, structure, size range, and surface proteins of exosomes.[78,82,83]

6. WHY EXOSOMES FROM IMSCS?

Several advantages make iMSCs worth considering as a source of exosomes. iMSCs can be obtained noninvasively (ie, from peripheral blood cells), and transplantation of patient-specific iMSCs can overcome potential problems related to ethical issues as well as the need for immunosuppression in recipients. In addition, autologous iMSCs may represent an unlimited source of MSCs that could be used to address unmet clinical needs.[84] iMSCs could also be a universal source that prevents the heterogeneity of MSCs isolated from different sources. Moreover, as a noncell-based form of therapy, exosomes-derived iMSCs could overcome immune rejection following cellular transplantation, providing an alternative approach to avoid the potential risks arising from cellular therapy.

7. IMSC-DERIVED EXOSOMES IN DISEASES

Researchers have reported positive outcomes regarding the role of iMSC-derived exosomes in diseases, namely hind limb ischemia, cutaneous and skin wound healing, osteoarthritis, osteonecrosis, osteoporosis, bone regeneration, hepatic ischemia–reperfusion injury, renal ischemia–reperfusion injury, and corneal disease (Table 3).[85–91] Zhang et al[92] evaluated cutaneous wound healing and revealed that administration of iMSC-derived exosomes in rats led to the transplantation of iMSC-derived exosomes around the wound site, resulting in re-epithelialization, promotion of collagen maturation, reduction in scar width, and acceleration of new vessel generation and maturation in wound sites. To examine collagen synthesis and angiogenesis, the authors

Table 3

iMSC–derived exosomes in diseases

No.	Disease	Aim	Study subjects	Findings	Mechanism	References
1	Hind limb ischemia	To determine the therapeutic effects of iMSC–Exo in a mouse hind–limb ischemic model	Mouse model	iMSC–exo administration resulted in the attenuation of limb injury due to the stimulation of angiogenesis in the ischemic muscle and the enhancement of the blood perfusion in the ischemic limb. Also, iMSC–Exo groups showed an increase in the microvessel density at 7, 14, & 21 days with a significant promotion of proliferation, migration, tube formation, and expression of tube molecules in HUVECs cells	–	85
2	Hepatic ischemia	Effect of iMSC–exo in hepatic I/R injury	Rat model	iMSC–exo improved the hepatic I/R injury, and stimulated the proliferation of hepatocytes following the I/R injury	Inhibiting inflammatory cell infiltration, alleviating oxidative stress, reducing the release of inflammatory factors (TNF–α, IL–6, and HMGB1), elevating antioxidant proteins (SOD, GSH, GSH–Px), reducing liver damage, and inhibiting the apoptotic response of hepatocytes (Caspase3 and Bax)	86
3	Renal I/R injury	Therapeutic potential of iMSC–exo in mouse	Rat model	Improvement in tissue damage and renal function	Reduced inflammatory cytokines, markers of oxidative stress, and apoptosis. Also, iMSC–exo activated ERK1/2 phosphorylation signaling pathway that plays crucial role in enhancing cellular growth and survival	87
4	Osteoarthritis	Comparison between therapeutic potentials of IMSC–exo vs SM–MSC–exo	Mouse model	iMSC–exo were more prominent in stimulating chondrocyte proliferation and migration compared to SV–MSC–exo resulted in a better therapeutic potentials of iMSC–exo.	–	88
5	Osteonecrosis	Role of transplantation of iMSC–exo in ONFH	Rat model	Prevented bone loss, increased microvessel density in the femoral head compared to control group. Also, iMSC–exo improved the proliferation, migration and tube formation in endothelial cells	Activation of PI3K/AKT signaling pathway	89
6	osteoporosis	Effect of iMSC–exo in promoting bone regeneration	Rat model	Increased cell proliferation, alkaline phosphatase, and protein expression of osteoblast–related genes (OPN, OCN RUNX2). iMSC–exo induced regeneration of bone and angiogenesis in crucial–sized calvarial defects	–	90
7	Corneal disease	iMSC–exo thermosensitive chitosan–based hydrogel (CHI–hydrogel) system in corneal epithelium injury and healing	Rat model	CHI–hydrogel sustained the release of iMSC–exo, enhanced the repair mechanism of stromal layer and corneal epithelium damage, reduced the expression of mRNA targeting the three most enriched collagens in corneal stroma, decreased scar formation in vivo	Secretion of miR–432–5p within iMSC–exo that suppress the TRAM2 which is a collagen biosynthesis modulator in the corneal stromal stem cells	91

The table represents the therapeutic roles of iMSC–exosomes in diseases.

Bax = BCL2–associted X protein; ERK1/2 = extracellular signal-regulated kinase 1/2; GSH = glutathione; GSH–Px = glutathione peroxidase; HMGB1 = high mobility group box protein 1; IL6 = interleukin 6; iMSC–exo = induced mesenchymal stem cells–exosomes; I/R = ischemia/reperfusion; miR–432–5p = microRNA–432–5p; OCN = osteocalcin; OPN = osteopontin; PI3K/AKT = phosphatidylinositol 3-kinase/protein kinase B; RUNX2 = runt–related transcription factor 2; SOD = superoxide dismutase; TNF–α = tumor necrosis factor alpha; TRAM2 = translocation–associated membrane protein 2.

used human dermal fibroblasts (HDFs) and human umbilical vein endothelial cells (HUVECs) as an in vitro model in the same study. Their in vitro work revealed that an increased dosage of iMSC-derived exosomes enhanced the proliferation and migration of HDFs and HUVECs. The dosage of iMSC-derived exosomes was positively correlated with collagen I and III production, elastin secretion, and messenger RNA (mRNA) expression by fibroblasts, and tube formation by HUVECs.[92] Kim et al[93] compared the impact of iMSC-derived exosomes and MSC-derived exosomes in stimulating skin proliferation in vitro and in vivo. They found that both iMSC- and MSC-derived exosomes enhanced migration, proliferation, survival, and cell cycle progression of human keratinocytes (HaCaT cells) and HDFs. However, the proliferation rate was higher in HaCaT cells treated with iMSC-derived exosomes compared with MSC-derived exosomes. In addition, fibronectin levels were higher in HaCaT cells treated with iMSC-derived exosomes compared to that of MSC-derived exosomes. Furthermore, the administration of iMSC-derived exosomes enhanced extracellular signal-regulated kinase (ERK1/2) phosphorylation. Hence, the authors suggested that iMSC-derived exosomes increased the proliferation of skin cells via ERK1/2 stimulation.[93]

Another study on bone regeneration in a rat model revealed that the administration of iMSC-derived exosomes combined with tricalcium phosphate (B-TCP) enhanced osteogenesis in rats compared with B-TCP scaffold alone, resulting in newly formed bone tissues within the defect areas.[94] In the in vitro model, osteogenesis occurred due to the activation and recruitment of preexisting exosomes in the bone tissue and the iMSC-derived exosome/B-TCP scaffold combination was internalized into the BM-MSCs. Thus, the internalization stimulated proliferation, migration, and osteogenic differentiation of bone BM-MSCs. Microarray results from the same study revealed that iMSC-derived exosomes significantly altered genes predominantly involved in the phosphoinositide 3-kinase (PI3K)/AKT pathway, thereby potentiating bone regeneration. However, PI3K inhibition did not completely alter the pro-osteogenic effects of iMSC-derived exosomes, suggesting that the PI3K/AKT pathway is not the only mechanism that resulted in the pro-osteogenic effect post-iMSC-derived exosomes

Table 4

iMSC–derived extracellular vesicles in diseases

No.	Disease	Aim	Study type	Study subjects	Findings	Mechanism	References
1	Tendinopathy-related acute pain	Explore the analgesic effect of iMSC–EV in acute pain	In vitro and in vivo	Rat model	Redued inflammation in tendinopathy, inhibited the activation of mast cell infiltration and interaction with nerve fibers	iMSC–EV reduced the proinflammatory cytokines and degranulation of mast cells. Reduced the expression of certain genes that are involved in HIF–1 pathway	96
2	Tendinopathy-related pain	Therapeutic effect of iMSC–EV on tendinopathy-related pain	In vitro and in vivo	Rat model	Alleviated tendinopathy-related pain compared to control group. Reduced proinflammatory cytokines expression, increase tenocyte proliferation	Upregulation in genes related to cellular proliferation and downregulation of genes involved in inflammation and collagen degeneration	97
3	Renal ischemia injury	To determine the potential mechanism of iMSC–EV for renal protection	In vitro and in vivo	Rat model	Renal cell protection against I/R–induced necrosis	Necrotic protection occurs due to the involvement of SP1–SK1–S1P. Activation of SK1 expression, and increment in S1P formation	98
4	Ischemic stroke	To determine the effect of iMSC –EV on angiogenesis in ischemic stroke	In vitro in vivo	Rat model	Reduced infract volume significantly and enhanced angiogenesis. Alleviated the migratory and tube formation of endothelial cells	Proangiogenic mechanism was correlated to inhibition of autophagy. Activation of STAT3	99
5	Sjogren's syndrome	Identify molecules responsible for the therapeutic effects of iMSC–EV on Sjogren's syndrome	In vitro and in vivo	Mouse model	Early passage iMSC–EV resulted in a better immunomodulatory effect compared to late passage in TLR4–stimulated splenocytes and primary SS mouse	Effectively suppressed Th1 and Th16 in splenocyte culture as well as increased the regulatory cytokine TGF–B1 and miR–21 that are mediated via iMSC–EV	100
6	Sjogren's syndrome (onset of sialadeitis)	Therapeutic potentials of iMSC and iMSC–EV on sjogren's syndrome	In vitro and in vivo	Mouse model	Inhibition of the onset of lymphocyte infiltration into the salivary gland.	Inhibited the activation of APCs and Tfh cells likely via the suppression of local mRNA expression of ICOSL and CD40. Suppress immune cells activation and proinflammatory factors expression that are important for Sjogren's syndrome progression	101
7	Rejuvenate senescent nucleus pulposus cells	Therapeutic effects of iMSC–EVs on intravertebral disc degeneration (IVDD)	In vitro and in vivo	Rat model	Significantly improved nucleus pulposus cells senescence and IVDD	Activation of Sirt6 pathway that responds to DNA repair and promote longevity. miR–105–5p mediated via iMSC–EVs resulted in suppressing cAMP–specific hydrolase PDE4D that led to the activation of Sirt6	102

APC = antigen–presenting cells; cAMP = cyclin adenosine monophosphate; CD40 = cluster of differentiation 40; DNA = deoxyribonucleic acid; HIF–1 = hypoxia–inducible factor–1; ICOSL = induced T cell costimulatory ligand; iMSC–EV = induced mesenchymal stem cells–extracellular vesicles; I/R = ischemia/reperfusion; miR–105–5p = micro ribonucleic acid–105–5p; miR–21 = micro ribonucleic acid–21; mRNA = messenger ribonucleic acid; PDE4D = phosphodiestrease–4D knock–out; S1P = sphingosine–1–phosphate; SP1–SK1–S1P = specificity protein1–sphingosine kinase1–sphingosine–1–phosphate; Sirt6 = sirtuin 6; STAT3 = signal transducer and activator of transcription; Tfh = T follicular helper cells; TGF–β1 = transforming growth factor beta 1; Th1 = T helper type 1; Th16 = T helper type 16; TLR4 = toll–like receptor 4.

administration.[94] Du et al[95] evaluated a hepatic ischemia–reperfusion injury model and showed that iMSC-derived exosomes suppressed hepatocyte necrosis and sinusoidal congestion with low histopathological scores. Hepatic injury markers (aspartate aminotransferase and alanine aminotransferase) were significantly reduced, and proliferative markers (proliferating cell nuclear antigen and Phosphohistone-H3) were effectively induced upon iMSC-derived exosome administration compared with the control group. In the same study, the in vitro model showed that the therapeutic effect occurred due to the fusion of iMSC-derived exosomes with HL7702 cells, resulting in higher sphingosine kinase (SK) activity and sphinginosine-1-phosphate (S1P) synthesis. Inhibition of SK1 and S1P receptors abolished the proliferative and protective effects of iMSC-derived exosomes on hepatocytes (in vitro and in vivo).[95]

Apart from iMSC-derived exosomes, other EVs from iMSCs have been tested in disease models, including tendinopathy-related acute pain, renal ischemic injury, ischemic stroke, and Sjogren's syndrome, and to rejuvenate senescent nucleus pulposus cells. The authors observed the therapeutic potential of these EVs, such as in reducing inflammation, downregulating proinflammatory cytokines, increasing the expression of tenocyte proliferation, cellular protection against induced necrosis, enhancing angiogenesis, tube formation, and migration, inhibiting lymphocyte infiltration, and senescence (Table 4).[96–102]

Owing to their function as cargo, exosomes may be utilized as a therapeutic tool for cellular communication. For example, in the context of osteoporosis, Cui et al[103] showed that loading small interfering RNA (siRNA) targeting Shn3 and iMSC-derived exosomes via electroporation resulted in the silencing of Shn3 gene that improved the osteogenic differentiation, promoted type H vessel formation (via the production of SLIT3), and inhibited osteoclasts (via decreasing autologous RANKL expression), thereby enhancing the osteoporotic therapeutic mechanism. The study revealed that iMSC-derived exosomes enhanced the efficiency of the delivered siShn3.[103]

In conclusion, iMSC-derived exosomes have shown important advantages due to their safety, low immunogenicity, and the inability to form tumors. Exosomes also potentiate cell-replacement therapy, an action that may prevent immunological reactions including rejection following stem cell administration in patients. However, there are certain points to consider when using exosomes instead of stem cell–based therapies. First, stem cells are capable of traveling to the site of injury or inflammation due to signals obtained from recipient cells, resulting in an accurate response at the targeted site; in exosomes, however, such an accurate response has not yet been shown. Second, exosomes derived from iMSCs using the conventional cell culture methods are not sufficient for producing large amounts of exosomes. Third, there is no standardized protocol for the isolation, characterization, and validation of exosomes, and there is only limited clinical translation of exosomal therapy in patients. Fourth, due to the heterogeneity of EVs, it is hard to determine the uniformity among batches of exosomes, as each batch will have donor and clone-specific differences. Finally, the dosage, mode of injection, safety, and toxicity must be standardized before exosomes can be used as a therapy. Overall, more studies and clinically relevant models are required to determine the full therapeutic potential when utilizing exosomes secreted from iMSCs for noncell-based therapy in diseases.

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