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Editorial Board Member of *World Journal of Clinical Cases*, Salma Ahi, MD, Assistant Professor, Research Center for Noncommunicable Diseases, Jahrom University of Medical Sciences, Jahrom 193, Iran. salmaahi.61@gmail.com

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Mesenchymal stem cell-derived exosomes: An emerging therapeutic strategy for normal and chronic wound healing

Qin-Lu Zeng, De-Wu Liu

ORCID number: Qin-Lu Zeng 0000-0001-9602-9622; De-Wu Liu 0000-0001-6617-8715.

Qin-Lu Zeng, De-Wu Liu, Burns Institute, The First Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi Province, China

Qin-Lu Zeng, First Clinical Medical College, Nanchang University, Nanchang 330006, Jiangxi Province, China

Corresponding author: De-Wu Liu, MD, PhD, Chief Doctor, Professor, Burns Institute, The First Affiliated Hospital of Nanchang University, No. 17 Yongwaizheng Street, Donghu District, Nanchang 330006, Jiangxi Province, China. dewuliu@126.com

Abstract

Skin wound healing is a complex biological process. Mesenchymal stem cells (MSCs) play an important role in skin wound repair due to their multidirectional differentiation potential, hematopoietic support, promotion of stem cell implantation, self-replication, and immune regulation. Exosomes are vesicles with diameters of 40-100 nm that contain nucleic acids, proteins, and lipids and often act as mediators of cell-to-cell communication. Currently, many clinical scientists have carried out cell-free therapy for skin wounds, especially chronic wounds, using exosomes derived from MSCs. This review focuses on the latest research progress on the mechanisms of action associated with the treatment of wound healing with exosomes derived from different MSCs, the latest research progress on the combination of exosomes and other biological or nonbiological factors for the treatment of chronic skin wounds, and the new prospects and development goals of cell-free therapy.

Key Words: Mesenchymal stem cells; Exosomes; Mesenchymal stem cell-exosomes; Wound healing; Therapeutics

Core Tip: We have mainly sorted out the reported mechanisms by which different mesenchymal stem cells (MSC)-derived exosomes play a promoting role in the hemostatic stage, inflammatory stage, proliferative stage, and remodeling stage of skin wound healing. The clinical prospect of MSC-exosomes as a cell-free therapy, such as the addition of carriers, combination of drugs, combined physical treatment, and

development as an immunosuppressive agent, is also summarized.

INTRODUCTION

Skin wound healing is a complex biological process that includes cell proliferation, differentiation, epithelization, migration, and matrix synthesis and deposition. Mesenchymal stem cells (MSCs) are important members of the stem cell family and come from many sources, such as adipose tissue, dental pulp, placenta, amniotic fluid, and umbilical cord blood[1]; these cells have the common characteristics of self-replication and multidirectional differentiation. The mechanism of action of stem cell therapy has been demonstrated to primarily involve paracrine actions mediated by stem cell secretion factors[2,3]. It has been reported that exosomes play a major paracrine role among certain secretory bodies associated with stem cells[3-5]. The small vesicles formed by reverse budding are called exosomes after they are released from cells[6], and these vesicles contain nucleic acids, proteins, and lipids and often serve as mediators of communication between cells. Currently, many clinical scientists have carried out research on these small exosomes. The commercial use of cell-free therapy based on exosomes has already begun[7-9]. In fact, in various disease models, including cardiovascular disease, respiratory disease, liver disease, kidney disease, nerve disease, musculoskeletal disease, eye disease, skin diseases, and cancer, MSC-derived exosomes have been used as cell-free substitutes for MSCs[4,10-13]. In the field of skin wound healing, researchers have attempted to explore the mechanism by which exosomes promote wound healing while developing cell-free therapy to promote the healing of normal skin wounds and chronic refractory wounds. Researchers have used different exosomes from MSCs to study the four stages of wound healing (hemostasis, inflammation, proliferation, and remodeling) from different angles and have made good progress.

BIOGENESIS AND BIOLOGICAL CHARACTERISTICS OF MSC-EXOSOMES

MSCs are described as pluripotent nonhematopoietic adult stem cells that express the surface markers CD73, CD90, and CD105 but do not express CD14, CD34, or CD45[1]. MSCs were first discovered by Freeden Stein in the 1960s through studies of bone marrow[14]. MSCs can also be isolated from other adult tissues, such as adipose tissue, dental pulp, placenta, amniotic fluid, umbilical cord blood, Wharton's jelly[1], and even the brain, spleen, liver, kidney, lung, thymus, and pancreas.

In 1983, the secretion of extracellular vesicles (EVs) was identified during the maturation of reticulocytes[15]. EVs are a general term that is currently used to refer to all secreted membrane vesicles. However, these vesicles are highly heterogeneous and are cell-derived membrane-bound structures secreted by various cell types, including T cells, B cells, dendritic cells, platelets, mast cells, epithelial cells, endothelial cells, neurons, cancer cells, oligodendrocytes, Schwann cells, embryonic cells, and MSCs[16].

EVs, including exosomes, microvesicles, and apoptotic bodies[17-19], can be found in physiological fluids such as normal urine, blood, bronchial lavage fluid, breast milk, saliva, cerebrospinal fluid, amniotic fluid, synovial fluid, and malignant ascites. Exosomes play a relatively major role among EVs[15,16].

Exosomes are 40-100 nm diameter membranous vesicles of endocytic origin that are released by a variety of cell types into the extracellular space[20]. Exosomes were first reported in 1983 by Johnstone and colleagues while culturing reticulocytes. Inward budding of endosomal membranes results in the progressive accumulation of intraluminal vesicles (ILVs) within large multivesicular bodies (MVBs). Transmem-

brane proteins are incorporated into the invaginating membrane, while cytosolic components are engulfed within ILVs[21].

Part of the MVB is degraded after entering the lysosome; the remaining part sags inward again to form small granular vesicles by fusing with the plasma membrane and then releasing these vesicles into the extracellular environment. These vesicles that are released from the cell are called exosomes[6].

The biological characteristics of exosomes are mainly reflected in three aspects. First, exosomes function to transport material and transmit information. As a component of the intercellular microenvironment, exosomes regulate cell-to-cell communication by carrying a diverse array of signaling molecules, including lipids, proteins, and nucleic acids. Studies have shown that intercellular communication appeared during the early stage of evolution and could influence the behavior of target cells in many ways[22]. After the exosomes are absorbed by the target cells, the components such as lipids, proteins, mRNA, and microRNAs in the exosomes can affect the modification and positioning of proteins by changing the transcription and translation procedures, and ultimately regulate the cell phenotype and function of the receptor cells by regulating signal cascade pathways and key enzyme reactions to affect cell self-regulation, as well as directly participating in various physicochemical reactions in cells. Second, exosomes act as tools for transporting and removing components from cells. In addition, exosomes are also involved in immunoregulation. *In vitro* experiments have shown that exosomes can inhibit the proliferation and differentiation of T cells and reduce the release of IFN-γ from T cells[23].

PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES OF WOUND HEALING

Normal cutaneous wound healing

The normal cutaneous wound healing process includes four stages: Hemostasis, inflammation, proliferation, and remodeling[24].

Hemostasis occurs immediately after an injury. Vasoconstriction and platelet aggregation proceed simultaneously. A blood clot covering the wound will form at the wound site. This clot not only reduces blood loss but also provides a scaffold-like structure for the migration of resident skin cells and immune cells[25]. Additionally, platelets in blood clots secrete or release various platelet-specific proteins; growth factors; adhesion molecules; fibrinogen and thrombin; pro- and antiangiogenic factors; and cytokines/chemokines such as NAP-2 and platelet-derived SDF-1α[26-28]. Subsequently, neutrophils, macrophages, fibroblasts, endothelial cells, smooth muscle cells, and circulating BMSCs can be induced by these secreted molecules to migrate to the wound site and become activated.

Inflammation is driven by secreted chemokines/cytokines, bacterial byproducts, and platelet-derived mediators. After vascular permeability increased, monocytes/macrophages and neutrophils gradually infiltrated the wound surface. The cells not only kill bacteria but also remove debris and damaged matrix proteins[29]. After reaching the wound, neutrophils release proteases such as MMP, ROS, growth factors, and antimicrobial peptides, while monocytes arrive within 24 hours and transform into M1 macrophages. Lymphocytes are the last inflammatory cells that are attracted to the wound site. Among them, γδ+ T cells can participate in the growth and survival of fibroblasts, immune cells, and keratinocytes by producing mediators such as IGF-1, FGFs, or KGFs. αβ+ T cells are also present in this inflammatory stage and have an important effect on pathogenic microorganisms[26,30,31].

In the later stage of inflammation, M2 macrophages transform from M1 macrophages or monocytes. On the one hand, the release of IL-10, VEGF, PDGF, FGFs, and IGF-1 by M2 macrophages plays a role in inducing proliferation, cell migration, and matrix formation. On the other hand, these cells also produce TIMP1 to counteract MMPs[32].

The focus of the proliferation phase is the development of granulation tissue, which covering the exposed wound surface while helps to restore the vascular network[33]. At this stage, fibroblasts can play a role in reducing the interstitial space by depositing a large amount of ECM. This process is affected by cytokines and growth factors, and the regulation of these factors can also induce the release of additional cytokines such as VEGF, FGFs, IGFs, IFNs, and HGF[24,28,34,35]. In addition, keratinocytes also release growth factors. Keratinocytes proliferate and migrate to facilitate wound coverage, form layers and differentiate, ultimately achieving the effect of rebuilding the epidermal barrier of the skin[24].

Remodeling is the final stage and is characterized by a gradual decrease in cells and blood vessels[29]. Fibroblasts are the main factors at this stage, and their main role is to increase the amount of collagen I and other extracellular matrix (ECM) components, while the role of MMPs is to decompose the disordered collagen that was used as a template, similar to the more common collagen III[24,36,37]. The decomposition of granulation tissue and the formation of scars are driven by a variety of MMPs and their inhibitors. Any disturbance in the balance of matrix metalloproteinases and their inhibitors can trigger the development of hypertrophic scars or the formation of chronic wounds.

Chronic wound healing

When tissue repair is ineffective, the skin may suffer from ulcerative injuries, such as venous ulcers in the lower limbs, pressure ulcers, and diabetic foot ulcers[30]. Although there is widespread controversy, wounds lasting more than 3 mo are generally considered chronic wounds[38].

Persistent inflammation is an important feature of chronic wounds, which manifests as dysregulated cytokine/growth factor levels and/or increased protease activity, impaired angiogenesis, and difficult re-epithelialization of the wound[38]. At the cellular level, many disorders occur in neutrophils and macrophages in chronic wounds. Neutrophils show phenotypic changes, reduced infiltration and migration across endothelial cells, and stay in the wound for longer periods of time. In addition, chronic wounds also showed reduced induction of the M2 macrophage profile and reduced antibacterial activity[39,40]. The function of macrophages in chronic wounds is also disturbed and may cause abnormal repair. This effect is specifically reflected in the uncontrolled production of inflammatory mediators and growth factors by macrophages, as well as the imbalance in the M1:M2 profile ratio. There is also communication failure between macrophages and other crucial participants (including fibroblasts, epithelial cells, endothelial cells, and stem or tissue progenitor cells)[41].

CUTANEOUS WOUND HEALING BY MSC-EXOSOMES

According to the existing literature, MSC-exosomes or MSC-EVs can accelerate wound healing at various phases of wound healing, and even have the ability of improving scars (Table 1).

Hemostasis phase

During the initial hemostatic stage of wound healing, the physiological mechanism for restoring skin barrier function mainly involves reducing blood loss through vasoconstriction and platelet aggregation in blood clots. As mentioned previously, platelets in blood clots can induce the migration of a series of cells to the wound site by releasing specific proteins. To date, there is no direct evidence that MSC-derived exosomes are involved in hemostasis or blood clotting. According to reports, human umbilical cord (hUC)-MSC-EVs can induce blood coagulation *in vitro*[42]. This result indicates that MSC-derived exosomes may have potential benefits in the coagulation process of wound healing, but further studies are needed to analyze the role of MSC-EVs or MSC-derived exosomes in the coagulation process under healthy or diseased conditions.

Inflammatory phase

The inflammatory stage is very important in the process of skin repair. As mentioned previously, this stage mainly relies on the infiltration of neutrophils and monocytes/macrophages at the wound site to complete tasks such as killing bacteria and removing debris. In late inflammation, with the release of M2-type macrophages and other mediators, wound healing transitions from the inflammatory phase to the proliferative phase, which is a critical step during normal wound healing[43]. However, studies have shown that prolonged inflammation can lead to excessive scarring of the wound[37], mainly in burns and other chronic wounds[44,45]. Based on this mechanism in the inflammatory phase of wound healing, we believe that macrophages play a crucial role in the later stages of inflammation, especially through proper transformation of M1-type macrophages into M2-type macrophages. A series of studies have shown that MSC-derived exosomes are involved in the promotion of macrophage polarization through a variety of regulatory mechanisms. This phenomenon is critical for transitioning from the inflammatory phase to the proliferative phase during the wound healing process, especially for the promotion of

Table 1 Effects of mesenchymal stem cell-exosomes on cutaneous wound healing

Phase	Exosome source	Nomenclature	Related exosomal cargo	Secreted factors or expressed genes affected	Outcome	Ref.
Hemostasis Phase	Human mesenchymal stem cells (MSCs) from the umbilical cord	EVs	-	Phosphatidylserine(+)	Umbilical MSCs and extracellular vesicles derived from them have a reasonably high procoagulant potential	[42]
Inflammatory Phase	Human jaw bone marrow-derived MSCs and bone marrow MSCs	Exosomes	miR-223	TNF-α↓ IL-10 ↑	Accelerated wound healing in mice	[46]
					Induced M2 macrophage polarization (CD206+ macrophage ↑)	
	Human umbilical cord (UC)-MSCs	Exosomes	let-7b	TLR4, p-p65, iNOS ↓ p-STAT3, p-AKT, ARG1 ↑	Alleviated inflammation and enhanced diabetic cutaneous wound healing in rats	[47]
					Induced M2 macrophage polarization Inhibited TLR4 signaling pathway	
	Human UC-MSCs	Exosomes	miR-181c	TNF-α, IL-1β, TLR4, p65, p-p65↓ IL-10 ↑	Reduced burn-induced inflammation in rats	[48]
					Reduced neutrophil and macrophage infiltration (MPO+ cell,CD68+ cell↓) Inhibited TLR4 signaling pathway	
	Human menstrual blood derived MSCs (MenSCs)	Exosomes	-	iNOS ↓ ARG1, VEGF ↑	Resolved inflammation and ameliorate cutaneous non healing wounds in diabetic mice	[49]
					Induced M2 macrophage polarization	
Proliferative Phase	Human bone marrow MSC-derived exosomes	Exosomes	TGF-β/Smad	TGF-β1, Smad2, Smad3, Smad4 ↓TGF-β3, Smad7↑	Effectively promoted the cutaneous wound healing by inhibiting the TGF-β/Smad signal pathway	[59]
	Human adipose MSCs (ASCs)	Exosomes	-	N-cadherin, cyclin 1, PCNA, collagen I/III, elastin ↑	Facilitated cutaneous wound healing *via* optimizing the characteristics of fibroblasts	[62]
	Human ASCs	Exosomes	-	Collagen I/II, TGF-β1/3, MMP1/3 α-SMA ↓	Promoted ECM reconstruction in cutaneous wound repair by regulating the ratios of collagen type III: type I, TGF-β 3:TGF-β1, and MMP3:TIMP1, and by regulating fibroblast differentiation to mitigate scar formation	[63]
	Human fetal dermal MSCs	Exosomes	Jagged 1	Collagen I/III, elastin, fibronectin mRNA ↑	Promoted wound healing by activating the ADF cell motility and secretion ability *via* the Notch signaling pathway	[64]
	Human UC-MSCs	Exosomes	Wnt4	CK19, PCNA, collagen I ↑	Stimulated the AKT pathway to protect immortalized keratinocytes from heat-induced apoptosis	[65]
					Stimulated the AKT pathway to protect immortalized keratinocytes from heat-induced apoptosis	
	Human UC-MSCs	Exosomes	Akt, ERK, STAT3	HGF, IGF1, NGF, SDF1↑	Promoted the proliferation and migration of fibroblasts in normal and chronic wounds. This effect was positively correlated with the dose of exosomes	[66]

Induced pluripotent stem cell-derived MSCs	Exosomes	-	Collagen ↑	Increased the secretion of collagen by HaCaT cells to accelerate skin cell proliferation	[67]
Adipose mesenchymal stem cells (ADSCs)	Exosomes	AKT/HIF-1α	-	Promoted the proliferation and migration of HaCaT cells by regulating the activation of the AKT/HIF-1α signaling pathway, thus promoting wound healing	[68]
Human UC-MSCs	Exosomes	-	PARP-1, PAR↑	Suppressed HaCaT cell apoptosis induced by H_2O_2 by restraining the nuclear translocation of apoptosis-inducing factor (AIF) and promoting poly (ADP-ribose) (PAR) and poly ADP ribose polymerase 1 (PARP-1) expression	[69]
Human adipose-derived MSCs (adMSC-Exo)	Exosomes	miR-125a	Angiogenic inhibitor delta-like 4 (DLL4)↓	Transferred miR-125a to endothelial cells and promoted angiogenesis by repressing DLL4	[70]
Mouse BM- MSCs	Exosomes	miR-17 miR-23a miR-125b	TNF-α, IL-1β, iNOS, TLR4, IRAK1, p65↓ ARG1, IL-10, TGF-β↑	Decreased the threshold for thermal and mechanical stimuli in mice Increased nerve conduction velocity, the number of intraepidermal nerve fibers, myelin thickness, and axonal diameters	[71]
Rat BM-MSCs	Exosomes	-	MDA, HIF1α, NOX2, Caspase 3, BAX, PARP1, MPO, ICAM1, IL-1β, NF-κB↓SOD, CAT, GPX, HO-1, BCL2, IL-10, bFGF, HGF, SOX9, VEGF↑	Decreased histopathological score of kidney injury in rats Reduced the levels of blood urea nitrogen (BUN) and creatinineReduced the level of oxidative stress Increased anti-oxidant status Reduced apoptosis and inflammation Improved regeneration and enhanced angiogenesis	[72]
Human endometrial MSCs	Exosomes	-	Tie2, VEGF, Ang1, Ang2↑	Increased the expression of angiogenesis markers, including Tie2, VEGF, Ang1, and Ang2, and increased the proliferation, migration, and angiogenesis of HUVECs	[73]
Human umbilical cord mesenchymal stem cells (hUCMSCs)	Exosomes	-	Ang2↑	hucMSC-Ex-derived Ang-2 plays a significant role in tube formation of HUVECs and promotion of angiogenesis	[74]
Human UC blood-MSCs	Exosomes	-	Ang, Ang1, HFG, VEGF↑	Human umbilical cord blood (UCB)-MSC-derived exosomes pretreated with thrombin could accelerate skin wound healing in rats with full-thickness wounds. Exosomes from human UCB-MSCs increased angiogenesis factors, such as VEGF, HGF, and Ang1, and decreased TNFα and IL-6	[75]
Human UC-MSCs	Exosomes	Wnt4	β-catenin, N-cadherin, PCNA, Cyclin D3↑	Enhanced angiogenesis in rats through the Wnt4/β-catenin pathway. When the expression of Wnt4 was knocked out by shRNA, the proangiogenic effect of hUC-MSC-derived exosomes was eliminated	[76]

	Human UC-MSCs	Exosomes	-	α-SMA, collagen I↓	Increased the formation and maturation of new blood vessels at the wound site, although the mechanism is still unclear	[77]
	Human UC-MSCs	Exosomes	GSK3β-Wnt/β-catenin	-	Alleviated hepatic IRI by transporting miR-1246 *via* regulating GSK3β-mediated Wnt/β-catenin pathway	[78]
Remodeling Phase	Human gingival MSCs	Exosomes	-	Collagen↑	Reduced the formation of scars by inhibiting the accumulation of mouse myofibroblasts	[79]
	Adipose mesenchymal stem cells (ASCs)	Exosomes	-	N-cadherin, cyclin-1, PCNA collagen I, III↑	Facilitates cutaneous wound healing *via* optimizing the characteristics of fibroblasts	[62]
	ASCs	Exosomes	ERK/MAPK	Matrix metalloproteinases-3 (MMP3)↑	APromoted ECM reconstruction in cutaneous wound repair by regulating the ratios of collagen type III: type I, TGF-β3:TGF-β1, and MMP3:TIMP1, and by regulating fibroblast differentiation to mitigate scar formation	[63]
	MenSCs	Exosomes	-	iNOS↓ ARG1, VEGF↑	Resolved inflammation and ameliorated cutaneous non-healing wounds in diabetic mice	[49]
					Induced M2 macrophage polarization	

MSCs: Mesenchymal stem cells; EVs: Extracellular vesicles; ILVs: Intraluminal vesicles; IL: Interleukin; MVBs: Multivesicular bodies; PF-4: Platelet factor 4; EGF: Epidermal growth factor; PDGF: Platelet-derived growth factor; TGF-β: Transforming growth factor; VEGF: Vascular endothelial growth factor; NAP-2: Neutrophil activating peptide-2; SDF-1α: Stromal-cell-derived factor-1; BMSC: Bone marrow-derived stem cells; MMP: Matrix metalloproteinases; ROS: Reactive oxygen species; IGF-1: Insulin growth factor 1; FGFs: Fibroblast growth factors; KGFs: Keratinocyte growth factors; TIMP1: Tissue inhibitor of metalloproteinase 1; TNF-a: Tumor necrosis factor alpha; CTGF: Connective tissue growth factor; IFNs: Interferons; HGF: Hepatocyte growth factor; ECM: Extracellular matrix; hUC: Human umbilical cord; hBM: Human bone marrow; BMMSC: Bone marrow MSC; JMMSC: Jaw bone marrow MSC; HDFs: Human dermal fibroblasts; HaCaTs: Human keratinocytes; LPS: Lipopolysaccharide; ASC: Human adipose mesenchymal stem cell; FD: Human fetal dermis; iPSC: Induced pluripotent stem cell; AIF: Apoptosis-inducing factor; HUVECs: Human umbilical vein endothelial cells; DLL4: Delta-like 4; Ang: Angiopoietin; iNOS: Inducible nitricoxide synthase.

chronic wound healing: (1) MiR-223 produced by human bone marrow MSC (hBM-MSC)-derived exosomes and jaw bone marrow MSC-derived exosomes regulates the polarization of macrophages by targeting pknox1, thereby promoting the healing and metastasis of skin wounds in mice[46]; (2) Lipopolysaccharide (LPS)-preconditioned exosomes may mediate the regulation of macrophage polarization and chronic inflammation regression by shuttling let-7b, thus promoting the healing of diabetic skin wounds[47]; (3) Burns significantly increased the inflammatory response in rats or macrophages exposed to LPS, while hUC-MSC-derived exosomes overexpressing miR-181c inhibited the TLR4 signaling pathway effectively, thus reducing inflammation in burned rats[48]; (4) The miRNA let-7b carried by MSC-EXOs pretreated with LPS can regulate the polarization of macrophages by inhibiting the TLR4/NF-κB pathway and activating the STAT3/AKT signaling pathway, thereby promoting wound healing[47]; and (5) Menstrual blood-derived MSC exosomes can resolve inflammation by inducing M1–M2 macrophage polarization[49].

In fact, there have been a large number of reports on the anti-inflammatory and immunoregulatory effects of MSC-derived exosomes[18,50-58].

Proliferative phase

In the proliferation stage of wound healing, whether MSC-derived exosomes can promote the development of granulation tissue and the recovery of the vascular network is a concern. Studies have shown that hBM-MSC-derived exosomes could effectively promote the proliferation of two types of skin cells *in vitro*: Human dermal fibroblasts (HDFs) and human keratinocytes (HaCaTs). *In vivo*, hBM-MSC-derived exosomes could accelerate skin wound healing by inhibiting the TGF-β/Smad signaling pathway[59]. This pathway is critical in the pathogenesis of wound healing [60]. TGF-β1 seems to be the key mediator of tissue scarring and mainly antagonizes acellular (Smad) signal transduction by activating its downstream factors[61].

According to related studies, MSC-derived exosomes are able to promote the migration and proliferation of dermal fibroblasts and can produce collagen, elastin, and fibronectin: (1) Human adipose MSC (ASC)-derived EVs or ASC-derived exosomes induce the migration and proliferation of dermal fibroblasts or keratinocytes *in vitro*[62,63]; (2) Human ASC-derived exosomes induce type I/III collagen and elastin production in HDFs to promote the healing of skin wounds in mice[62,63]; (3) Human fetal dermis (FD)-MSC-derived exosomes activate the Notch pathway by transmitting Jagged1 protein and induce the mRNA expression of collagen I/III, elastin, and fibronectin[64]; (4) hUC-MSC-derived exosomes containing Wnt4 accelerated the re-epithelialization of burned skin in rats. The wound healing effect was inhibited when the expression of Wnt4 in hUC-MSC-derived exosomes was knocked out with siRNA[65]; and (5) hUC-MSC-exosomes can promote the proliferation and migration of fibroblasts in normal and chronic wounds. This effect was positively correlated with the dose of exosomes[66].

The positive effects of MSC-derived exosomes on keratinocytes have also been reported: (1) hUC-MSC-derived exosomes can stimulate the AKT pathway to protect immortalized keratinocytes from heat-induced apoptosis[65]; (2) Induced pluripotent stem cell (iPSC)-derived MSC (iMSC) exosomes could increase the secretion of collagen by HaCaT cells to accelerate skin cell proliferation[67]; (3) ASC-derived exosomes activate the AKT/HIF-1α axis to accelerate the migration and proliferation of keratinocytes to promote wound healing[68]; and (4) Human umbilical cord MSC (hUCMSC)-derived exosome treatment suppressed HaCaT cell apoptosis induced by H_2O_2 by restraining the nuclear translocation of apoptosis-inducing factor (AIF) and promoting poly (ADP-ribose) (PAR) and poly ADP ribose polymerase 1 (PARP-1) expression[69].

Regarding the recovery of the vascular network, it has also been reported that MSC-derived exosomes can induce angiogenic activity in endothelial cells: (1) Human ASC-derived exosomes induce HUVEC tube formation by delivering miR-125a, thereby inhibiting the expression of the angiogenesis inhibitor delta-like 4[70]; (2) Human BM-MSC-EVs or rat BM-MSC-derived exosomes enhance angiogenesis in stroke mice[71] or in rats with renal ischemia-reperfusion (IR) injury[72]; (3) Exosomes from human endometrial MSCs can increase the expression of angiogenesis markers, including Tie2, vascular endothelial growth factor (VEGF), angiopoietin 1 (Ang1), and Ang2, and increase the proliferation, migration, and angiogenesis of human umbilical vein endothelial cells (HUVECs)[73]; and (4) In a rat burn model, treatment with hUCMSC-derived exosomes promoted Ang-2 protein expression in wounds and HUVECs through exosome-mediated Ang-2 transfer. The overexpression of Ang-2 in hUCMSC-derived exosomes further promoted the migration and tube formation of HUVECs, while knockout of Ang-2 in hUCMSC-derived exosomes eliminated these therapeutic and proangiogenic effects[74].

In addition, there have been reports confirming the proangiogenic effect of MSC-derived exosomes *in vivo*: (1) Human umbilical cord blood (UCB)-MSC-derived exosomes pretreated with thrombin could accelerate skin wound healing in rats with full-thickness wounds. Exosomes from human UCB-MSCs increased angiogenesis factors, such as VEGF, HGF, and Ang1, and decreased TNFα and IL-6[75]; (2) hUC-MSC-derived exosomes enhanced angiogenesis in rats through the Wnt4/β-catenin pathway. When the expression of Wnt4 was knocked out with shRNA, the proangiogenic effect of hUC-MSC-derived exosomes was eliminated[76]; (3) Human iMSC-derived exosomes increased the formation and maturation of new blood vessels at the wound site, although the mechanism is still unclear[77]; and (4) hUCB-MSC-derived exosomes alleviated hepatic ischemia/reperfusion injury by transporting miR-1246 by regulating the glycogen synthase kinase 3β (GSK3β)-mediated Wnt/β-catenin pathway[78].

Remodeling phase

MSC-derived exosomes may help to further reduce scar formation. Typically, uncontrolled accumulation of myofibroblasts in the wound leads to scar formation. Recently, it has been reported that hUC-MSC-derived exosomes reduced the formation of scars by inhibiting the accumulation of mouse myofibroblasts[79]. It has also been reported that human ASC-derived exosomes accelerate skin healing by optimizing the characteristics of fibroblasts. In the early stage of wound healing, ASC-derived exosomes increased the production of collagen I and III, and exosomes could inhibit the expression of collagen to reduce the formation of scars in the later stage[62]. It is well known that various proteases, such as MMPs, are necessary for all stages of skin wound healing[80]. In the remodeling phase, macrophages, fibroblasts, endothelial cells, and epidermal cells control the release of MMPs to degrade the majority of type III collagen fibers[81]. However, the levels of MMPs and their inhibitors should be kept in a relatively stable balance; otherwise, hypertrophic scars or chronic wound outcomes may occur. Studies have shown that ASC-derived exosomes promote ECM reconstruction in cutaneous wound repair by regulating the collagen III:collagen I, MMP3:TIMP1, and TGF-β3: TGF-β1 ratios and by regulating fibroblast differentiation to mitigate scar formation[63]. However, whether ASC-derived exosomes prevent excessive ECM degradation in chronic wounds through the same regulatory mechanism remains to be investigated. Another study also showed that exosomes derived from human menstrual blood mesenchymal cells can reduce scar formation by affecting the Col1:Col3 ratio[49].

PERSPECTIVES ON CLINICAL APPLICATIONS OF MSC-EXOSOMES

Advantages and disadvantages

In the past few years, MSC-derived exosomes have been used as a new cell-free treatment for wound healing and regeneration. This new treatment method has unique advantages. First, exosomes with diameters of 40-100 nm are more likely to participate in blood circulation than MSCs. Some MSCs cannot circulate easily through capillaries, but exosomes can. Second, the treatment also benefits from the small sizes of exosomes. Compared with injection of MSCs, injection of exosomes can achieve higher efficacy with a lower dose. In addition, MSC-derived exosomes can be produced and sterilized as a commercial product, while MSCs cannot be produced. Last but not least, the use of exosomes for cell-free therapy can largely avoid the transfer cells whose DNA may have been damaged or mutated. In cell-based therapy, MSC-derived exosomes are considered to have no safety issues, such as the tumori-genicity potential of cell-mediated drug delivery[11,82]. However, this method of treatment also has some shortcomings. The exosomes injected into the body are static and cannot produce more exosomes. In addition, compared with using MSCs, exosome treatment has increased manufacturing costs and difficulty associated with laboratory preparation.

New prospects of MSC-EXOs in treating wounds

Adding carriers and combined stents to treat wounds: It is well known that uncontrolled protease activity can hinder wound healing[63]. In addition, some studies have shown that the delayed healing of chronic wounds is related to prolonged, high levels of protease activity[80,81,83,84]. The proteolytic environment of chronic wounds may also affect the therapeutic efficacy of MSC-derived exosomes because MSC-derived exosome surface proteins are susceptible to proteolysis, which in turn affects the interaction between exosomes and recipient cells[85]. Based on the natural biocompatibility and cell targeting ability of exosomes, many clinical researchers have focused their attention on using exosomes as new drug delivery systems[86]. Some studies have mixed exosomes with hydrogel or chitosan dressings, allowing the hydrogel or chitosan dressing to act as a stent for sustained release. This strategy can stabilize the local concentration of exosomes, thereby enhancing the ability of exosomes to heal chronic wounds. For example, the FHE@exo hydrogel is prepared by mixing FHE hydrogel (F127/OHA-EPL) and AMSC-derived exosomes to treat diabetic full-thickness skin wounds and can increase the wound closure rate and accelerate angiogenesis, re-epithelialization, and collagen deposition at the wound site. This treatment can significantly improve the healing of chronic diabetic wounds[87]. Similarly, Pluronic F-127 (PF-127) hydrogel containing hUCMSC-derived exosomes can also promote the healing of chronic diabetes wounds and complete skin regeneration[88]. In addition, gingival MSC-derived exosomes combined with a

chitosan/silk hydrogel sponge are also helpful for healing chronic wounds in diabetic patients[79]. Furthermore, studies have shown that exosomes derived from microRNA-126-overexpressing synovial MSCs mixed with chitosan dressing can also promote the healing of chronic diabetic wounds[89,90].

Combined with other medications: It has been reported that the exosomes from BMSCs pretreated with atorvastatin could promote angiogenesis through the AKT/eNOS pathway, thereby accelerating the repair of diabetic wounds[91]. Melatonin-stimulated MSC-derived exosomes regulate the polarization of M1 and M2 macrophages by targeting the PTEN/AKT pathway to improve wound healing in diabetes[92].

Combined treatment with physical factors: It has been reported that the exosomes derived from hUC-MSCs exposed to blue light at 455 nm have a robust ability to promote angiogenesis[93]. Labeling human BMSC-derived exosomes with iron oxide nanoparticles and guiding this treatment with a magnetic field could significantly promote the proliferation and migration of endothelial cells and the formation of angiogenic tubules *in vivo*. Moreover, this treatment could also reduce scar formation and PCNA, CK19, and collagen expression *in vivo*[94].

Development as an immunosuppressant: Based on the inhibitory effect of exosomes on immune regulation, exosomes can be used as immunosuppressive therapeutic agents in clinical practice. However, because exosomes carry a small amount of allogeneic protein, which can stimulate an autoimmune response, their application in immune regulation remains to be verified[95].

Quality control of extracellular vesicle therapy
Cell-free therapy using exosomes has great development prospects, but the quality control and production specifications should be controlled to some extent for clinical use. The existing preparation guide is minimal information for studies of extracellular vesicles 2018 (MISEV2018), which was proposed by the International Society of Extracellular Vesicles in 2018 and is a series of minimal information on extracellular vesicle research (MISEV)[96-98]. The Korean Ministry of Food and Drug Safety also issued guidelines for EV therapeutic products, entitled "Guidelines for the Quality, Nonclinical and Clinical Evaluation of Extracellular Vesicle Therapeutic Products"[99]. There are certain specifications on the identity, quantity, size, and purity of EVs. Unfortunately, there is currently no evaluation standard for exosomal therapeutic products.

CONCLUSION
In the field of traditional regenerative medicine, although high-dose single factors or cells are used to treat diseases, which can take effect to a certain extent, their regulation mechanism is single and they carry a certain potential risk, such as the formation of ectopic tissue and cell rejection. The clinical application of stem cells in wound repair is greatly limited. Compared with direct reaction of stem cells, exosomes can be extracted on a large scale, with high activity, safe use, more suitable for the internal environment, and no ethical controversy involved, thus having good application potential in wound repair. With the recent explosion in the number of studies on MSC-derived exosomes, various studies have shown anti-inflammatory, anti-aging, and wound healing effects of MSC-exosomes *in vivo* and *in vitro* models. MSC-exosome factors are now widely accepted as a new generation of drugs for cell-free therapy. Undeniably, this treatment can obtain good therapeutic effects and has many advantages with practical significance. However, transforming this treatment from a clinical experiment into a mature medical commodity still faces many challenges. For example, we need to set certain standards for exosomal therapeutic products in terms of the identity, quantity, size, purity, and the content of exosomes derived from MSCs.

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