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Mesenchymal stem cell-derived exosomes: Versatile nanomaterials for skin wound treatment

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ABSTRACT

Accumulating studies reveal that mesenchymal stem cells (MSCs) promote skin wound healing mainly through the paracrine effects. Exosomes, one of the crucial paracrine mediators in wound healing, are cell-derived nanosized membranous vesicles containing diverse bioactive cargoes. With the potent ability of modulating skin cell behaviors, MSC-derived exosomes (MSC-Exos) are regarded as a promising nanomaterial for regenerative wound therapy. Under hostile conditions, MSC-Exos are efficient in protecting skin cells from severe damage and restoring their function. According to recent studies, MSC-Exos possess remarkable pro-healing effects in a variety of skin wounds, typically resulting in increased wound closure, inhibited scar tissue formation, and better restoration of skin function. To further enhance the therapeutic potential of MSC-Exos, the development of applicable pretreatment strategies and the optimization of exosome delivery are under intensive investigation. Herein, we summarize current research progress of MSC-Exos for skin wound treatment, with an emphasis on the biological effects of these nanovesicles, the repair mechanisms, and future challenges in clinical translation.

KEYWORDS

exosomes, mesenchymal stem cells, diabetic wounds, burn wounds, scar, wound healing

1 Introduction

A variety of causes including trauma, diabetic ulcers, and burns can lead to severe skin injuries. The healing of skin wounds requires sophisticated coordination of fibroblasts, keratinocytes, endothelial cells, and immunocytes. Normal wound healing comprises four well-orchestrated stages, namely the hemostasis, inflammation, proliferation, and extracellular matrix (ECM) remodeling stages [1, 2]. Any destruction in these stages can lead to chronic wounds or excessive scarring, both of which seriously affect the life quality of patients [3, 4]. Furthermore, inadequate regeneration of skin appendages remains a significant obstacle to fully restore the normal function of skin [5].

Mesenchymal stem cells (MSCs) are adult stem cells that encompass a broad differentiation potential. They express a series of cell surface markers (cluster of differentiation 73 (CD73), CD90, CD105, etc.), and do not express hemopoietic stem cell markers such as CD14, CD34 and CD45 [6]. In the past few decades, MSCs have been isolated and characterized from almost all of the human tissues (e.g., bone marrow, fat, placenta, and umbilical cord) [7]. Owing to the low immunogenicity and prominent regenerative potential, MSCs have received widespread attention in the field of regenerative medicine, such as cartilage regeneration [8] and chronic wound healing [1]. A large number of animal studies have confirmed their immense promise for wound treatment. Particularly, recent clinical trials have shown improved repair outcomes in the skin wounds receiving MSC transplantation [1].

Although MSCs play diverse roles in skin wound healing, their paracrine effects are widely recognized as the primary healing mechanism [1, 9]. As a key mediator for intercellular communication, exosomes are extracellular vesicles released by living cells through a series of biological processes, typically including the endocytosis, integration and efflux actions [10]. Besides the isolation from human body fluids, exosomes can be enriched from cell culture medium, providing an avenue for producing tailored exosomes. They contain diverse contents (e.g., functional proteins, phospholipids, nucleic acids, etc.) and can be efficiently phagocytized by target cells to modulate the cell behaviors.

A wealth of studies report that exosomes enriched from MSC culture medium can modulate the function of skin cells *in vitro* and facilitate wound repair *in vivo*. When confronting with harsh conditions (e.g., oxidative stress, photodamage, and heat stress), MSC-Exos can restore the repair function of skin cells. Notably, when compared with the transplantation of MSCs, MSC-Exos

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show comparable healing outcomes in various skin wound models, such as diabetic wounds, burns, and artificial skin flap transplantation [11–14].

From a clinical point of view, the administration of MSC-Exos can get rid of some potential risk of MSC transplantation such as the maldifferentiation or tumor formation of grafted cells [15, 16], making it a more attractive choice for regenerative wound therapy. Particularly, the storage and administration of MSC-Exos are convenient to practice. Based on these advantages, increasing researches have been carried out to explore the potential of MSC-Exos as an alternative to traditional stem cell transplantation. This review focuses on current research progress of MSC-Exos for skin wound treatment. After an overview of the isolation and basic properties of MSC-Exos, the influence of MSC-Exos on skin cell behaviors is detailed and the underlying mechanisms are summarized. Animal studies about the application for different kinds of skin wounds are systematically reviewed. To facilitate future clinical translation, challenges regarding the clinical application of MSC-Exos are discussed as well.

2 MSC-Exos: Characterization and properties

MSC-Exos are nano-size membranous vesicles with a round or oval morphology, usually 30–150 nm in diameter (Fig. 1). They express a set of common markers of exosomes (e.g., CD63, CD81 and tumor susceptibility gene 101 (TSG101)) [17]. Furthermore, several MSC markers such as CD90 and CD73 are also expressed on the surface of MSC-Exos [18]. It is noteworthy that MSC-Exos contain a variety of bioactive cargoes related to skin wound healing, including growth factors, noncoding RNAs and cytokines [19]. The ultracentrifugation of cell culture medium is the most common method to isolate MSC-Exos [18]. Other approaches such as the utilization of commercially available exosome isolation kits and the tangential flow filtration method are also reported in the literatures [20, 21]. Some variations in the yield, purity and protein abundance have been observed among different isolation methods [22, 23].

The proteomic profile of MSC-Exos differs significantly among cell sources [19, 24]. Hoang et al. reported that exosomes from different MSC sources showed obvious differences in the profile of growth factors. For instance, umbilical cord MSC-Exos contain transforming growth factor-β (TGF-β), whereas it is absent in bone marrow- or adipose tissue-derived MSC-Exos [19]. Particularly, the influence of MSC-Exos on skin cell function is relevant to the tissue origin. Compared with exosomes derived from umbilical cord- or adipose tissue-derived MSCs, those enriched from bone marrow-derived MSCs possessed a greater capability to promote the proliferation of fibroblasts [19]. In addition to tissue origin, cell culture condition greatly affects the property of MSC-Exos [25, 26]. In xeno-free culture medium, umbilical cord MSCs produce extracellular vesicles, which encompass exosomes and microvesicles, with a higher content of pro-angiogenetic miRNAs than those enriched from a commonly used medium [26]. Similarly, in another study, Kim et al. reported that human umbilical cord MSCs cultured in a serum-free chemically defined culture medium generated exosomes with higher levels of regeneration-related cytokines and less pro-inflammatory cytokines than those cultured with a serum containing medium [27]. To ensure the reproducibility and accuracy of researches, it is crucial to exclude other particles during the isolation of MSC-Exos, such as chylomicrons, ribonucleoproteins, and fetal bovine serum-derived exosomes.

Compared to traditional MSC transplantation, MSC-Exos have several advantages. Firstly, they possess a much smaller range of particle size (30–150 nm) than that of MSCs (with a diameter around 25 μm), and thus reduce the risk of vascular embolism which has been reported in the intravascular administration of

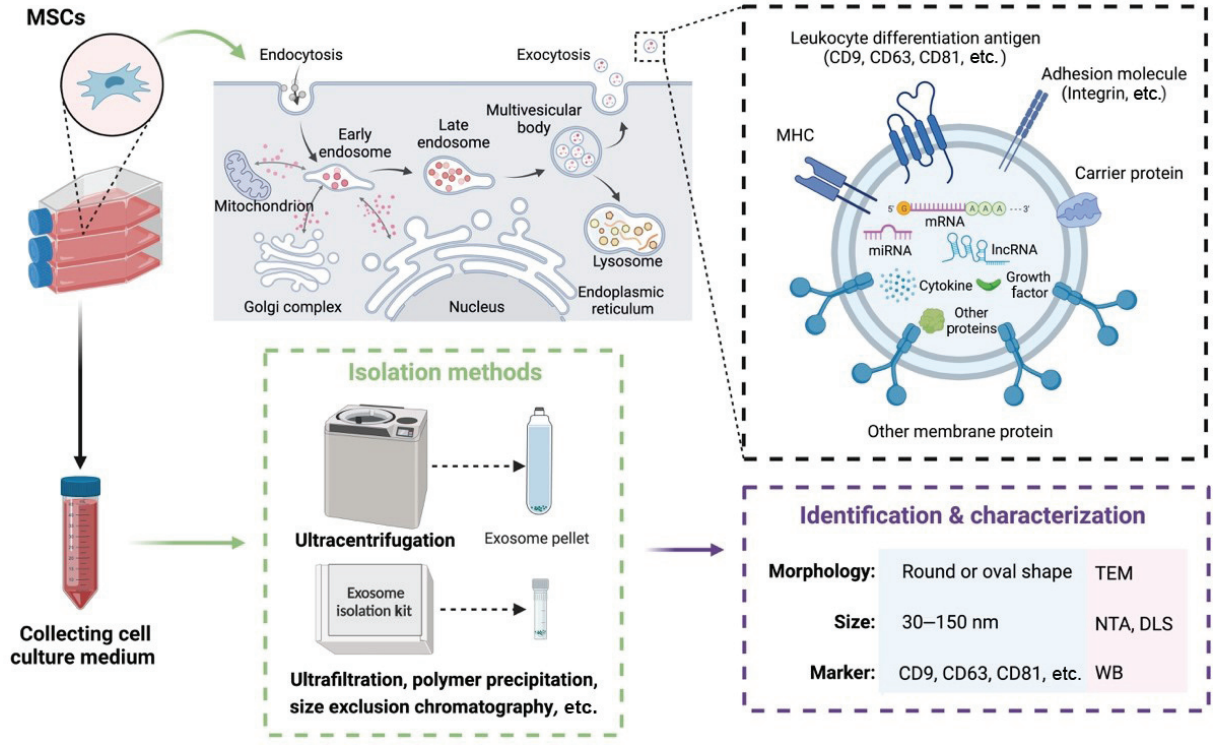

Figure 1 Schematic illustration of MSC-Exos and the isolation and characterization procedures. MSC-Exos are nano-size membranous vesicles with a round or oval morphology, containing diverse contents and a variety of membrane proteins such as leukocyte differentiation antigens, adhesion molecules, and carrier proteins. After the collection of cell culture medium, the dead cells and cell debris can be removed by low-speed centrifugation. Then, exosomes can be isolated by different methods, such as the ultracentrifugation method, the usage of commercially available exosome isolation kits, and other approaches. Afterwards, to identify MSC-Exos, the morphology, size and typical markers of exosomes should be characterized by different methods. TEM: Transmission electron microscope; NTA: Nanoparticle tracking analysis; DLS: Dynamic light scattering; WB: Western blot.

MSCs [28–31]. Secondly, MSC-Exos lack self-replicating capability; therefore, the potential risk of tumor formation or maldifferentiation of grafted cells is eliminated. It has been reported that murine MSCs underwent spontaneous transformation *in vitro* and formed sarcoma *in vivo* [32, 33]. Furthermore, several studies have noted the maldifferentiation of MSCs, which resulted in unfavorable ectopic tissue formation, such as ossification in infarcted myocardium [34], bone formation at the repair site of tendon [35], and maldifferentiation into glomerular adipocytes after intrarenal injection [36]. Thirdly, compared with the method for long-term storage of MSCs, more preservation methods are applicable for MSC-Exos. Besides cryopreservation that is frequently utilized in cell preservation [37], freeze-drying is suitable for exosome storage [38].

Due to the presence of various bioactive substances such as proteins and RNAs, the storage environment can alter the physicochemical and biological properties of exosomes, thereby affecting the therapeutic efficacy. At room temperature (37 °C), exosomes maintained in phosphate-buffered saline exhibited a significant decrease in the particle size within 24 h, which indicates a structural change or degradation [39]. It is widely believed that exosomes can be stored at −80 °C for long-term preservation [38, 40]; nevertheless, several studies reported that freezing at −80 °C changed the size, RNA content, protein content, and surface characteristics of exosomes [41, 42]. These studies highlight an urgent need to explore the applicable strategies for long-term storage of exosomes with good stability from multiple perspectives, including the size, morphology, exosomal cargoes, surface markers, and biological functions.

In view of the fact that exosomes are often delivered to skin wounds at room temperature, it is necessary to develop convenient methods that can preserve the properties of exosomes at room temperature. Recently, it is demonstrated that, with the addition of trehalose as a cryoprotectant, lyophilization is an effective method for room temperature storage of exosomes, showing good protection of protein and RNA content, the physicochemical characteristics, and pharmacokinetics properties [43]. However, long-term stability of lyophilized exosomes still needs further investigation.

3 Effects of MSC-Exos on skin cell behaviors

Fibroblasts, keratinocytes, endothelial cells, and immunocytes are all indispensable in normal skin wound healing. Understanding the influence of MSC-Exos on the biology of skin cells and revealing the underlying mechanisms are essential to enhance the repair ability of MSC-Exos. Through the transferring of bioactive cargoes (Fig. 2), MSC-Exos can alter the behaviors of skin cells through a variety of mechanisms (Table 1).

3.1 Fibroblasts

Fibroblasts play a central role in collagen deposition, ECM remodeling, and scar tissue formation during wound healing [67, 68]. *In vitro*, MSC-Exos can be internalized by fibroblasts in a time-dependent manner [69]; furthermore, they can stimulate the proliferation and migration of fibroblasts *in vivo* [14, 47, 70, 71], regulate the remodeling of ECM at the wound bed [72, 73], and reduce scarring [74, 75].

Multiple mechanisms are involved in the regulation of fibroblast behaviors by MSC-Exos. It is well documented that MSC-Exos can upregulate the expression of genes related to cell migration and proliferation, such as the *N-cadherin*, *Cyclin-1*, and *proliferating cell nuclear antigen* genes [76]. Also, they can activate several signaling pathways such as the Akt, extracellular regulated protein kinases (ERK) 1/2, and signal transducer and activator of transcription 3 (STAT3) signaling pathway to induce the expression of cell cycle-related genes [49]. By transmitting Jagged-1 to fibroblasts, fetal dermal MSC-Exos are found to activate the Notch signaling pathway to promote the migration of cells [50]. Additionally, MSC-Exos can transfer noncoding RNAs including microRNAs [44, 47, 70, 77] and lncRNAs (e.g., miR-135a [47], miR-21-5p [44] and H19 [52]) to promote the migration of fibroblasts.

At the early stage of wound healing, MSC-Exos can promote the synthesis of collagens in fibroblasts [73, 75], which is regulated by the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway [75]; meanwhile, they can inhibit the expression of matrix metalloproteinases, which reduce the degradation of ECM. At the late stage of wound healing, MSC-Exos improve the deposition of collagens by increasing the expression of metalloproteinase-3 [74]. To attenuate hypertrophic fibrosis, miR-192-5p in adipose MSC-Exos can directly target to receptor of interleukin-17A (IL-17A, IL-17RA) that downregulates the expression of pro-fibrotic proteins in hypertrophic scar fibroblasts [48]. Additionally, MSC-Exos can alleviate scar tissue formation by reversing the transdifferentiation of dermal fibroblasts into

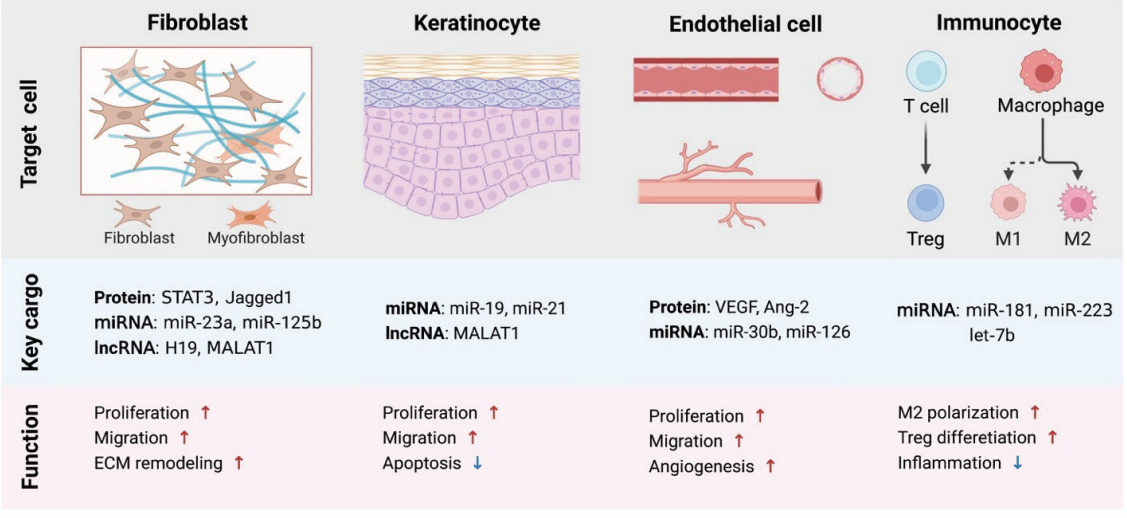

Figure 2 MSC-Exos improve the repair ability of skin cells through the transferring of bioactive cargoes. MSC-Exos contain a variety of bioactive cargoes such as growth factors and miRNAs that can modulate the behaviors of skin cells. For instance, MSC-Exos can promote the proliferation and migration of dermal fibroblasts, keratinocytes, and endothelial cells, enhance the angiogenesis of endothelial cells, promote the polarization of macrophages toward an M2 phenotype, and induce Treg cell differentiation to alleviate the excessive inflammatory reaction. Ang-2: Angiopoietin-2.

Table 1 Key cargoes of MSC-Exos with the ability of modulating of skin cell behaviors[a]

Target cell	Cargo	Molecule	Biological function	Ref.
Fibroblast	miRNA	miR-19b	Rescue cell viability and enhance cell migration in oxidative stress	[14]
		miR-21-5p	Promote cell proliferation and migration by targeting SPRY2	[44]
		miR-21, -23a, -125band -145	Suppress myofibroblast formation by inhibiting the TGF-β2/Smad2 signaling pathway	[45]
		miR-126-3p	Promote cell proliferation and migration	[46]
		miR-135a	Promote cell migration by downregulating LATS2	[47]
		miR-192-5p	Target IL-17RA to reduce the expression of pro-fibrotic proteins	[48]
	Protein	STAT3	DNA-binding activity	[49]
		Jagged1	Activate the Notch signaling pathway to enhance cell proliferation and migration	[50]
	lncRNA	MALAT-1	Reduce cell apoptosis and attenuate the impairment of cell proliferation and migration in oxidative stress	[51]
		H19	Inhibit miR-19b to promote cell proliferation and migration	[52]
Keratinocyte	miRNA	miR-19b	Target CCL1 to active the TGF-β signaling pathway; reduce oxidative stress-induced apoptosis; promote cell migration	[14]
		miR-21	Enhance cell migration and proliferation	[53]
		miR-150-5p	Target PTEN to activate the PI3K/AKT signaling pathway; promote cell proliferation and migration and reduce cell apoptosis in oxidative stress	[54]
	lncRNA	MALAT-1	Relieve cell apoptosis and promote the proliferation and migration of cells under oxidative stress	[51]
Endothelial cell	miRNA	miR-21-5p	Enhance cell proliferation, migration and angiogenesis by targeting SPRY2	[44]
		miR-30b	Target DLL4 to enhance the formation of tube-like structure	[55]
		miR-125a	Inhibit DLL4 to promote angiogenesis	[56]
		miR-125a-3p	Inhibit PTEN to promote cell viability, migration and angiogenesis	[57]
		miR-126	Enhance angiogenesis	[58]
		miR-126-3p	Enhance cell migration by activating the PI3K/AKT and MAPK/ERK signaling pathway	[46]
		miR-146a	Promote angiogenesis	[59]
		miR-135b-5p, -499a-3p	Promote angiogenesis	[60]
	circRNA	mmu_circ_0000250	Absorb miR-128-3p and upregulate SIRT1 to restore the function of endothelial cells in high glucose	[61]
	Protein	Ang-2	Promote cell migration and tube formation	[62]
		VEGF	Enhance angiogenesis	[58]
		DMBT1	Promote cell proliferation, migration and angiogenesis	[63]
Immunocyte	miRNA	miR-181c	Target TLR4 to reduce LPS-induced inflammation in macrophages	[64]
		miR-223	Target pknox1 to promote M2 macrophage polarization	[65]
		let-7b	Promote M2 macrophage polarization in inflammatory conditions	[66]

[a]PTEN: phosphatase with tensin homology; Ref.: reference.

myofibroblasts [48]. For instance, they suppress myofibroblast formation through the inhibition of TGF-β1/Smad2/3 signaling pathway [45, 48, 78]. Some exosomal microRNAs (miR-21, -23a, -125b, and -145) play critical roles in the suppression of myofibroblast formation [45].

When confronted with harsh conditions, MSC-Exos exert a protective effect on fibroblasts [79–82]. For instance, they can alleviate the hydrogen peroxide (H_2O_2)-induced damage to fibroblasts [51, 79]. Under the condition of H_2O_2-induced oxidative stress, the down-regulated expression of longevity protein sirtuin 1 (SIRT1) leads to the senescence of fibroblasts. However, administration of MSC-Exos effectively reverses this cellular senescence process [79]. In other studies, MSC-Exos improve the survival of fibroblasts under oxidative stress conditions [14, 79]. He et al. reported that MSC-Exos can transmit lncRNA metastasis-associated lung adenocarcinoma tran 1 (MALAT1) to activate the Wnt/β-catenin signaling pathway in fibroblasts, which confers a significant protection against H_2O_2-induced damage [51]. Apart from oxidative stress, MSC-Exos can protect fibroblasts from ultraviolet damage [80]. They contain a variety of growth factors for skin rejuvenation [73], which can penetrate the skin tissues and restore the expression of genes interfered by ultraviolet radiation. They may reduce the level of metalloproteinases, increase the expression of type I collagen, and protect the skin from ultraviolet-induced photoaging [81, 82]. In diabetic wounds, the regenerative potential of fibroblasts decreases sharply. However, Shabbir et al. reported that MSC-Exos could enhance the proliferation and migration of fibroblasts isolated from diabetic patients [49]. Besides, through the activation of the protein kinase B (AKT) signaling pathway, MSC-Exos can reduce heat stress-induced apoptosis in fibroblasts [12].

3.2 Keratinocytes

Re-epithelization is essential to restore the integrity of skin after injury, which involves the proliferation and migration of keratinocytes at the wound bed [83]. Previous studies have shown that MSC-Exos not only improve the repair function of keratinocytes *in vitro* but also enhance the wound re-epithelization process *in vivo* [11, 84–87]. They can effectively alleviate the oxidative stress-induced apoptosis in keratinocytes [11, 14, 51, 88],

promote the antioxidant activity of cells, and reduce the oxidative responsiveness in H_2O_2-stimulated keratinocytes [89].

After the treatment with MSC-Exos, various signaling pathways, such as the Wnt/β-catenin signaling pathway [88], the AKT/hypoxia inducible factor (HIF)-1α signaling pathway [86] and the PI3K/AKT signaling pathway [53] are activated to promote the proliferation and migration of keratinocytes. Interestingly, after a regenerative response, MSC-Exos will inhibit the excessive proliferation of keratinocytes at the late stage of wound healing [90]. Zhang et al. reported that, when keratinocytes were at a high cell density *in vitro*, MSC-Exos could inhibit the proliferation of keratinocytes through the inhibition of the Wnt/β-catenin signaling pathway, which was medicated by exosomal 14-3-3ζ [90].

With a therapeutic effect on keratinocytes, noncoding RNAs are critical components of MSC-Exos [14, 51, 53, 91]. Cao et al. reported that miR-19b derived from human adipose MSC-Exos could promote the proliferation and migration of HaCaT cells by regulating the chemokine CC motif ligand 1 (CCL1)/TGF-β signaling pathway [14]. Similarly, in a H_2O_2-induced damage model, Shen et al. observed that miR-93-3p in human bone marrow MSC-Exos could downregulate the expression of apoptotic peptidase activating factor 1 in HaCaT cells, which promoted the proliferation and migration of cells [91]. In another study, He et al. demonstrated that MALAT1, a lncRNA in adipose MSC-Exos, could activate the Wnt/β-catenin signaling pathway in HaCaT cells, which enhanced the proliferation and migration of cells [51].

Several studies have demonstrated that MSC-Exos are potent at protecting keratinocytes from environmental damages, such as the photoaging conditions and the heat-induced injuries [12, 85]. In an ultraviolet light-induced photoaging model, Liu et al. observed that human umbilical cord MSC-Exos could inhibit the photoaging of HaCaT cells, which was evidenced by a reduced level of intracellular reactive oxygen species (ROS), fewer apoptotic cells, reduced cell senescence, and increased expression of collagen type I [85]. Through the activation of the AKT signaling pathway, human umbilical cord MSC-Exos are capable of reducing the heat stress-induced apoptosis in keratinocytes; meanwhile, they can promote the proliferation and migration of keratinocytes, which is mediated by the Wnt4/β-catenin signaling pathway [12].

3.3 Endothelial cells

As the constitutive cellular component of blood vessels, endothelial cells are key player in the process of wound angiogenesis. The formation of new blood vessels from preexisting vasculature relies considerably on the proper interactions between endothelial cells and the local microenvironment [92]. It is known that, in human organs, MSCs reside in a perivascular niche [93] and are capable of facilitating the revascularization of injured tissues [94]. Studies have reported that MSCs can secrete exosomes to communicate with endothelial cells *in vitro*. After taken up by endothelial cells, MSC-Exos could promote the proliferation, migration, and tube-formation of endothelial cells [25, 56, 95–98], which is considered as a result of the activation of multiple cell signaling pathways, such as the mitogen-activated protein kinase (MAPK)/ERK [46], PI3K/AKT [46, 64, 99, 100], and AKT/endothelial nitric oxide synthase (eNOS) pathway [101].

In endothelial cells, the internalization of MSC-Exos increased the expression of pro-angiogenic factors (e.g., vascular endothelial growth factor (VEGF) [58, 96], Ang1 [56] and Flk1 [56]), cell proliferation related proteins (e.g., cyclin A2 [96], cyclin D1 [96] and cyclin D3 [102]), and chemokines (e.g., C-X-C motif chemokine ligand 12 (CXCL12) [96]). However, the expressions of anti-angiogenic genes (e.g., *Vash1* and *Thrombospondin-1 (TSP1)* [56]) were down-regulated. Several studies have identified the key molecules underlying the pro-angiogenic effect of MSC-Exos [62, 63]. After screening the proteomic profile of exosomes derived from urine-derived stem cells, Chen et al. discovered that deleted in malignant brain tumors 1 (DMBT1) was highly expressed in the exosomes; particularly, the inhibition of DMBT1 markedly attenuated the pro-angiogenic ability of exosomes [63]. Likewise, in human umbilical cord MSC-Exos, Liu et al. noted that the overexpression of angiopoietin-2 protein improved the pro-angiogenic effect of exosomes, whereas knockdown of the angiopoietin-2 protein abrogated the therapeutic effect [62].

Apart from proteins, exosomal microRNAs have been verified to promote the angiogenic performance of endothelial cells [44, 55, 56, 100, 103, 104]. To modulate the behaviors of endothelial cells, exosomal microRNAs can trigger the intracellular signaling pathways. For example, to enhance the angiogenic potential of endothelial cells, exosomal miR-30b and miR-125a can downregulate the expression of delta-like 4 (DLL4), a ligand of Notch signaling pathway with anti-angiogenic function [55, 56]. By transferring miR-21-5p, bone marrow MSC-Exos can promote the proliferation and migration of endothelial cells, which is mediated by the downregulation of sprouty RTK signaling antagonist 2 (SPRY2) protein, a negative regulator in cell proliferation and migration [44]. Ding et al. demonstrated that miR-126 in deferoxamine preconditioned bone marrow MSC-Exos could activate the PI3K/AKT signaling pathway in endothelial cells to stimulate their angiogenesis activities [100]. Other microRNAs enriched in MSC-Exos such as miR-135b-5p [60], miR-499a-3p [60], miR-125a-3p [57], miR-125b [105] and miRNA-21-5p [106] also promote the proliferation and migration of endothelial cells.

3.4 Immunocytes

Normal wound healing will be significantly delayed if macrophages are depleted prematurely [65, 107, 108]. The wound healing effect of MSC-Exos relies largely on their ability to modulate the phenotype of macrophages. After the internalization in target cells, MSC-Exos can modulate the expression of pknox1, a transcription factor, in macrophages, which further inhibits the induction of pro-inflammatory pathways and promotes the anti-inflammatory responses. The polarization of macrophages from M1 to M2 phenotype results in a reduced expression of TNF-α and an increased level of IL-10 [65]. In monocytes, compared with the lipopolysaccharide (LPS)-induced M1 polarization of cells through the Toll-like receptor (TLR) signaling pathway, exosomes from huES9.E1 human ESC derived MSCs activate the TLR signaling pathway to promote the M2 polarization of cells, which exerts the immunosuppressive effects [109]. Furthermore, MSC-Exos can promote the Treg differentiation of CD4 positive T cells, thereby weakening the activation of immune system [109, 110]. A broad array of bioactive molecules, such as let-7a, are associated with the immunomodulation function of MSC-Exos [66].

4 Wound healing mechanism of MSC-Exos

As a complex biological process to restore injured skin, wound healing can be divided into four stages, including hemostasis, inflammation, proliferation, and remodeling. They are overlapped and tightly coordinated to secure skin function. During the wound healing process, many cell types interact with each other in sophisticated manners. As described in previous section, MSC-Exos allow for the transferring of therapeutic cargoes to damaged skin, which influences the wound healing progress by changing the performance of resident cells. They can modulate wound

inflammation, activate the proliferation, migration and function of various skin cell types, and reduce scarring. Multiple mechanisms are involved in the repair of MSC-Exos, which can be broadly categorized into the following: (1) Dampening excessive inflammation, (2) promoting wound angiogenesis, (3) enhancing wound re-epithelization, and (4) modulating ECM deposition (Fig. 3).

4.1 Hemostasis stage

When a skin injury occurs, the body promptly controls bleeding to establish a favorable environment for wound repair. The platelets initiate the formation of blood clots that protect the injured site. No direct evidence has showed the involvement of MSC-Exos in blood clotting. Further studies are needed to uncover their roles in the hemostasis stage.

4.2 Inflammatory stage

After hemostasis, the wounds initiate the inflammatory stage to clear bacteria and cell debris. Neutrophils, monocytes, and macrophages are major players in this stage [111]. For instance, neutrophils release chemotactic factors to attract monocytes; macrophages secret growth factors, chemokines and cytokines to activate the next phase of wound healing. Regulation of inflammation is crucial in wound repair. Moderate inflammation is essential for normal wound repair, while prolonged inflammation inhibits normal wound healing and can lead to chronic wounds or scarring [112, 113]. It is critical to change the excessive inflammatory microenvironment of chronic wounds, which helps to achieve a transition from the inflammation stage to the proliferative phase [114, 115].

Macrophages are crucial in normal skin wound healing, which should appropriately switch from the M1 to M2 phenotype. M2 macrophages promote wound repair in the later stages due to their anti-inflammatory properties. Containing a rich source of anti-inflammatory mediators, MSC-Exos show a potent regulatory activity on immune cells [116]. Particularly, MSC-Exos can induce macrophage polarization towards a M2 phenotype at the inflammation stage [65, 117–119], which secretes the anti-inflammatory factors such as IL-10 and TGF-β to facilitate wound healing.

4.3 Proliferative stage

During the proliferative stage, the angiogenesis and vascularization of endothelial cells support the oxygen and nutrition need of repair cells at the wound site. Fibroblasts from surrounding tissues migrate into the injured site, produce extracellular matrix proteins to strengthen the newly formed tissue, differentiate into myofibroblasts to facilitate wound contraction, and secrete growth factors to activate the migration and proliferation of keratinocytes. Meanwhile, keratinocytes migrate to the wound bed to generate new epidermis. When the migration of keratinocytes is stopped by contact inhibition, the wound re-epithelization process is completed.

MSC-Exos are reported to promote the proliferation, migration, and angiogenesis of endothelial cells *in vitro*, resulting in an increased expression of angiogenic factors (hepatocyte growth factor, VEGF, etc.) and more tube formation [49, 120, 121]. Moreover, the pro-angiogenic effect of MSC-Exos has been demonstrated *in vivo*, showing more new blood vessel formation and more blood vessel maturation at the wound site [60, 120, 121]. For dermal fibroblasts and keratinocytes, MSC-Exos also enhance their proliferation, migration and ECM production (collagen, elastin, fibronectin, etc.) [11, 122, 123]. To sum up, at the proliferative phase of wound healing, MSC-Exos stimulate vascular regeneration and the function of keratinocytes and fibroblasts [47, 124], resulting in better granulation tissue formation and faster wound re-epithelization.

4.4 Remodeling stage

The remodeling stage begins two to three weeks after injury and can last for one or more years. In this stage, the repair tissue is remodeled by cell apoptosis (fibroblast, myofibroblast, and other skin cells) and the degradation of extracellular matrix [125]. Controlled release of proteases from skin cells contributes to the degradation of collagen fibers. Uncontrolled accumulation of myofibroblasts at the wound sites can lead to scar tissue formation [126]. Currently, there is not effective treatment for adverse scarring such as hypertrophic scars and keloids [127], and thus scar prevention or reduction is a major medical issue to solve in wound healing.

MSC-Exos are beneficial to reduce scarring. Fibroblasts are of ultimate importance in the remodeling stage, since they are

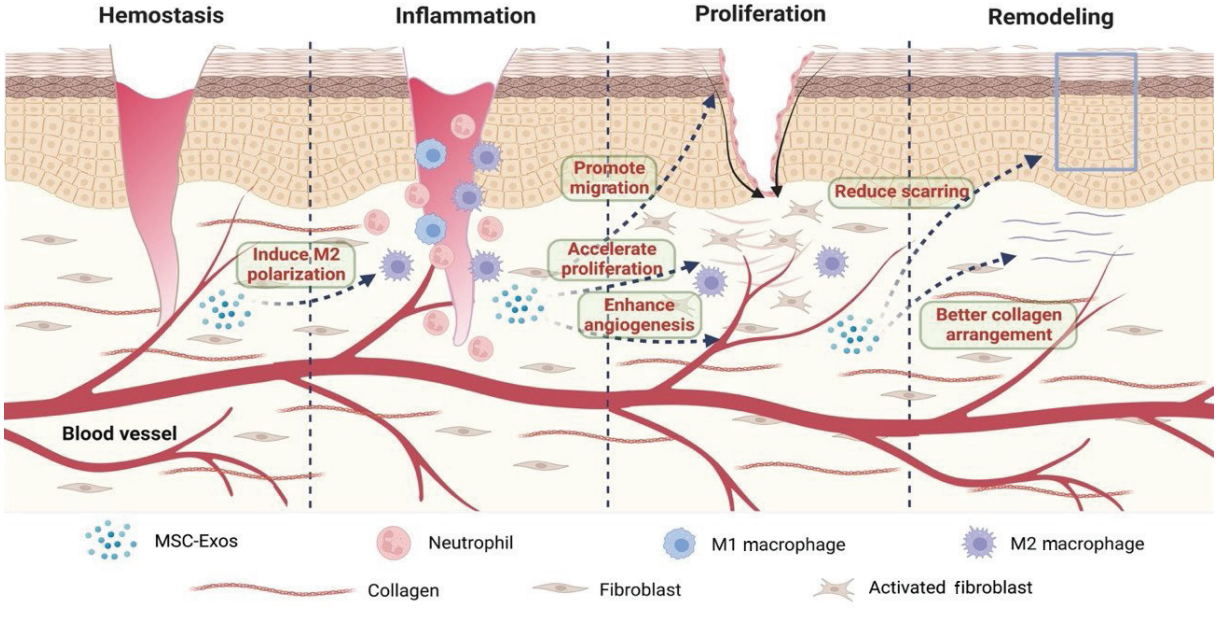

Figure 3 Schematic illustration of the wound healing mechanism of MSC-Exos. MSC-Exos have multiple functions during skin wound healing process. At the inflammation stage, MSC-Exos can promote M2 polarization to alleviate excessive inflammation. At the proliferation stage, MSC-Exos accelerate wound angiogenesis, re-epithelization and granulation tissue formation. At the remodeling stage, MSC-Exos improve the collagen arrangement and reduce scarring.

responsive for collagen deposition. If fibroblasts do not function properly or produce excessive collagen fibers, they may eventually lead to scar tissue formation. At the remodeling phase of wound healing, MSC-Exos increase the ratio of type III collagen to type collagen I in the repair tissue [13, 74]. The antifibrotic properties of MSC-Exos are important for scar prevention [48, 128], but how they regulate the function of fibroblasts is not fully understood. It has been illustrated that exosomes derived from adipose MSCs can attenuate the collagen deposition, the trans-differentiation of fibroblasts-to-myofibroblasts, and the hypertrophic scar formation in mice, which was mediated through the miR-192-5p/IL-17RA/Smad axis [48].

5 Applications of MSC-Exos for skin wound treatment

5.1 Normal full-thickness skin wounds

Despite of some regenerative potential of human skin, large full-thickness skin wounds remain a great challenge even in patients without any serious chronic diseases. Full-thickness skin wounds cannot heal themselves like the epidermal lesions, and often result in the formation of scars which lack skin appendages (sweat glands, sebaceous glands, etc.) and subsequently their pivotal physiological functions. Due to the ease of operation and the convenience of observation, full-thickness skin wounds created in normal animal models have been widely used for assessing the healing potential of stem cells, cell-derivates, and biomaterials [129].

When delivered to normal full-thickness skin wounds, MSC-Exos show remarkable repair capability, which has been demonstrated in many animal studies (Table 2). Typically, the administration of MSC-Exos, either alone or along with biomaterials, could significantly enhance the process of wound healing [11, 47, 48, 130], resulting in faster re-epithelization [75, 130], promoted angiogenesis [11, 75], better restoration of skin barrier function [11], and reduced scarring [11, 71]. For instance, Zhang et al. reported that adipose MSC-Exos significantly accelerated wound healing in a mouse model of full-thickness incision wound [75]. Likewise, in a rat full-thickness skin wound model, Zhang et al. observed that umbilical cord blood MSC-Exos accelerated wound closure, ameliorated scar tissue formation, improved skin appendage regeneration, regulated the distribution of collagen fibers, and suppressed the excessive formation of myofibroblasts (Fig. 4), which were closely related to the miR-21-5p- and miR-125b-5p-mediated inhibition of the TGF-β receptor [131].

At the early stage of wound healing, MSC-Exos can alleviate wound inflammation through the downregulation of pro-inflammatory factors (TNF-α, IL-1β, IL-6, etc.) and the upregulation of anti-inflammatory factors such as IL-10 [124, 128]. To enhance the formation of granulation tissues, they increase the expression of collagen I and III [76]. However, at the ECM remodeling stage, MSC-Exos can inhibit excessive collagen synthesis [76], which reduces scarring [11, 71]. Meanwhile, they increase the ratio of collagen III to collagen I [74], which results in a better arrangement of the ECM in the repair tissue [132]. Additionally, to reduce the fibrosis of wounds, MSC-Exos can inhibit the aggregation of myofibroblasts [45, 55, 74] and reduce the expression of α-smooth muscle actin (α-SMA), which is correlated with a lower ratio of TGF-β3 to TGF-β1 around the wounds [55, 74]. Importantly, MSC-Exos can improve the arrangement of collagens [132], resulting in a neatly arranged distribution similar to that of normal skin.

Several molecular mechanisms have been identified to regulate the repair of full-thickness skin wounds *in vivo* [14, 47, 53]. For instance, MSC-Exos could increase the level of Smad7, which promoted wound healing by inhibiting the TGF-β/Smad signaling pathway [84]. Furthermore, MSC-Exos contain a plethora of miRNAs with repair function. These include miR-19b, which regulates the TGF-β signaling pathway to promote the wound healing process [14]; miR-135a, which inhibits the expression of large tumor suppressor 2 (LATS2) to increase the migration of fibroblasts [47]; and miR-21, which enhances the migration and proliferation of keratinocytes through the upregulation of MMP-9 expression [53]. Zhao et al. proposed that the effect of MSC-Exos on the arrangement of wound collagens was mainly mediated by the exosomal RNA components [77]. Nevertheless, other studies have reported the critical role of exosomal proteins in this process. For example, Jiang et al. demonstrated that the overexpression of tumor necrosis factor stimulated gene 6 (TSG-6) protein in MSC-Exos could significantly suppress scar tissue formation, which was achieved through the reduction of wound inflammation and the inhibition of collagen deposition [128].

5.2 Burns

Large deep burn wounds, one of the most extensive soft tissue injuries to human being, can lead to severe organ dysfunction and high mortality, with about 300,000 deaths worldwide each year [145]. They are often challenging to treat because of the prone to severe infection and excessive inflammation. Although skin grafts effectively reduce the deaths from infection, burn patients usually suffer from chronic pain for a long period of time [146, 147]. Additionally, burn wounds pose an enormous economic burden on the health care system. The cost for caring burn patients takes more than $573 million in the United States annually [148, 149].

Despite of much progress in reducing the mortality of burn patients, current treatment is far from ideal, necessitating the search for cost-effective and reliable treatment to overcome the limitations. MSCs and their derivates, especially MSC-Exos have gained increasing interest, as they possess excellent anti-inflammatory and pro-healing abilities [150]. In recent years, several animal studies have revealed the positive effect of MSC-Exos on burn wound treatment. For example, in a deep second-degree burn model, Liu et al. observed that subcutaneous injection of the exosomes derived from human umbilical cord MSCs obviously improved the angiogenesis, re-epithelization, and closure of wounds, which was mediated by the delivery of angiopoietin-2 protein [62]. In another study, Li et al. demonstrated that systemic administration of human umbilical cord MSC-Exos via tail vein injection significantly alleviated burn-induced excessive inflammation in rats (Fig. 5(a)), which was related to the transferring of exosomal miR-181c that can suppress the toll-like receptors 4 (TLR4) signaling pathway in burn wounds. Furthermore, *in vitro* studies revealed that human umbilical cord MSC-Exos significantly downregulated LPS-induced TLR4 protein expression in macrophages, which suppressed the LPS-induced macrophage inflammation [64].

Besides the inhibition of severe inflammation, MSC-Exos accelerate burn wound healing by promoting vascular regeneration, which has been confirmed by an increased expression of CD31 or α-SMA at the wounds [60, 62, 102]. Zhang et al. reported that the activation of the Wnt4/β-catenin signaling pathway was critical in the proangiogenic effect of human MSC-Exos [102]. Interestingly, exosomes isolated from blue light pre-stimulated MSCs showed enhanced angiogenetic potential (Fig. 5(b)) [60]. In addition to the pro-angiogenetic ability, MSC-Exos can reduce the heat stress-induced cell apoptosis at burned skin, which is associated with the activation of the AKT signaling

Table 2 The application of MSC-Exos for normal full-thickness skin wounds[a]

Cell origin	Animal	Dose	Delivery	Condition/scaffold	Outcome	Ref.
Umbilical Cord	Mouse	NA	Local injection	Hydrogel	Reduce scarring and myofibroblast formation	[45]
	Rat	100 μg	Intravenous injection	Oxide nanoparticles pretreatment	Reduce scarring; promote re-epithelization, angiogenesis and collagen maturity	[96]
	Rat	6 μg	SA	Genipin crosslinked hydrogel	Promote re-epithelization, angiogenesis, skin appendage regeneration, and collagen deposition; reduce inflammation	[133]
	Rat	20 μg	Intravenous injection	None	Promote the regeneration of skin appendages, vessels and nerves; suppress scarring	[131]
	Mouse	100 μg	Local administration	None	Promote wound closure and skin nerve fiber regeneration	[134]
	Rat	30 μg	SI	3D spinner flask culture	Accelerate re-epithelization and granulation tissue formation	[124]
	Mouse	100 μg	SA	Silk fibroin/sericin hydrogel	Promote angiogenesis and scarless wound healing	[135]
	Mouse	100 μg	SI	None	Promote re-epithelization and collagen synthesis; increase wound healing	[136]
	Mouse	NA	SA	Gelatine methacryloyl	Promote cell proliferation, re-epithelization, angiogenesis, and collagen deposition; reduce inflammation	[137]
Bone marrow	Dog	NA	SI	None	Promote wound re-epithelization, collagen synthesis, angiogenesis, and skin appendages regeneration	[138]
	Mouse	200 μg	Intravenous injection	None	Accelerate wound healing, promote angiogenesis and collagen deposition	[65]
	Mouse	100 μg	SI	Modifying TSG-6	Promote scarless healing; reduce wound inflammation	[128]
	Rat	250 μg	SI	None	Promote wound angiogenesis and skin appendage regeneration; increase wound healing rate	[84]
	Rat	100 μg	SI	Stimulation with Fe_3O_4 nanoparticles and a static magnetic field	Enhance re-epithelization, angiogenesis and collagen deposition; reduce scarring; regenerate sebaceous gland and hair follicle	[44]
	Mouse	200 μg	SI	Modifying miR-126	Promote scarless wound healing; enhance wound angiogenesis	[139]
Adipose tissue	Mouse	200 μg	Intravenous injection	None	Promote scarless wound healing; inhibit myofibroblast differentiation	[74]
	Mouse	200 μg	SI	None	Promote wound angiogenesis, re-epithelization, and scarless healing	[75]
	Mouse	100 μg	SI	None	Promote granulation formation; reduce inflammation; promote wound healing	[14]
	Mouse	400 μg	NA	None	Promote wound healing	[86]
	Mouse	NA	SI	None	Accelerate wound healing; reduce inflammation; promote collagen synthesis and epidermis tissue formation	[52]
	Rat	100 μg	SI	None	Promote re-epithelization, collagen deposition; reduce inflammation	[140]
	Mouse	200 μg	SA	None	Promote re-epithelization, angiogenesis, and collagen synthesis; restore skin barrier function; reduce inflammation and scarring	[141]
	Rat	100 μg	SI	Modifying miR-146a	Reduce scarring; enhance angiogenesis	[142]
Fetal skin	Mouse	200 μg	SI	None	Promote cell proliferation, re-epithelization and ECM deposition	[50]
Teeth	Mouse	10 mg/kg	SI	LPS-induced wounds	Reduce inflammation; relieve itch response during wound healing	[143]
Placenta	Rat	50 μg	Local injection	None	Reduce scarring; enhance angiogenesis and skin appendage regeneration	[144]

[a]NA: not available; SA: smearing administration.

pathway [12]. Additionally, MSC-Exos can promote the proliferation and function of keratinocytes and fibroblasts around the burn wounds, thus enhancing the wound re-epithelialization and collagen deposition [12].

Excess collagen deposition results in a pathologic scar, which underlies post-burn physical and psychosocial morbidity. To improve the rehabilitation of burn patients and enhance their reintegration into society, a few studies have explored the possibility of MSC-Exos for post-burn scar treatment. Recently, Yuan et al. demonstrated that adipose MSC-Exos with the overexpression of miR-29a reduced excessive scar formation in burn wounds (Fig. 5(c)), which was associated with the inhibition of the TGF-*β*2/Smad3 signaling pathway, an important regulator in pathological scar formation [151].

5.3 Diabetic wounds

Diabetes is a multifaceted metabolic disease with high incidence, which has become a great health and economic concern worldwide. It is estimated that 20%–35% of diabetic patients will develop chronic wounds in their lifetime [152], and approximately one-third of the medical cost of diabetes is attributed to the management of chronic wounds [153], which can lead to amputation, disability, and even early mortality. Unlike the local microenvironment of normal skin wounds, diabetic wounds show

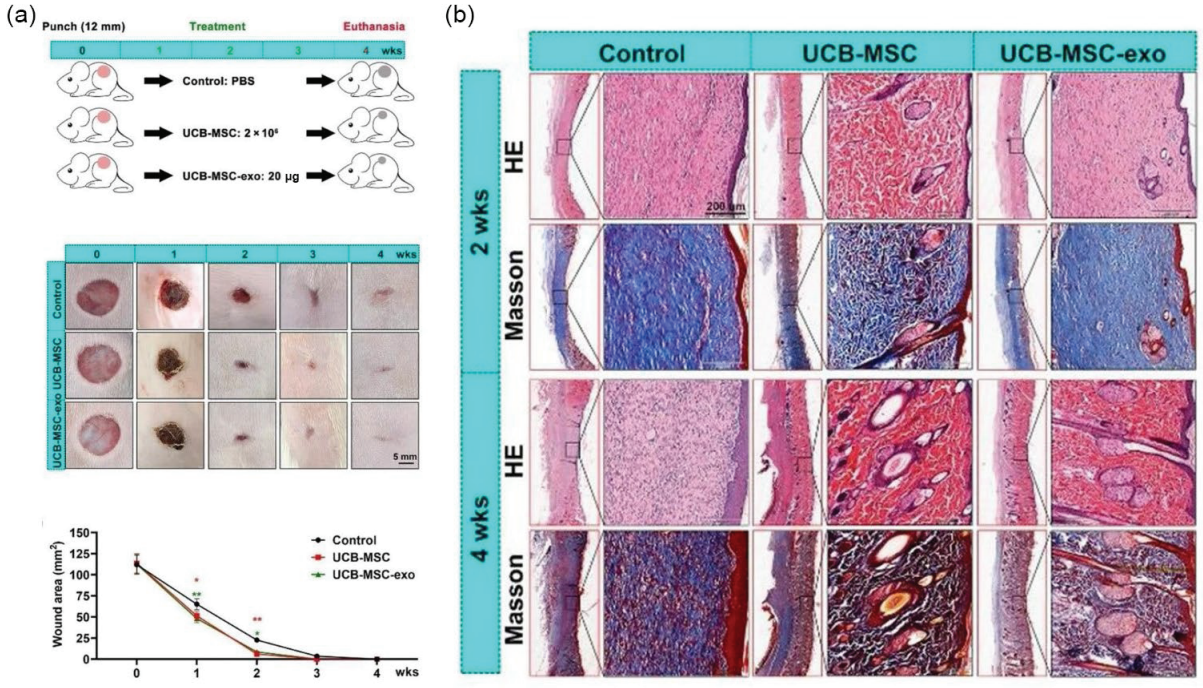

Figure 4 Umbilical cord blood MSC-Exos enhanced the healing and suppressed scar tissue formation in normal full-thickness skin wounds of rats. (a) Schematic illustration of the animal experiment. Full-thickness skin wounds were created on the dorsal region of normal rats. The rats received tail vein injection every week: PBS (control group), UCM-MSCs (2×10^6 cells), or UCB-MSC-exo (20 μg). Morphological changes during the wound healing process were observed, and the changes in the wound area were compared. Scale bar: 5 mm; $^*P < 0.05$; $^{**}P < 0.01$. (b) Hematoxylin and eosin (HE) and Masson staining of the healing skin, which revealed that UCB-MSC-exo improved regenerative wound healing in rats, showing more appendages (HE staining), less collagen fibers (blue in Masson staining), and fewer myofibers (red in Masson staining) than those in the control group. Scale bar: 200 μm. Reproduced with permission from Ref. [131], © Zhang, Y. et al. 2021.

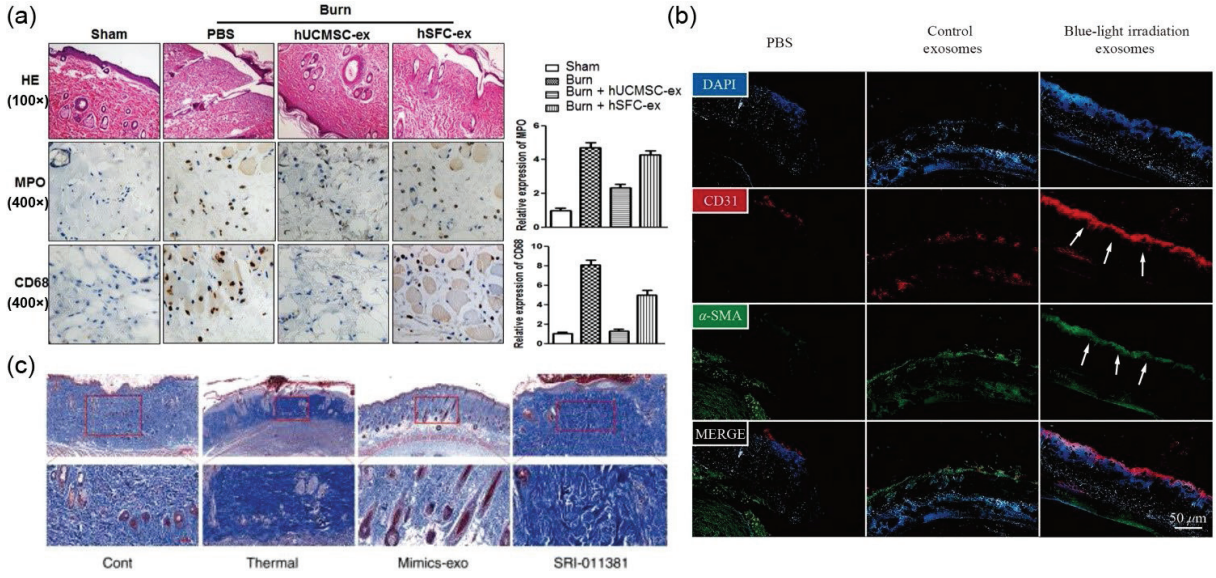

Figure 5 MSC-Exos enhanced burn wound healing and reduced scarring. (a) MSC-Exos suppressed burn-induced excessive inflammation. The histological micrographs show that the inflammatory cells in burn rats, such as the neutrophils (MPO) and macrophages (CD68) were much more abundant than the sham group. Administration of human umbilical cord MSC-Exos (hUCMSC-ex) significantly reduced the number of inflammatory cells. hSFC-ex, exosomes derived from human skin fibroblasts. Reproduced with permission from Ref. [64], © Li, X. et al. 2016. (b) MSC-Exos promoted wound angiogenesis in a rat model of second-deep burn injury. Representative immunofluorescence staining images show the increased expressions of CD31 and α-SMA in the wound area. Scale bar: 50 μm. Reproduced with permission from Ref. [60], © Yang, K. et al. 2019. (c) MSC-Exos reduced scar tissue formation in burn wounds. Masson staining reveals that miR-29a-overexpressing adipose MSC-Exos reduced scarring in burn wounds. Scale bars in the upper panel: 200 μm, in the lower panel: 50 μm. Cont: control; mimics-exo: miR-29a overexpressed adipose MSC-Exos; SRI-011381: mimics-exo co-treated with TGF-β agonist SRI-011381 hydrochloride. Reproduced with permission from Ref. [151], © Yuan, R. et al. 2021.

distinct properties, typically with severe infection, prolonged inflammation, impaired wound angiogenesis, increased degradation of ECM and growth factors, and inferior repair function of resident cells, all of which are closely associated with the poor healing outcome [112, 154].

A number of preclinical studies have revealed that MSC-Exos provide a novel powerful therapeutic tool to promote the healing of diabetic wounds (Table 3). Generally, the administration of MSC-Exos reduces the infiltration of inflammatory cells at the wound bed [66, 155], promotes the polarization of macrophages towards an M2 phenotype [13, 156], and stimulates the progress of wound healing from the inflammation stage to the proliferation stage [156]. The level of pro-inflammatory factors (IL-1β, IL-6, TNF-α, etc.) at the wounds is reduced after the treatment of MSC-

Table 3 The application of MSC-Exos for diabetic wounds[a]

Cell origin	Animal	Dose	Delivery	Pretreatment	Scaffold	Outcome	Ref.
Synovial membrane	Rat	NA	SA	None	Hydroxyapatite/chitosan hydrogel	Promote wound re-epithelization, angiogenesis, collagen synthesis, and the regeneration of follicles and sebaceous glands	[46, 99]
Gingival tissue	Rat	150 μg	SA	None	Chitosan/silk hydrogel sponge	Enhance re-epithelization, angiogenesis, nerve regeneration, and collagen deposition	[163]
	Mouse	100 μg	SA	None	Microsphere	Promote wound re-epithelization, angiogenesis and collagen deposition	[164]
Urine	Mouse	200 μg	SI	None	None	Promote wound angiogenesis, re-epithelization, collagen deposition, and the regeneration of follicles	[63]
Bone marrow	Rat	100 μg	SI	Deferoxamine	None	Accelerate wound closure; enhance angiogenesis	[100]
	Rat	NA	Local injection	Atorvastatin	None	Promote re-epithelization, ECM remodeling and vascularization	[103]
	Rat	NA	SA	None	Hydrogel	Promote wound re-epithelization, angiogenesis, collagen deposition and fibrin regeneration	[165]
	Mouse	NA	Intravenous injection	None	None	Enhance wound angiogenesis and follicle regeneration; alleviate inflammation	[159]
Adipose tissue	Mouse	10 μg	SA	None	Polypeptide-based FHE hydrogel	Enhance wound angiogenesis, re-epithelization, granulation tissue formation, skin appendages regeneration, and collagen deposition	[162]
	Mouse	200 μg	SI	Modifying mmu_circ_0000250	None	Accelerate wound closure with smaller scars; enhance angiogenesis; suppress apoptosis of skin tissue	[61]
	Mouse	100 μg	NA	None	Acellular amniotic membrane	Promote wound vascularization, re-epithelization and ECM deposition; alleviate inflammation by inducing M2 macrophage polarization; skin attachment regeneration	[166]
	Mouse	200 μg	NA	None	None	Reduce inflammation and apoptosis; accelerate wound cell proliferation, re-epithelization, angiogenesis, collagen synthesis, and tissue remodeling	[72]
	Mouse	200 μg	SI	Hypoxia	None	Alleviate inflammation by inducing M2 macrophage polarization; enhance angiogenesis; accelerate wound healing	[167]
	Mouse	50 μg	SI	None	None	Promote wound closure, angiogenesis, collagen deposition and ROS scavenging	[168]
	Mouse	NA	SA	None	Metformin-loaded self-healing conductive hydrogel	Promote wound healing, re-epithelization, angiogenesis, collagen deposition and follicles regeneration; reduce inflammation and scarring	[169]
	Mouse	200 μg	SI	None	None	Promote re-epithelization and angiogenesis; reduce inflammation	[170]
Menstrual blood	Mouse	10 μg	Intradermal injection	None	None	Alleviate inflammation; enhance re-epithelization, angiogenesis, and collagen synthesis	[13]
Umbilical Cord	Rat	100 μg	SA	None	Pluronic F127 hydrogel	Promote angiogenesis, hair follicle regeneration, granulation formation and collagen deposition; reduce inflammation	[158]
	Rat	NA	SA	None	Alcohol/alginate nanohydrogel	Enhance re-epithelization, angiogenesis and orderly collagen deposition	[160]
	Rat	10 μg	SI	None	None	Promote angiogenesis and collagen deposition; alleviate inflammation	[118]
Hair follicle	Mouse	100 μg	SI	Modifying lncRNA H19	None	Promote wound closure and granulation tissue formation; reduce inflammation	[171]
Placenta	Mouse	NA	SA	Modifying MiR-146a	Silk fibroin patch	Promote hemostasis, re-epithelization, angiogenesis, granulation tissue formation and collagen deposition; reduce scar formation	[172]
Amniotic tissue	Rat	10 μg	SI	None	None	Promote wound closure; enhance angiogenesis	[173]

[a]NA: not available; SI: subcutaneous injection.

Exos, while the expression of anti-inflammatory factors (IL-4, IL-10, etc.) is upregulated, which is mediated by various molecular mechanisms such as the Nrf2 anti-oxidant pathway [157].

After administration, MSC-Exos can stimulate the proliferation of resident skin cells at diabetic wounds and reduce cell apoptosis [72], which are evidenced by an increase number of Ki67- or proliferating cell nuclear antigen (PCNA)-positive cells [72, 158] and a decreased number of caspase-3-positive cells [72]. It is reported that the increased level of TGF-β1 at the wound bed promotes the proliferation of fibroblasts [158]. Also, MSC-Exos can up-regulate the expression of VEGF at the wound bed, which promotes angiogenesis [158–160] and increases local blood perfusion [161]. Shi et al. reported that adipose MSC-Exos promoted wound angiogenesis in diabetic mice through inducing autophagy in endothelial cells, which was associated with the mmu_circ_0000250/miR-128-3p/SIRT1 axis [61]. Similarly, Zhang et al. showed that umbilical cord MSC-Exos encapsulated in a nanohydrogel obviously improved the angiogenesis of diabetic wounds, which was related to the ERK1/2 signaling pathway that can up-regulate the expression of VEGF (Fig. 6) [160].

The restoration of skin structure and function is a key consideration in diabetic wound treatment. It has been verified that the collagen fibers in the diabetic wounds received MSC-Exos were arranged more neatly than those without treatment [160], and that the scar tissue was narrower and smaller [61].

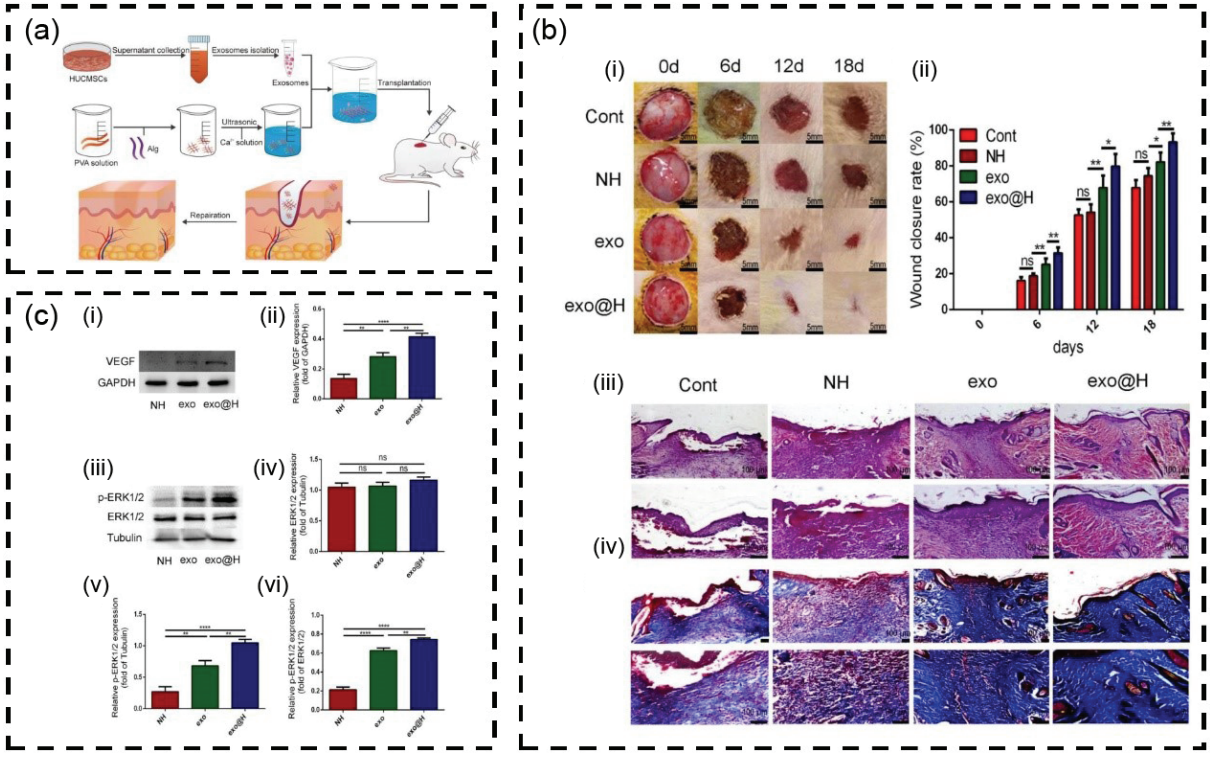

Figure 6 Human umbilical cord MSC-Exos accelerated diabetic wound healing. (a) Schematic illustration of exo@H preparation, exosome administration, and the repair outcomes. exo@H: Human umbilical cord MSCs-derived exosomes (exo) encapsulated in a polyvinyl alcohol (PVA)/alginate (Alg) nanohydrogel. (b) Gross and histological observation of the wounds: (i) Gross images of the diabetic wounds treated with NH, exo, or exo@H. Scale bars: 5 mm. NH: PVA/Alg nanohydrogel; (ii) Wound closure rates; (iii) HE and (iv) Masson staining of the wounds. Scale bar: 100 μm. (c) The repair mechanism of exo@H: (i) VEGF expression and (ii) statistic analysis in the wounds. (iii) Total ERK1/2 and phosphorylated ERK1/2 in the wounds. The relative expression of (iv) total ERK1/2 and (v) phosphorylated ERK1/2 compared with tubulin, and (vi) phosphorylated ERK1/2 compared with ERK1/2 in the wounds. $^{**}P \leq 0.01$, $^{****}P \leq 0.0001$, ns: lack of significance. Reproduced with permission from Ref. [160], © 2020 Elsevier B.V. All rights reserved.

Particularly, MSC-Exos promoted the regeneration of skin appendages in diabetic wounds, such as the regeneration of hair follicles and sebaceous glands [160, 162]. After the treatment of MSC-Exos, the expressions of *Filaggrin*, *Loricrin*, and *Aquaporin 3 (AQP3)* genes were elevated, which implies the restoration of skin barrier function in diabetic wounds [72].

5.4 Other applications

In addition to the aforementioned applications, MSC-Exo have been explored for the treatment of other skin wounds (Table 4). They are effective in promoting the survival of skin flaps, ameliorating allergic dermatitis, and reducing photoaging (Fig. 7). In a rat model of skin flap transplantation, Bai et al. demonstrated that subcutaneous injection of MSC-Exos promoted the survival of skin flaps, increased the wound revascularization, and reduced the inflammation and apoptosis of flaps after ischemia reperfusion injury (Fig. 7(a)) [174]. Increased expression of VEGF was recognized as the key factor to improve the survival of ischemic flaps [175]. Likewise, Xiong et al. observed that adipose MSC-Exos significantly promoted the vascularization of prefabricated flaps, which was associated with the enhanced proliferation of vascular endothelial cells and was regulated by exosomal microRNAs [176].

Several studies have investigated the potential of MSC-Exos for skin aging treatment [80, 89]. In the skin of ultraviolet-irradiated mice, MSC-Exos showed a protective role against the oxidative stress-induced injury, which was partially mediated by the nuclear factor-erythroid 2-related factor-2 (NRF2) defense system [89]. Similarly, Zhang et al. reported that topical application of human umbilical cord MSC-Exos, which was used in a combination with marine sponge *Haliclona* sp. spicules, effectively rejuvenated photoaged skin (Fig. 7(b)), showing reduced micro-wrinkles, alleviated histopathological changes, and increased ECM constituents [80].

In a mouse specific dermatitis model, Cho et al. reported that intravenous or subcutaneous injection of MSC-Exos ameliorated the pathological symptom of skin lesions (Fig. 7(c)), which was achieved by reducing the expression of inflammatory cytokines. The level of serum IgE, the number of eosinophils in the blood, and the infiltration of mast cells in the skin lesions were decreased. Meanwhile, the expression of inflammatory cytokines in the diseased skin showed a dose-dependent reduction with the treatment of MSC-Exos [177]. Similarly, in a mouse eczema model, MSC-Exos accelerated wound healing by inhibiting the infiltration of inflammatory cells and promoting wound revascularization; compared with the control group receiving corticosteroid therapy, the MSC-Exos group demonstrated more epidermis and dermis formation and less scarring at the lesions [110].

6 Strategies to enhance the therapeutic potential of MSC-Exos

The function of MSC-Exos is strongly influenced by the status and the culturing condition of their parental cells [27, 183, 184]. Based on this, various cell pretreatment strategies, such as hypoxia preconditioning, the addition of specific chemical agents, and physical stimulation of cells, have been developed to enhance the healing effect of MSC-Exos. In addition to the modification of exosomes, optimizing their delivery *in vivo*, especially the use of biomaterials for prolonged release, offers a feasible avenue to promote the therapeutic effect (Fig. 8).

6.1 Preconditioning with chemical agents

Many studies have demonstrated that preconditioning with proper chemical agents *in vitro* could promote the therapeutic

Table 4 The application of MSC-Exos for other skin wounds[a]

Animal model	Cell origin	Animal	Dose	Delivery	Pretreatment	Scaffold	Outcome	Ref.
Skin flap transplantation	Adipose tissue	Rat	100 μg	SI	H_2O_2	None	Enhance re-epithelization, angiogenesis, blood perfusion and skin flap survival; reduce inflammation and apoptosis	[174]
	Bone marrow	Rat	135 μg	Local injection	None	None	Enhance skin flap survival; promote angiogenesis and blood supply; reduce flap edema	[175]
	Adipose tissue	Rat	200 μg	Local injection	None	None	Increase the thickness and collagen deposition of skin flap; promote angiogenesis	[176]
	Bone marrow	Rat	500 μg	Intravenous injection	None	None	Reduce necrosis, apoptosis, inflammation and oxidative stress of skin flap; enhance angiogenesis	[178]
	Dental pulp	Rat	100 μg	SI	None	None	Enhance skin flap survival; reduce apoptosis and inflammation; promote re-epithelization, angiogenesis, collagen deposition and hair follicle regeneration	[179]
Photoaging	Adipose tissue	Rat	NA	SI	None	None	Relieve skin aging by reducing epidermis layer thickness and increasing skin layer thickness	[82]
	Umbilical cord	Mouse	150 μg	Smearing administration	None	Sponge *spicules*	Relieve skin aging; less micro wrinkles	[80]
	Adipose tissue	Mouse	5×10^9 particles	Intravenous injection	None	None	Reduce UVB-irradiation-induced thickening of epidermis; deposit collagen in dermis	[180]
	Adipose tissue	Mouse	5×10^{10} particles	SI	Modifying lncRNA H19	None	Reduce collagen degradation; ameliorate UVB-induced skin senescence	[181]
	Umbilical cord	Mouse	10 μg	Intracutaneous injection	None	None	Reduce epidermal thickness, inflammation, and oxidative injury; prevent UV-induced cell proliferation and collagen deposition	[89]
Atopic dermatitis	Adipose tissue	Mouse	0.14, 1.4 or 10 μg	Intravenous injection or SI	None	None	Ameliorate atopic dermatitis symptoms; alleviate mast cell filtration; reduce serum IgE; decrease the expression of IL-4, IL-31, IL-23 and TNF-α	[177]
	Adipose tissue	Mouse	10 μg	SI	None	None	Normalize stratum corneumhydration; reduce epidermal hyperplasia and erythema; alleviate itch; decrease skin allergic inflammation; increase the production of epidermal ceramides; improve epidermal barrier function	[182]
Eczema	Umbilical cord	Mouse	NA	NA	None	None	Promote scarless healing and the regeneration of epidermis and dermis; reduce intradermal lymphocyte infiltration	[110]

[a]NA: not available; SI: subcutaneous injection; UV: ultraviolet; UVB: ultraviolet radiation b.

potential of MSCs *in vivo* [185]. Similarly, preconditioning with small molecules, drugs or other chemical agents provides a convenient, feasible and efficient way to produce exosomes with enhanced wound healing potential [103, 174, 186]. In a rat model of skin flap transplantation, Bai *et al.* reported that, compared with exosomes without any preconditioning treatment, exosomes derived from adipose MSCs that were pretreated with a low concentration of H_2O_2 attained a higher ratio of skin flap survival and a greater density of capillary [174]. Interestingly, in the senescence MSCs pretreated with high concentrations of H_2O_2, the pro-angiogenic ability of exosomes was significantly reduced, which may be partially attributed to the decreased expression of miR-146a [59]. The distant results highlight the critical role of pretreatment condition, even with the same chemical agent.

Without the use of hypoxia incubators or chambers, hypoxia-mimicking agents such as cobalt chloride, dimethyloxalyl glycine and deferoxamine can create a hypoxic-like cell culture condition through the activation of HIF-1 signaling pathway [187, 188]. Ding et al. demonstrated that exosomes originated from deferoxamine-preconditioned MSCs exhibited a superior pro-angiogenic property in diabetic wounds, which was related to the transferring of exosomal miR-126 [100]. In another study, Shi et al. found that the treatment with 3,3'-diindolylmethane, a naturally occurring small-molecule compound, could raise the level of Wnt11 in human umbilical cord MSC-Exos, which further activated the Wnt/β-catenin signaling pathway in parent MSCs to enhance the wound healing ability [189].

Some studies have investigated the possibility of drug preconditioning to improve the repair function of MSC-Exos [103, 156, 186]. It is reported that exosomes from MSCs pretreated with atorvastatin, a drug used to reduce blood lipid in the clinic, showed better neovascularization in diabetic wounds [103]. Similarly, the preconditioning with beglitazone, a common drug for diabetes, enhanced the angiogenetic potential of MSC-Exos and accelerated the healing of diabetic wounds [186]. Others reported that pretreatment with melatonin or LPS greatly

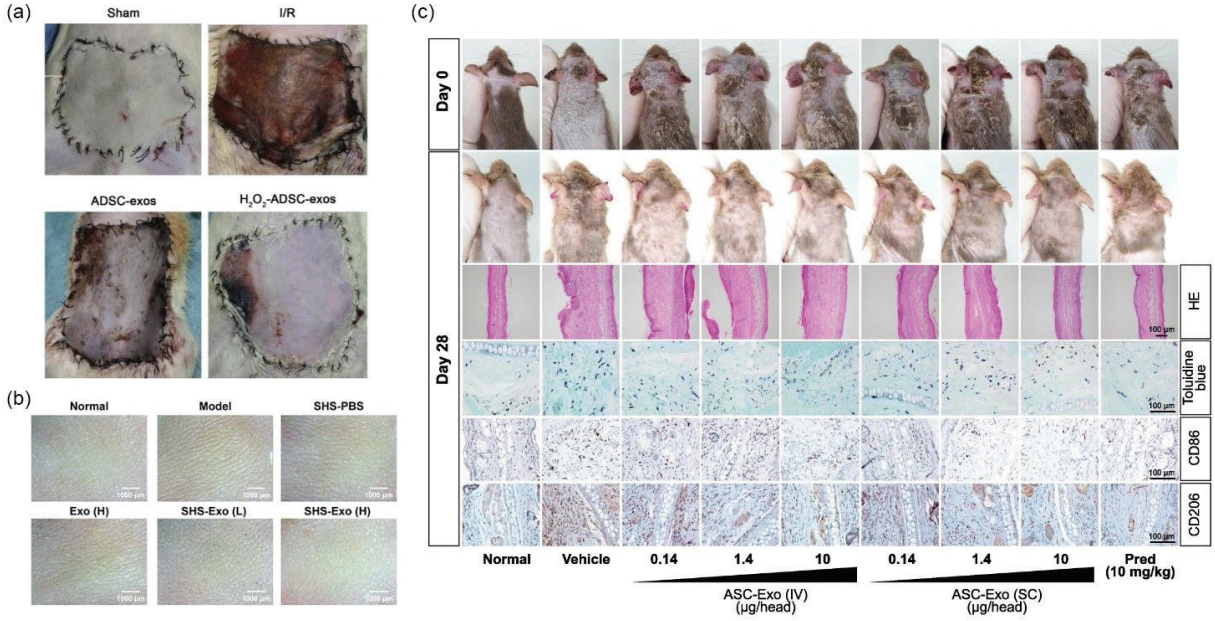

Figure 7 The application of MSC-Exos for skin flap transplantation, photoaging and atopic dermatitis. (a) Adipose MSC-exosomes (ADSC-exos) enhanced the survival of skin flaps in an ischemia-reperfusion (I/R) injury model. The images show skin flaps 5 days after treatment. Reproduced with permission from Ref. [174], © 2018 Elsevier Inc. All rights reserved. (b) Human umbilical cord MSC-Exos exerted an anti-aging effect on photoaged skin. The images demonstrate the micro-wrinkles of photoaged skin after treatment. Model: No treatment. Exo (H): MSC-Exos (1 mg/mL); SHS-Exo(L), SHS-Exo(H) and SHS-PBS groups: MSC-Exos (100 μg/mL), MSC-Exos (1 mg/mL) or PBS combined with marine sponge *Haliclona* sp. spicules (SHSs) respectively. Reproduced with permission from Ref. [80], © Zhang, K. et al. 2020. (c) Adipose MSC-Exos ameliorated atopic dermatitis. Exosomes were administered intravenously (IV) or subcutaneously (SC) thrice a week for 4 weeks. The images show representative skin manifestations in atopic dermatitis mice at days 0 and 28, together with HE, toluidine blue, and immunohistochemical staining of ear skin samples. Reproduced with permission from Ref. [177], © Cho, B. S. et al. 2020.

promoted the ability of MSC-Exos to reduce the inflammation of diabetic wounds, which was achieved by the promotion of M2 macrophage polarization [66, 156].

6.2 Hypoxia preconditioning

Hypoxia can enhance the angiogenetic potential of MSC-Exos [95, 190–193]. Han et al. reported that exosomes isolated from hypoxic-preconditioned adipose MSCs significantly enhanced the proliferation, migration and tube-formation of human umbilical vein endothelial cells *in vitro*, which may be attributed to the high level of exosomal angiogenetic factors such as VEGF, epidermal growth factor, and fibroblast growth factor [95]. Similarly, in diabetic wounds, exosomes from hypoxia-pretreated adipose MSCs showed better therapeutic effects, which may be attributed to the delivery of exosomal circ-Snhg11 that can increase the survival and function of endothelial cells [190]. Additionally, using high-throughput sequencing technique, Wang et al. discovered that the microRNAs correlated with skin wound healing were differently expressed in hypoxic exosomes, which effectively promote diabetic wound healing through the PI3K/AKT signaling pathway [193].

6.3 Physical stimulation

Physical cues considerably affect the behaviors of MSCs, such as their fate decision and the secretion of bioactive factors [194]. The effect of physical stimulation on the wound healing potential of MSC-Exos has been investigated in several studies. For instance, when exposed to blue monochromatic light, human umbilical cord MSCs could produce exosomes with improved angiogenic ability, which was due to the elevated level of miR-135b-5p and miR-499a-3p. In a mouse burn wound model, the delivery of blue light irradiation MSC-Exos not only promoted the proliferation of epidermal and dermal cells but also enhanced wound angiogenesis [60]. Similarly, with the stimulation of Fe_3O_4 nanoparticles and a static magnetic field, bone marrow MSCs produced exosomes that can promote the proliferation, migration and angiogenesis of endothelial cells to a greater extent *in vitro*. Furthermore, local administration of such exosomes into full-thickness wounds in rats resulted in a faster wound closure and better angiogenesis than the wounds received exosomes without stimulation [44].

6.4 Genetic modification

Due to the endogenous delivery property and high cargo loading ability, MSC-Exos are acknowledged as a potent carrier to transport specific molecules [195, 196]. Using genetic modification technologies, the overexpression of specific non-coding RNAs provides a practical method to enhance the therapeutic potential of MSC-Exos [61]. In addition, the alteration of specific protein level possesses great value to improve the wound healing potential of MSC-Exos [128].

6.5 Biomaterial-assisted delivery

In animal studies, MSC-Exos are usually administrated by direct injection into the wounds. However, due to the ease of disperse and the rapid clearance of exosomes, this method may not be efficient enough to achieve the optimal therapeutic effect. Particularly, in some circumstances such as diabetic patients with severe peripheral nerve and vascular lesions, small injuries may increase the risk of infection and even lead to the development of refractory ulcers. It is necessary to explore simple, effective and non-invasive methods to deliver exosomes. To fulfill this purpose, biomaterial-assisted delivery strategy has attracted much interest, for it enables the enrichment of exosomes at the wounds, maintains the stability of exosomes, and controls their release *in vivo*.

A wide range of biomaterials have been employed to promote the therapeutic efficacy of exosomes. Because of the biocompatible, biodegradable and hydrophilic properties, various kinds of hydrogels, such as the self-healing hydrogels, the thermosensitive hydrogels, and the pH-responsive hydrogels have been engineered to enable a sustained release of MSC-Exos *in vivo* [158, 197, 198]. Notably, these hydrogels can protect the wound

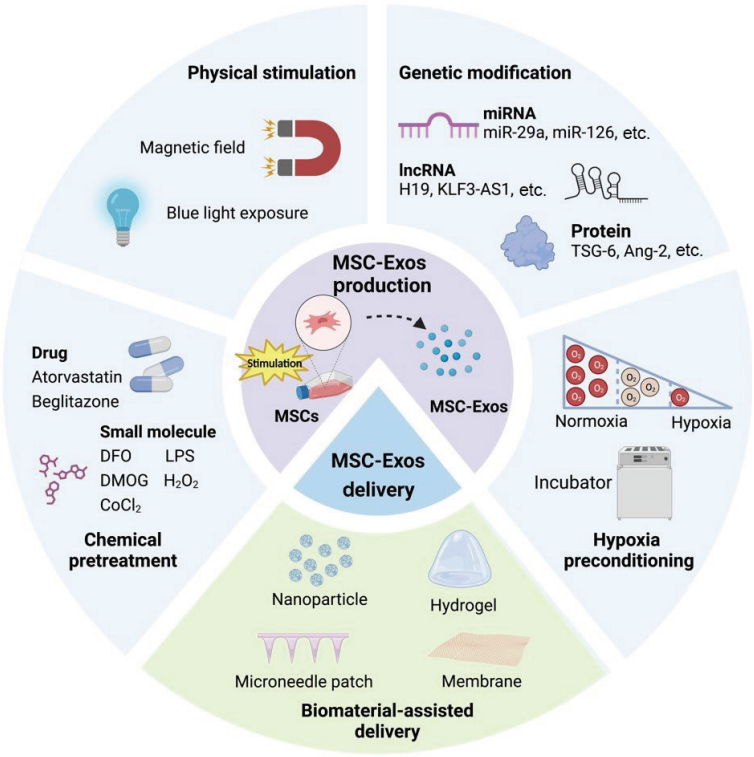

Figure 8 Strategies to enhance the therapeutic potential of MSC-Exos. Various approaches, including physical stimulation, pretreatment with chemical agents, hypoxia preconditioning and genetic modification have been utilized to produce MSC-Exos with improved repair ability. In addition, the optimization of exosome delivery by using biomaterials has proven to be feasible to increase the healing potential of MSC-Exos.

bed from unfavorable external stimuli and maintain a moist microenvironment around the wounds, both of which are beneficial to wound healing. Recent studies have validated that the healing potential of MSC-Exos was obviously increased through the use of hydrogels, showed better granulation tissue formation [99], promoted wound angiogenesis, accelerated re-epithelialization, improved collagen arrangement [46, 64, 158], and significantly enhanced regeneration of nerves [163].

Membranous biomaterials, composite scaffolds, microspheres, and microneedle patches also prolong the release of MSC-Exos *in vivo*, which results in a faster regeneration of skin tissues [164, 166, 172, 199–202]. Particularly, microneedle patches have emerged as an effective and safe approach for transdermal delivery of MSC-Exos. For instance, Gan et al. have reported an adhesive microneedle patch that can continuously deliver MSC-Exos to the wound bed, which relieves the inflammation, promotes the angiogenesis, and suppresses the bacterial infection of diabetic wounds [201].

Additionally, some nanoparticles have been utilized to enhance the therapeutic potential of MSC-Exos [44]. For instance, to improve the organ-targeting ability of MSC-Exos, Li et al. have fabricated iron oxide nanoparticle-labeled exosomes from umbilical cord MSCs, in which the iron oxide nanoparticles were served as the magnet-guided navigation tool. After systemic injection of the nanoparticle-labeled exosomes in a skin burn injury model, magnetic guidance greatly enhanced the accumulation of exosomes at the wounds, which significantly accelerated the wound healing rate, improved the wound angiogenesis and re-epithelization, and reduced scarring [96].

7 Challenges and future perspective

Despite the excellent pro-healing effect of MSC-Exos in animal skin wound models, only one clinical trial has been registered to investigate the repair potential of MSC-Exos for skin wounds at the public website of the Clinical Trials Data Bank at the National Institutes of Health (www.clinicaltrials.gov; as at August 2, 2023). The identified clinical trial "Mesenchymal stem cells derived exosomes in skin rejuvenation" (ClinicalTrials.gov ID: NCT05813379) aimed to slow down skin aging by local injection of MSC-Exos. Similarly, in the literature, only three studies have reported the clinical application of MSC-Exos for skin disorders, including sensitive skin-related disease [203], acne scars [204], and hyperpigmentation [205]. Although improved clinical symptoms are reported in these studies, high-quality clinical trials with long-term follow-up are needed to further confirm the therapeutic efficacy.

Before the large-scale clinical translation of MSC-Exos, there are many challenges needed to be overcome. First, the safety of MSC-Exos is critical for clinical application, which has been explored in rodent models [206], yet data from large animal models are rare. Second, considering the relatively complicate isolation of exosomes and the low yield from MSCs, it is difficult, time-consuming and costly to obtain sufficient MSC-Exos with high-quality for therapeutic purposes. Compared with MSCs, the process of exosome isolation (ultracentrifugation, chromatography, ultrafiltration, etc.) requires specialized and expensive devices or equipment [38, 207, 208]. Furthermore, under traditional monolayer cell culture conditions, the yield of exosomes is low, making it challenging to scale up for clinical application. Interestingly, Kim et al. reported that, compared with conventional monolayer culture, three-dimensional spheroid culture improved the production efficiency of MSC-Exos, which was related to the non-adherent round cell morphology [209]. Similarly, Haraszti et al. observed that a combination of microcarrier-based three-dimensional culture and tangential flow filtration significantly increased the yield of exosomes from umbilical cord-derived MSCs, providing a promising strategy for scalable production of biologically active exosomes [210]. Despite of some progress, the development of applicable approaches for

mass production of exosomes, such as the use of bioreactors, microcarriers, and advanced isolation methods, needs to be addressed [211, 212].

Third, there is no standard to control the quality of exosomes, which may hinder a repeatable repair outcome in the clinic. It is known that the quality of exosomes is influenced by cell sources, culture conditions, and purification approaches. Owing to the differences in the cell origin, MSC-Exos show different molecular characteristics and result in highly variable effects on skin cell behaviors [24, 213, 214]. The correlation between the origin of MSC-Exos and their therapeutic potentials warrants further investigation. Key exosomal cargo that significantly contributes to the therapeutic effect of MSC-Exos deserves further exploration. It is necessary to uncover the mechanisms by which MSC-Exos orchestrate the behaviors of resident skin cells to accomplish tissue regeneration. Particularly, to minimize batch-to-batch variance in the properties of MSC-Exos, quality control criteria must be established, including the purity, physicochemical properties, and bioactivity of products. For clinical translation, the manufacturing process of MSC-Exos, including the cell cultivation, exosome purification, and quality control, should comply with the principles of good manufacturing practice [215–218].

Furthermore, previous researches reveal that the storage conditions influence the physiochemical properties of exosomes [41, 42]. Common storage strategy for exosomes includes freezing at −80 °C and lyophilization. During cryopreservation, cryoprotectants are employed to increase the stability of exosomes [38]. Similarly, in the process of freeze-drying, lyoprotectants such as trehalose improve the preservation of exosomes [43]. This underlines the need of future optimization of the storage approaches to protect the bioactivity of MSC-Exos and make them convenient to transport and use in the clinic. Taken together, despite the limitations and challenges mentioned above, MSC-Exos hold immense promise for future clinical applications, especially due to the merit of avoiding the potential risk of maldifferentiation or tumor formation of MSC transplantation.

8 Conclusion

MSC-Exos are small membrane vesicles containing diverse bioactive cargoes that can be stably transported to skin cells to modulate the cell behaviors. As a promising nanomaterial for skin wound treatment, MSC-Exos avoid the potential risk of MSC transplantation such as the neoplastic transformation of grafted cells. Recent researches have demonstrated that MSC-Exos are effective in promoting the repair function of fibroblasts, keratinocytes and endothelial cells *in vitro*. In animal studies, MSC-Exos reduce the excessive inflammation of chronic wounds, promote the wound angiogenesis, re-epithelization and ECM remodeling in various types of skin wounds. Such effects are partially attributed to the activation of multiple signaling pathways in the recipient cells, and a number of key exosomal components have been verified. Along with the biomaterial-assisted strategy to optimize the delivery of exosomes, cell preconditioning and genetic modification strategies have showed great potential to boost the therapeutic potential of MSC-Exos. Based on current researches, MSC-Exos can serve as versatile nanoparticles for skin wound healing, which merits future studies for clinical translation.

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