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Mesenchymal Stromal Cell-Derived Extracellular Vesicles in the Treatment of Diabetic Foot Ulcers: Application and Challenges

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Abstract

Diabetic foot ischemia and ulcer (DFU) persists as a serious diabetes mellitus complication in spite of increased understanding of the pathophysiology and the cellular and molecular responses. Contributing to this pessimistic situation is the lack of effective treatments that are slow to heal the deep chronic wounds and microvascular obstruction. Mesenchymal stromal cells (MSCs) have been tested as a promising cell-based therapy for diabetes in vitro and in vivo, which is able to accelerate wound closure with increased epithelialization, granulation tissue formation and angiogenesis by differentiation into skin cells and paracrine pathways to repair injured cells. The secretomes of MSCs, including cytokines, growth factors, chemokines, and extracellular vesicles containing mRNA, proteins and microRNAs, have immunomodulatory and regenerative effects. This review will shed new light on the therapeutic potential of MSC-derived extracellular vesicles (MSC-EVs) for the treatment of diabetes-induced lower limb ischemia and ulcers. The identification of underlying mechanisms for MSC-EVs regulation on impaired diabetic wound healing might provide a new direction for MSC-centered treatment for diabetic lower limb ischemia and ulcers.

Keywords Diabetic foot ulcers · MSC-derived extracellular vesicles · Exosomes

Introduction

With the growth of aging populations, dietary changes, and sedentary lifestyles, world-wide rates of diabetes mellitus (DM) have risen steadily. According to epidemiological studies as of the year of 2019, diabetes affects more than 463 million adults worldwide [1]. DM is a group of metabolic disorders and the pathophysiology of DM is underpinned by insulin-resistance and pancreatic β cell dysfunction leading to long-term hyperglycemia [2]. Patients with DM are prone to suffer from multiple complications, such as infections, diabetic nephropathy, diabetic retinopathy, diabetic peripheral neuropathy, and diabetic foot ulcers (DFU) [3]. It is estimated that, annually, between 9.1 million and 26.1 million people develop DFUs worldwide [4]. Moreover, the lifetime incidence of DFU ranges between 15 and 25% among persons with diabetes, but when the patient's history of foot ulcer is considered, 19% to 34% of persons with diabetes are likely to be affected [4]. DFU, which is mainly caused by ischemic, neuropathic, or combined neuro ischemic abnormalities [5], results in a huge illness burden both to society, and to the patients and their families. The risk of death at 5 years for a patient with a diabetic foot ulcer is 2.5 times higher than as the risk for a patient with diabetes who does not have a foot ulcer [6]. Diabetic ulcers are the most common cause of nontraumatic amputations and approximately 20% of moderate or severe diabetic foot infections lead to different levels of amputation [4, 6]. Decreased vascularization, elevated oxidative stress, and infection contribute to the pathological hallmarks of non-healing chronic diabetic wounds [7].

Therefore, the principles of management of DFU include increasing angiogenesis, removal of oxidative stress, and eradication of infection from the ulcer [8]. However, routine and advanced treatments, such as blood sugar control, debridement, hyperbaric oxygen therapy, electrical stimulation, negative pressure wound therapy and bioengineered skin still present difficulties in the treatment of DFU due to repeated

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infections, limb ischemia, and nerve damage [3]. Mesenchymal stromal cells (MSCs) obtained from a variety of tissues such as bone marrow, the umbilical cord, fat tissue, the placenta and menstrual blood etc., have been proven that as a promising regenerative therapy for DFU because of their multipotency and paracrine secretions [9–13]. MSCs are capable of secreting cytokines, chemokines, growth factors, and extracellular vesicles containing proteins, mRNA, microRNAs, and mitochondria, contributing to enhanced angiogenesis, fastened re-epithelialization, improved granulation, and contribute to chronic wound closure [14]. The secretome from MSCs has been recognized as the dominant mechanism for ameliorating the symptoms of DFU. In addition, emerging evidence has demonstrated that MSC-EVs are the trophic mediator modulating many biological processes, including anti-inflammation and anti-apoptosis. In this review, we summarize the updated knowledge about the mechanisms of MSC-EVs on DFU and explain the underlying potential for future clinical applications.

Mesenchymal Stromal Cells in DFU

MSCs are being investigated as possessing the peculiar characteristics of differentiating into keratinocytes and endothelial cells, and the secretion of trophic immunoregulatory and reparative factors [15]. The ability of MSCs to home and migrate into damaged sites contributes to the eradication of infections and orchestrates tissue homeostasis. Additionally, the properties of immunomodulation, regeneration in different damaged organs reveal the potential of MSCs as a promising therapeutic alternative that could be translated into clinical work.

The Ability of Homing to Injured Tissue

MSCs possess the ability to migrate and home through the expression of the receptors of cytokines and chemokines, and integrins on the cellular surface, such as C-X-C chemokine receptor ligand 4(CXCR4), C-C cytokine receptor type 2 (CCR2), CCR7, integrin β1 and Integrin α4, which contact with the vascular cell adhesion protein 1 (VCAM-1) on the endothelial cells [16–18]. MSCs secrete matrix metalloproteinase 2 (MMP-2) to transmigrate across endothelial monolayers to accelerate the process of homing [19]. In addition, MSCs pretreated with the pro-inflammatory cytokines, such as TGF-β1, IL-1β, and TNF-α could increase MMP-2, MT1-MMP, and/or MMP-9 production in MSCs, resulting in a strong stimulation of chemotactic migration through the extracellular matrix (ECM) [20]. However, the comprehensive mechanisms of MSC homing and how MSCs contact with endothelial cells remains speculative.

The Mechanisms of MSCs in DFU

The paracrine secretions of MSCs are recognized as the main molecular mechanisms in the treatment of DFU to facilitate diabetic wound healing. MSCs are capable of secreting various kinds of angiogenic growth factors, immunomodulatory factors, anti-microbial peptides, reparative molecules and chemokines to enhance granulation tissue formation, wound re-epithelialization, and promote wound angiogenesis and collagen metabolism **(**Table 1**).**

In the in vitro diabetic wound model, umbilical cord-derived MSCs (UC-MSCs) were demonstrated to promote diabetic wound repair by secreting VEGF and fibroblast growth factor (FGF), and transforming growth factor-β production (TGF-β), compared to a fibroblast group [24]. Specifically, UC-MSCs can be localized to the injured ulcerated tissue and enhance the tissue recover by upregulating cytokeratin 10 secretion from keratinocytes and accelerating extracellular matrix formation [29]. Additionally, UC-MSC-conditional medium (CM) has been shown to have a greater therapeutic potential than UC-MSCs in contributing to an increase in vascular density and the recovery of sensory function by secreting VEGF, keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF) [23]. More importantly, UC-MSCs have also shown the great therapeutic potential to recover diabetic femoral neural (FN) degeneration by increasing capillaries in FN-innervated gastrocnemius, serum nerve growth factor (NGF) expression, and restoring the slow conduction of FN in the model of diabetic ulceration rats [30]. In a mouse hind limb ischemia model, Wharton's Jelly MSCs have been also demonstrated that the proangiogenic actions were mediated by secretion of angiogenin, interleukin-8, monocyte chemoattractant protein-1 (MCP-1), and vascular endothelial growth factor (VEGF) [21].

Adipose-derived MSCs (ASCs) have been shown to have a rich secretome and multi-differentiation ability, whereby the critical role of ASCs is manifested in angiogenesis and immunoregulation [31, 32]. ASCs also produce angiogenic growth factors, such as VEGF, KGF, TGF-β1, but ASCs also secrete hepatocyte growth factor (HGF), epidermal growth factor (EGF) and insulin-like growth factor (IGF-1) to promote the wound healing [22, 26]. In a Zucker diabetic fatty rat model, transplantation of ASC sheets was able to decrease the wound area, and increase blood vessel density in full-thickness skin defects [22]. In addition, silk fibroin patches (SFP) cellularized with ASCs and SFP decellularized with ASCs accelerated the rate of wound healing by the significant upregulation of the angiogenic gene (Wnt5α, Wisp1 and TGFβγ3) and the genes involved in ECM deposition and remodeling such as MMP2, Col5α1, Col5α2, Col4α1 etc. [28]

Simultaneously, MSCs, which are identified as having profound immunosuppressive effects, can restore the balance of

Table 1 MSC-secreted soluble paracrine factors therapy in DFU

Paracrine factors	Study	Component	Main Function
Angiogenic growth factors	***Jiangbo* et al [21–25]**	VEGF	Enhancing wound vascular density and granulation tissue formation
	***QZ Zhang* et al [23, 25]**	PDGF	Enhancing wound angiogenesis and collagen metabolism
	***Kato, Y* et al [22, 26]**	HGF	Promoting wound angiogenesis, re-epithelialization, and granulation tissue formation
	***Kato, Y* et al [22]**	IGF-1	Promoting granulation tissue formation
	***K.Kinoshita* et al [26]**	EGF	Enhancing wound re-epithelialization and granulation tissue formation
	***You, H. J.* et al [24]**	FGF	Enhancing granulation tissue formation, restoring normal epidermal thickness
	***You, H. J.* et al [22, 24]**	TGF	Promoting wound vascular density and inhibiting macrophage and monocytes activation
	***Shrestha, C* et al [22, 23]**	KGF	Enhancing wound re-epithelialization
chemotactic factor	***Cao, Y* et al [27]**	IL-8	Promoting wound angiogenesis and recruit neutrophil to damaged tissue
	***HK kim* et al [21]**	MCP-1	Recruit monocytes, T cells and dendritic cells to inflamed sites
Immune factors	***Navone, S* et al [28]**	PEG2	Modulate macrophage polarization
	***Chuan Tong* et al [25]**	IL-10	Anti-inflammatoryfunction

the immune response in the process of chronic inflammation by modulating the cytokine network and other humoral and cellular effectors. MSC-secreted prostaglandin E2 (PGE2) mediated macrophage polarization from inflammatory macrophages (M1) toward anti-inflammatory macrophages (M2) [33]. Moreover, MSCs also can suppress T/B cell proliferation, and the maturation and differentiation of dendritic cells. However, the comprehensive understanding of MSC effects on the immune system is limited.

Human bone marrow-derived-MSCs, placental derived-MSCs, hair follicle dermal sheath-derived-MSCs and gingiva derived-MSCs were all demonstrated to promote angiogenesis and immunomodulation in similar DFU models [25, 27, 34]. Nevertheless, due to poor cell engraftment and inconsistent stem cell potency, accumulating evidence reported that EVs are one of the most important effectors in the MSC secretome to recapitulate the therapeutic effect of their parent MSCs.

Limitations of MSCs: Reasons to Investigate MSC-EVs

MSCs are not retained and do not survive in the injured tissue for long, normally disappearing gradually within 24 hours [35], and the lack of standardized and optimized criteria contributes to the main challenge of MSC application in DFU. Discrepancies from bench to bedside include donor-based MSC heterogeneity, the conditions of the cell isolation and culture, cryopreservation methods, dose, frequency and route of MSC infusion, timepoint of cell administration, and follow-up period. Moreover, the ambiguous impacts of MSC transplantation include potential tumorigenicity, untargeted tissue differentiation, and undesired immune responses, the most important of which is malignant promotion and transformation. MSCs exhibit tumor tropism [36] and the possibility of tumorigenicity increases if the MSCs are expanded in culture [37]. In addition, despite possessing immune-privileged properties, allogeneic MSCs can be recognized by the host immune system and induce anti-donor immune responses due to the alloantigen on the cell surface [38]. These issues of whole-cell administration initiate the investigation of MSC-EVs' effect on DFU.

MSC-EVs: Definition, Isolation, Characterization

Extracellular Vesicles (EVs) are small circular structures surrounded by a phospholipid membrane that are released by cells and are vital in intercellular communication as they can transport a variety of substances large distances across the body and influence recipient cell behavior via the delivery of functional bio-molecules [39]. According to the nomenclature recommendation from the Minimal information for studies of extracellular vesicles 2018 (MISEV2018), three different operational terms are used to define EV subtypes: 1) physical characteristics of EVs, such as size with ranges ("small EVs" (sEVs): less than 100 nm or 200 nm [small], and "medium/large EVs" (m/lEVs): more than 200 nm [large and/or medium]) or density (low, middle, high, with each range defined); 2) biochemical composition (CD63+/CD81 + - EVs, Annexin A5-stained EVs, etc.); or 3) descriptions of conditions or cell of origin (podocyte EVs, hypoxic EVs, large oncosomes, apoptotic bodies) [40]. Moreover, EVs transfer specific genetic information via microRNAs, proteins, mitochondria, coding RNAs and non-coding RNAs [41]. These biological and effective molecules have been shown to regulate cell proliferation, anti-inflammation, and angiogenesis in recipient cells, which play a fundamental role in normal physiological processes as

well as a pathological process as a result of altered gene regulatory networks and/or epigenetic programming [42].

Ultracentrifugation (differential centrifugation), density gradient centrifugation, and ultrafiltration are the most common isolation approaches towards MSC-EVs, of which ultracentrifugation is the most widely used and easily handled of separating EVs by size and can be handled [43]. In terms of distinct methods, the purity, yield, and the sedimentation efficiency of the isolation differ from each other. In more detail, the steps of ultracentrifugation are typically divided into two stages, including a low-speed spin at 300 g to eliminate apoptotic debris followed by a higher speed spin at 100,000 g to precipitate EVs [44]. However, ultracentrifugation washing procedures lead to the low yield EVs. Density gradient centrifugation is the second most commonly used means to isolate EVs from MSC-CM, which has a higher EV purity and a larger number of EV proteins and RNAs than ultracentrifugation [45]. In addition, Birke et al. have found that the yield and purity of ultrafiltration combined with size exclusion chromatography were superior to ultracentrifugation [46].

There are four distinct methods in checking the characterization of EVs, based on the minimal information for studies of EVs 2018 from the international society of extracellular vesicles 2018 (MISEV2018). First, the source of MSC-EVs and EV preparation are supposed to quantitatively describable. Next, EV general characterization should show at least three positive protein markers of EVs by flow cytometry and western blot, such as CD63, CD81 and CD82, and at least one negative protein marker, such as CD45 [47]. The third approach is to detect the characterization of single vesicles: using an electron or atomic force microscope or single-particle analyzers. The last method recommends that the topology of EV-associated components be assessed [40].

MSC-EVs Effects on DFU in Experimental Models

Multiple studies have presented MSC-EVs as being beneficial to the regeneration process of DFU, including the inflammation stage, angiogenesis stage, re-epithelialization, and remodeling stage [48]. miRNAs ("angiomiRs") and proteins, such as those deleted in malignant brain tumors 1 (DMBT1), OxOband, nuclear factor erythroid 2-related factor 2 (NRF2), miR-21, miR-23a, miR-124a, miR125b, miR126, lncRNA H19, let-7b, mmu_circ_0000250, miR-130a, miR-132 encapsulated in MSC-EVs play a vital role in promoting cell proliferation of connective tissues through activating targeting signaling pathways to modulate the immune response, autophagy, and neovascularization [49, 50] **(**Table 2 **and** Fig. 1**)**.

DMBT1 Exosomes isolated human urine-derived MSCs (USCs) induced a remarkable proliferation and migration of keratinocytes and fibroblasts, which were detected by CCK8 analysis, and by a scratch wound healing assay and a transwell assay [65]. In addition, USC-Exos stimulated human vascular endothelial cells (HMECs) to generate more capillary-like structures and motility. More importantly, after identifying the components of USC-Exos by bioinformatics analysis, DMBT1, a potent promoter of angiogenesis [63], and VEGFA, a positive mediator of physiological and pathological of angiogenesis, were enriched in the exosomes. In addition, DMBT1 is capable of increasing VEGFA expression through the PI3K-Akt signaling pathway. To confirm the DMBT1 effect on diabetic wound healing, shRNA-DMBT1 was transfected into USC to suppress DMBT1 expression, and the exosomes were isolated from DMBT1-silenced USCs ($USCs^{shDMBT1}$-Exos). Interestingly, the $USCs^{shDMBT1}$-Exos lost promising effect on tube formation partially and delayed cutaneous wound healing in a diabetic mice model.

OxOband ADSC-Exos labeled with Calcein AM dye endocytosed by keratinocytes and fibroblasts promoted the migration of these two kinds of cells, reduced the oxidative stress, and elevated the metabolic activity in neuronal cells in the hyperglycaemic microenvironment. Meanwhile, results of a Live/Dead viability/integrity assay using Calcein AM/PI staining demonstrated that Exos cultured keratinocytes promote their survival rate in an H2O2-induced oxidative stress condition, compared to the non-treated cells [66]. In addition, ADSC-Exos containing metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) can be transferred into keratinocytes and fibroblasts and targeted on miR124 to promote cell proliferation, migration, and inhibit cell apoptosis through stimulating the Wnt/β-catenin pathway [62]. Moreover, ADSC-Exos embedded into the scaffolds of oxygen-releasing antioxidant polymeric cryogel (PUAO-CPO-Exos) revealed a slower release than PUAO -Exo embedded in antioxidant polyurethane (PUAO) scaffolds, which meant PUAO-CPO-Exos had a prolonger effect on damaged tissue in different phases of chronic wound closure. Importantly, exosomes laden oxygen releasing antioxidant scaffolds PUAO-CPO-Exos (OxOband) are capable of reducing ROS release, supply adequate oxygen, generate hair follicles and sebaceous glands to enhance wound healing [66].

NRF2 NRF2 is a nuclear mediator of cellular resistance to oxidants that regulate the physiological and pathophysiological outcomes of oxidant exposure [67]. In the presence of ADSC-Exos, the apoptosis rate of endothelial progenitor cells (EPCs), which were stained with Annexin-V/propidium iodide (PI), was significantly decreased [68]. ADSC-Exos also enhances the tube formation capability of EPCs which were seeded in a Matrigel-coated plate. Moreover, the intracellular ROS levels also decreased, as determined by DCH-DA staining, and the pro-inflammatory cytokine (IL-1β, IL-6, and TNF-α) secretion of EPCs co-cultured with ADSC-Exos

Table 2 Studies investigating the effect of MSC-derived EVs in preclinical models of DFU

		Study	Source	Mechanisms
Exosomes	miR-21	***Madhyastha, R.* et al [51–53]**	UCB/Human circulating fibrocytes	Inhibit PTEN and SPRY I; stimulating PI3K/AKT signalling pathway, MMP9 expression DC differentiation; regulating collagen deposition
	miR-23	***Fang, S.* et al [54]**	UB-MSC	Targeting Sprouty2, Sema6A; SDF-1α/CXCL12;suppression of TGF-β/SMAD2 stimulation
	miR-124a/miR125b	***Geiger, A* et al [55]**	Human circulating fibrocytes	Anti-inflammatory function
	miR126	***Fish, J.*et al [56–58]**	SMSC/Human circulating fibrocytes	Stimulating VEGF and FGF signaling pathway, including SPRED1 and PIK3R2/p85-β stimulated the migration and tube formation of HMEC-1 and collagen maturity
	lncRNA H19	***Li, B.* et al [59]**	myeloid-derived MSCs	Suppression of apoptosis and inflammation of the fibroblasts through miR-152-3p/ PTEN axis
	let-7b	***Ti, D.* et al [60]**	UC-MSC	Regulating macrophage plasticity through activating TLR4/NF-κB/STAT3/AKT signaling
	mmu_circ_0000250	***Shi, R.* et al [61]**	ADSC	Suppressing miR-128-3p expression and enhanced autophagic plaque formation in EPCs
	miR-130a/miR-132	***Geiger, A* et al [55]**	Human circulating fibrocytes	Proangiogenic function
	OxOband	***Shiekh, P. A* et al [62]**	ADSC	Targeting on miR124 to promote proliferation and migration of keratinocytes and fibroblasts, stimulating the Wnt/β-catenin pathway
	DMBT1	***Chen.C.Y.* et al [63]**	USCs	Promoting angiogenesis, activating PI3K-Akt signaling pathway
	NRF2	***Li, X.* et al [64]**	ADSC	Decrease ROS production and pro-inflammatory cytokine expression, and promote collagen deposition and granulation tissue formation

was suppressed compared to the exosomes isolated from ADSCs pretreated GW4869, a neutral sphingomyelinase inhibitor that blocks exosome generation, leading to the conclusion that ADSC-Exos were able to attenuate the hyperglycemia-induced endothelial progenitor cells (EPCs) senescence. Additionally, exosomes isolated from NRF2 overexpressed-ADSCs also decrease ROS production and pro-inflammatory cytokine expression, and promote collagen deposition and granulation tissue formation, as measured by Masson Trichrome staining and HE staining [68].

miR-21 miR-21 is one of the most investigated miRNAs in the context of various diabetic complications, including DFU, myocardial ischemia/reperfusion, and diabetic kidney diseases [64, 69]. miR21 is a responsive protective molecule which has been reported to present different mechanisms in diabetic wound healing. For example, miR-21 was positively associated with MMP9 expression and DC differentiation through downregulating phosphatase and tensin homolog (PTEN) and stimulating PI3K/AKT signalling pathway in a full-thickness wound rat model [70]. In what could be another wound healing mechanism, that TGF-β activated NF-κB signaling pathway, and the CHIP assay demonstrated that TGF-β facilitated NF-κB p65 subunit to bind with miR-21 promoter directly in fibroblasts in a high glucose condition, which may promote fibroblast migration [51]. Remarkably, miR-21-3p enriched in UCB-Exos was able to inhibit PTEN and sprout homolog I (SPRY I), contributing to accelerated re-epithelialization, reduced scar widths, and angiogenesis [52].

miR-23 The miR-23 family (miR-23a/b/c) has been reported to enhance angiogenesis by activating angiogenic signaling through targeting Sprouty2 and Sema6A [53]. However, data from the tissue biopsies of DFU in patients reported that only miR23c regulates DFU healing by targeting stromal cell-derived factor-1α (SDF-1α/CXCL12) [71]. In a full-thickness skin defect mouse model, miR-23 carried by UB-MSC-Exos played a critical role in myofibroblast differentiation during scare formation via suppression of TGF-β/SMAD2 stimulation [72].

miR-126 miR-126 is recognized as a master regulator which mediates physiological angiogenesis and inflammation [54, 73, 74]. Jason et al. have shown that miR-126 regulated the response of endothelial cells to VEGF, and directly suppressed the negative mediators of the VEGF and FGF signaling pathway, including the Sprouty-related protein SPRED1 and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2/p85-β) [56, 75]. Exosomes derived from miR-126 overexpressed synovium MSCs (SMSC-126-Exos) activated the proliferation of human dermal fibroblasts and human

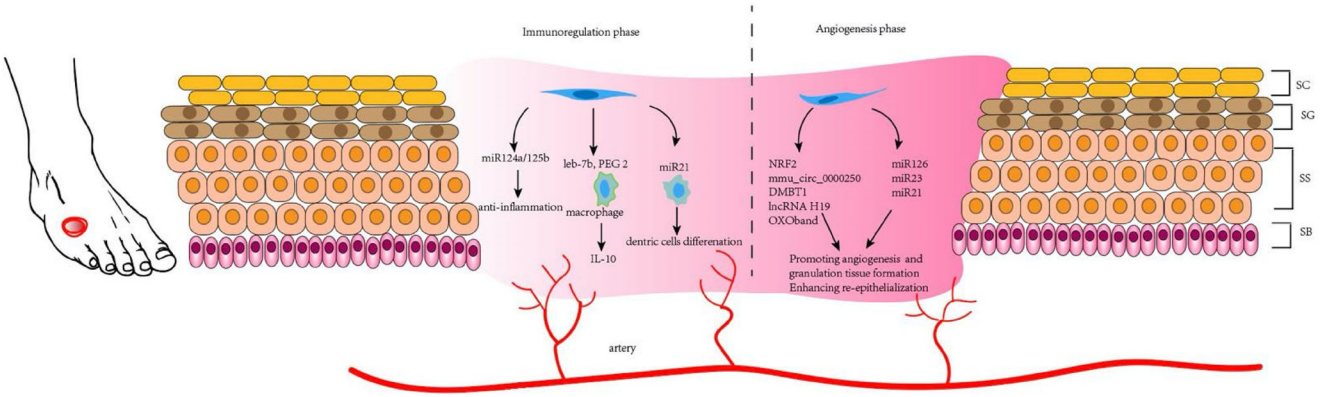

Fig. 1 MSCs show great therapeutic potential in immunomodulatory and angiogenesis phases of DFU. In immunoregulatory phase, MSCs are capable of secreting miR124a/125b to relieve inflammation response, producing leb-7b, PEG2 to modulate macrophage polarization, and secreting miR21 to regulate dendritic cell differentiation. In angiogenic phase, MSCs own the ability to produce NRF2, mmu_circ_0000250, DMBT1, lncRNA H19, OxOband, miR126, miR23, and miR21 to promote the process of angiogenesis, granulation tissue formation and re-epithelialization. SC: stratum corneum; SG: stratum granulosum; SS: stratum spinosum; SB: stratum basale

dermal microvascular endothelial cells (HMEC-1), and SMSC-126-Exos also stimulated the migration and tube formation of HMEC-1 and collagen maturity [57].

lncRNA H19 Long noncoding RNAs H19 (lncRNAs H19) have been reported to participate in the regulation of hepatic glucose production and insulin resistance in a hyperglycemia microenvironment [58]. Fibroblasts isolated from patients with DFU co-cultured with exosomes derived from lncRNA H19 overexpressed MSCs demonstrated that lncRNA H19 may exert a positive effect on proliferation, migration, and suppression of apoptosis and inflammation of the fibroblasts through miR-152-3p/ PTEN axis [76], contributing to a stimulated wound-healing process in DFU.

Let-7b LPS preconditioned UC-MSC exosomes showed a stronger effect on modulating macrophage polarization and increasing anti-inflammatory cytokine secretion than untreated UC-MSC exosomes. Moreover, miRNA microarray analysis exhibited that UC-MSC exosomes shuttled let-7b to macrophages and regulated macrophage plasticity through activating TLR4/NF-κB/STAT3/AKT signaling pathway in a high glucose (HG) condition [59].

mmu_circ_0000250 There is emerging evidence that circular RNAs (circ RNAs) exert important roles in mediating the microenvironment of wound healing [60]. In an in vitro tubule formation model of EPCs on Matrigel-coated culture wells, angiogenesis was inhibited in the HG microenvironment, and the inhibition was reversed by mmu_circ_0000250 modified ADSCs-Exos. Moreover, a luciferase reporter assay and qPCR revealed that overexpression of mmu_circ_0000250 suppressed miR-128-3p expression and enhanced autophagic plaque formation in EPCs in a HG microenvironment. In a streptozotocin-induced diabetic mice model, mmu_circ_0000250 was able to accelerate full-thickness cutaneous wound healing in the feet [77].

Recently, accumulating data have shown that human circulating fibrocytes ($CD34^+$ bone marrow-derived progenitor cells) possess the capacity to differentiate into osteoblasts and chondrocytes, which meet the definition of MSCs partially and are able to remodel extracellular matrix components and acquire myofibroblast-like properties in wounds [61, 78]. Geiger et al. have demonstrated fibrocyte-derived exosomes containing various kinds of miRNAs that possess distinct effects in accelerating wound healing, such as regulating collagen deposition (miR-21), proangiogenic (miR-126, miR-130, miR-132) and anti-inflammatory (miR-124a and miR-125b) [55].

Barriers in MSC-EVs for Clinical Application

Unfortunately, until now there are no clinical trials aimed at MSC-EV treatment in DFU. Even though MSC-EVs show therapeutic value in DFU models, the disparities between experimental results and potential actual outcomes of clinical trials still require a more profound view of the role of MSC-based therapy in regenerative medicine. Based on other ongoing and completed clinical trials of MSC treatment in DFU, some clinical challenges and underlying limitations need to be addressed before MSC-EVs can be administrated in humans.

The lack of standardized and optimized criteria contributes to the main challenge of MSC-EV application. Protocols from different institutes vary in relation to EV preparation, including EV isolation, characterization, and purification. Hence, the academic societies (ISEV, ISCT, and ISBT) should take on the responsibility of proposing consolidated and well-standardized criteria, and collaborate with biomedical centers worldwide to share cutting-edge improvements.

Finally, developing a safety and efficacy approach to generating large-scale MSC-EVs is the main headwind to deal with. Although some protocols have been established for biomanufacturing exosomes, knowledge about the biomanufacturing of microvesicles remains limited [79, 80].

Conclusions

Today millions of DM patients are still fighting with DFU, and controlling blood glucose level is recognized as the first step to fight this chronic complication. Pro-clinical data support the idea that MSC-EV therapy possesses immunomodulatory and reparative properties which accelerate diabetic wound healing. However, to overcome the barriers to use which exist from laboratory to hospitals, the mechanisms of MSC-EV's therapeutic potential must be fully understood and good manufacturing practice protocols found.

Despite the challenges that must be overcome, the evidence indicates MSC-EVs deserve further invsitigation due to their promising value in combating DFU.

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Yi Chen: Syntax checking. Yingchun Tu: Syntax checking.
Ping Lin: Writing- Reviewing and Editing.

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Compliance with Ethical Standards

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