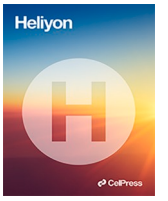
Heliyon 10 (2024) e37031

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

Advancements in diabetic foot ulcer research: Focus on mesenchymal stem cells and their exosomes

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ARTICLE INFO

Keywords:
Diabetic foot ulcers
Mesenchymal stem cells
Exosomes

ABSTRACT

Diabetes represents a widely acknowledged global public health concern. Diabetic foot ulcer (DFU) stands as one of the most severe complications of diabetes, its occurrence imposing a substantial economic burden on patients, profoundly impacting their quality of life. Despite the deepening comprehension regarding the pathophysiology and cellular as well as molecular responses of DFU, the current therapeutic arsenal falls short of efficacy, failing to offer a comprehensive remedy for deep-seated chronic wounds and microvascular occlusions. Conventional treatments merely afford symptomatic alleviation or retard the disease's advancement, devoid of the capacity to effectuate further restitution of compromised vasculature and nerves. An escalating body of research underscores the prominence of mesenchymal stem cells (MSCs) owing to their paracrine attributes and anti-inflammatory prowess, rendering them a focal point in the realm of chronic wound healing. Presently, MSCs have been validated as a highly promising cellular therapeutic approach for DFU, capable of effectuating cellular repair, epithelialization, granulation tissue formation, and neovascularization by means of targeted differentiation, angiogenesis promotion, immunomodulation, and paracrine activities, thereby fostering wound healing. The secretome of MSCs comprises cytokines, growth factors, chemokines, alongside exosomes harboring mRNA, proteins, and microRNAs, possessing immunomodulatory and regenerative properties. The present study provides a systematic exposition on the etiology of DFU and elucidates the intricate molecular mechanisms and diverse functionalities of MSCs in the context of DFU treatment, thereby furnishing pioneering perspectives aimed at harnessing the therapeutic potential of MSCs for DFU management and advancing wound healing processes.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No.8217081220), the Jilin Scientific and Technological Development Program (Grant No. 20240205001YY), the Changchun Science and Technology Development Plan Project (Grant No. 21ZGY29)

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https://doi.org/10.1016/j.heliyon.2024.e37031
Received 18 June 2024; Received in revised form 11 August 2024; Accepted 26 August 2024
Available online 29 August 2024

1. Introduction

Diabetes manifests as a metabolic disorder marked by diminished pancreatic function and insulin resistance, culminating in hyperglycemia, attributable to the intricate interplay of immune, genetic, psychological, and environmental factors. Moreover, individuals afflicted with diabetes present with the hallmark clinical manifestations coined as "polyuria, polydipsia, polyphagia, and weight loss. Diabetes is typically categorized into the following four types: type 1 diabetes (insulin-dependent diabetes), type 2 diabetes (non-insulin-dependent diabetes), other distinct variants of diabetes, and gestational diabetes [1]. Type 1 diabetes mellitus typically manifests in childhood or adolescence due to an autoimmune-mediated destruction of pancreatic β-cells, resulting in insufficient or completely absent insulin production. Consequently, patients must rely on exogenous insulin to regulate blood glucose levels. In contrast, Type 2 diabetes mellitus, the most prevalent form of diabetes, generally develops in adulthood. It is characterized by a dual pathophysiology of insulin resistance coupled with inadequate insulin secretion, leading to hyperglycemia. Other distinct forms of diabetes are attributable to specific etiologies, commonly encompassing genetic defects, drug or chemical-induced diabetes, and diabetes secondary to endocrine disorders. Gestational diabetes mellitus (GDM) is defined as glucose intolerance first identified during pregnancy. Although blood glucose levels usually normalize postpartum, these patients are at an elevated risk for progressing to Type 2 diabetes mellitus later in life. Presently, as a result of enhancements in quality of life and the exacerbation of the aging population conundrum, the global incidence of diabetes exhibits an annual upward trajectory. The prognostications from the International Diabetes Federation project a surge in the global diabetic populace to 643 million (constituting 11.3 % of the populace) by 2030, and a subsequent escalation to 783 million by 2045 (comprising 12.2 % of the populace) [2]. Over recent years, there has been a discernible trend towards a gradual reduction in the age of diabetes mellitus onset, concomitant with the protracted course of the condition and its myriad complications, which have exerted an augmented impact on both the health status and economic outlays of patients.

Owing to diabetes being characterized as a systemic metabolic disorder, as the disease advances, patients endure prolonged hyperglycemia, disrupting not only the internal milieu's equilibrium but also instigating chronic impairment and dysfunction across diverse bodily tissues, culminating in irreversible damage to assorted organs and tissues, consequently precipitating a myriad of complications. Common complications associated with diabetes encompass diabetic nephropathy, diabetic retinopathy, diabetic foot ulcers, cardiovascular disease, neuropathy, and cerebrovascular complications. As the disease progresses, sustained hyperglycemia exacerbates non-enzymatic glycation, leading to the accumulation of advanced glycation end-products (AGEs) that are detrimental to vascular endothelial cells and adjacent tissues. Simultaneously, elevated blood glucose levels contribute to increased oxidative stress, resulting in the generation of excessive free radicals that inflict damage on endothelial cells. Moreover, chronic inflammation is frequently observed in diabetic patients, which further compromises endothelial function and accelerates the onset of microvascular pathology, thereby contributing to the development of chronic wounds [3,4]. Among these, diabetic foot ulcers(DFU) represent one of the most prevalent complications. DFU primarily ensues from ischemic, neuropathic, or a confluence of ischemic-neuropathic aberrations [5]. Failure to promptly mitigate the condition post-incidence can instigate limb necrosis, culminating in a heightened disability quotient [6]. Studies elucidate that roughly 25 % of individuals afflicted with diabetes will progress to diabetic foot ulcers, with an additional 30 % of diabetic foot ulcer patients destined for amputation following disease advancement [7]. The malady is distinguished by its protracted trajectory, intricacy, dismal prognosis, exerting a profound toll on the quality of life of afflicted individuals, and exacting a substantial economic burden on both societal and individual levels [8]. Presently, conventional therapeutic modalities in clinical settings encompass blood glucose regulation, wound dressings alterations, hyperbaric oxygen therapy, negative pressure wound therapy, total contact casting, wound debridement, tissue grafting, among others [9]. However, Numerous constraints beset these therapeutic approaches, meriting them capable solely of mitigating patient symptoms or retarding disease advancement, devoid of any reparative effect on impaired blood vessels and nerves, thus perpetuating the recalcitrance of wound healing.

MSCs represent a cadre of cells distinguished by their remarkable secretion, immunomodulatory, and homing attributes. These cells can be derived from a plethora of tissues, encompassing bone marrow, umbilical cord, adipose tissue, amniotic fluid, placenta, menstrual blood, and beyond. Owing to their capacity to generate a plethora of biologically active substances including growth factors, cytokines, chemokines, and beyond, coupled with their immunomodulatory prowess and aptitude for differentiation into specialized tissue cells, these cells wield significant influence in stimulating vascular regeneration, mitigating inflammation, engaging in extracellular matrix remodeling, fostering wound healing, and beyond [10]. Through paracrine action, MSCs have the capacity to liberate exosomes laden with proteins, miRNA, long non-coding RNA(lncRNA), and other bioactive molecules. The secretome originating from MSCs is presently deemed the principal mechanism through which they mitigate DFU. Consequently, MSCs and their exosomes have garnered frequent utilization in investigations pertaining to diabetic wound healing. The present paper systematically expounds upon the role and mechanism of action of MSCs and their exosomes in DFU healing.

2. Pathophysiology of DFU

Upon the onset of diabetes, the organism experiences a state of metabolic disruption. The presence of a hyperglycemic and inflammatory microenvironment results in endothelial injury, microvascular hemorrhage, leakage, and microthrombus formation. Furthermore, it induces the accumulation of Advanced Glycation End-products (AGEs) within the body [11]. AGEs constitute a category of highly active end products resulting from the non-enzymatic glycation of proteins, fatty acids, and nucleic acids within the organism, involving their amino and reducing sugar residues, with a profound correlation observed between their generation and the myriad complications of diabetes [12]. The accrual of AGEs within the organism manifests a potent deleterious impact on neural tissues. The activation of specific transcription factors by AGEs escalates the organism's inflammatory cascade and oxidative stress levels, eliciting consequential functional derangements in tissue cells. This, in turn, suppresses monocyte-macrophage functionality

and diminishes cytokine secretion levels, thereby compromising vascular homeostasis, culminating in the manifestation of skin thinning in diabetic patients [13]. The thinning of the skin at specific regions renders the skin's protective barrier increasingly vulnerable to breach, thereby exposing the underlying deep tissues. Various infectious bacteria and viruses are more likely to invade the deeper tissues and further multiply and grow, eventually causing infection and suppuration of the wound. Moreover, as the condition advances, the wound exhibits gradual enlargement and ulceration [14]. Moreover, the inflammatory microenvironment in diabetic wounds obstructs the progression of wound healing during the proliferative phase and disrupts crucial processes such as angiogenesis, extracellular matrix (ECM) remodeling, and re-epithelialization [15].

In addition to disruptions in glucose metabolism, metabolic dysregulation in the organism extends to abnormalities in lipid

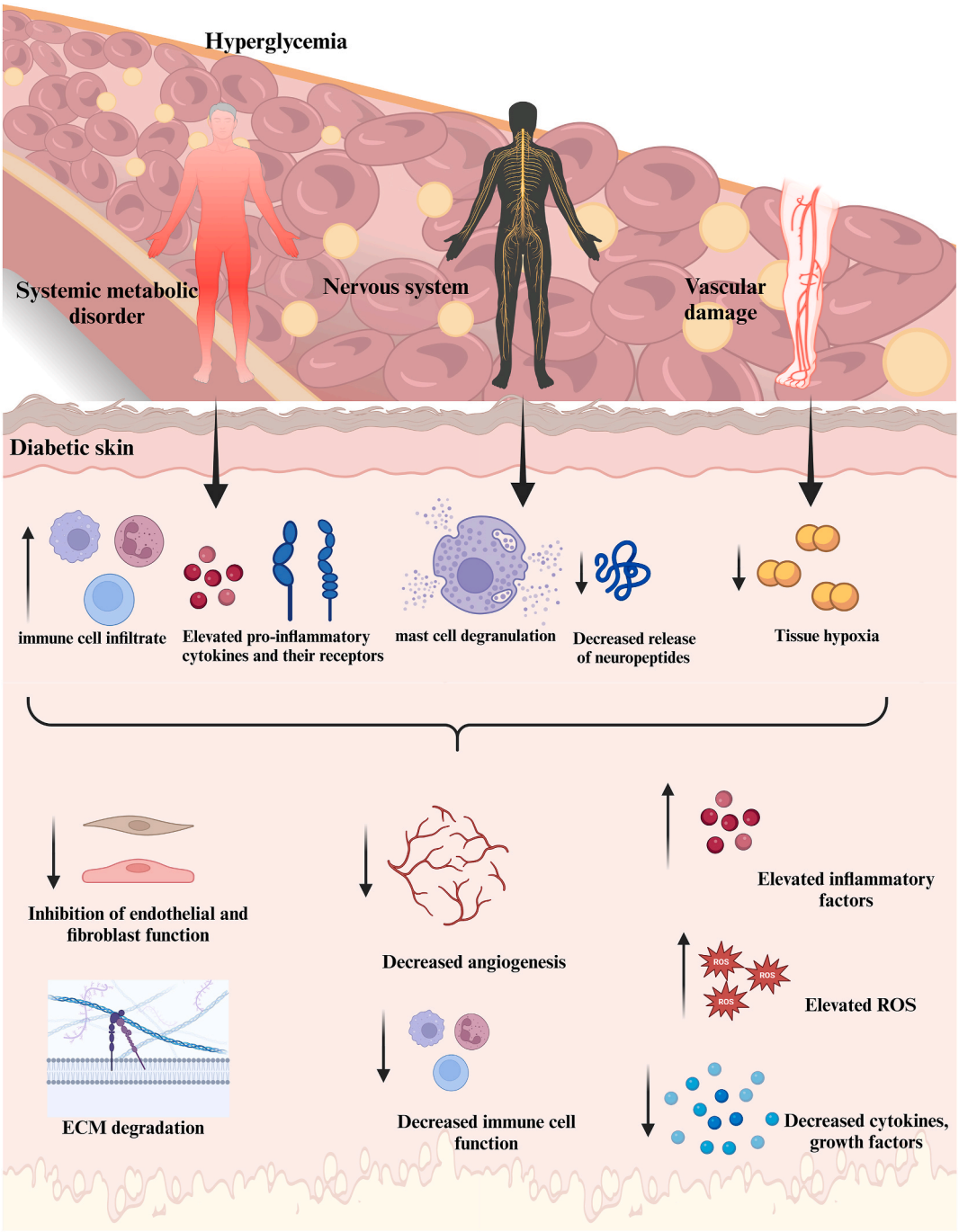

Fig. 1. Schematic pathophysiology of DFUs.

metabolism. The disorder in lipid metabolism facilitates the release of inflammatory mediators, consequently provoking infiltration by immune cells, predominantly macrophages [16]. Stimulated by elevated levels of blood glucose and blood lipids, the organism undergoes further release of inflammatory mediators, thereby perpetuating a state of heightened inflammation within the organism. In addition to the systemic increase in pro-inflammatory cytokines, diabetic skin also exhibits elevated concentrations of pro-inflammatory cytokines and their receptors [17]. Concurrently, diabetic patients experience a decrease in the phagocytic ability of immune cells, including white blood cells [18]. Within the region of the wound, there is a sustained elevation in local inflammation factor levels, concurrent with a reduction in white blood cell activity. This phenomenon imposes a significant inhibitory effect on the functionality of vascular endothelial cells and fibroblasts, thereby hampering the formation of granulation tissue. Owing to the persistent stimulation by high glucose levels, the organism experiences heightened levels of oxidative stress, culminating in an increase in oxygen species (ROS) expression [19]. This, in turn, diminishes the organism's antioxidant capacity, thereby exerting inhibitory effects on the release of cytokines and growth factors. Concurrently, the activity of fibroblasts, the synthesis of collagen fibers, and the formation of new blood vessels are subject to varying degrees of influence. Consequently, this culminates in capillary constriction, thereby exacerbating microcirculatory disturbances. In the protracted presence of high glucose stimuli, the nervous system is subject to significant impact [20], predominantly characterized by the degeneration of peripheral nerve axons and nerve membrane cells. Consequently, this suppresses the functionality of the organism's motor, sensory, and autonomic nervous systems, thereby impeding limb perfusion and culminating in muscle atrophy, as well as tendon and ligament sclerosis [21]. Studies have shown that in skin injuries of diabetic rabbits and mice induced by alloxan and streptozotocin, the expression of Substance P and other neuropeptides released by nociceptive C-fibers is reduced. This may result in decreased leukocyte recruitment following injury [22]. Additionally, studies have found that the skin of streptozotocin-induced diabetic mice exhibits mast cell degranulation, which correlates with a diminished ability to mount an inflammatory response to tissue injury [23]. With further progression of the disease, foot deformities may manifest. Owing to the buildup of metabolic byproducts within the organism, insufficient blood and oxygen delivery to the surrounding wound area, compounded by factors such as prolonged exposure to high glucose environments and inflammatory responses, diabetic wounds manifest features indicative of impaired healing. In the event of infection, it can instigate the exacerbation of the local wound, gradually evolving into necrosis [24].

The formation and progressive deterioration of DFU are intricately linked with numerous pathophysiological processes, which have the potential to interact, interconvert, and even synergistically compound each other, thereby underscoring the treatment of DFU as one of the pressing and pivotal issues that needs immediate attention [25].(Fig. 1).

The investigation into the mechanisms behind DFU formation motivates us to further explore the interplay between the local microenvironment of diabetes and the process of vascular formation. This exploration helps in offering more scientifically grounded and efficient avenues for clinical treatment, while also elucidating the root causes behind the delayed healing of diabetic wounds.

3. Mechanisms underlying wound healing

The process of wound healing encompasses four dynamic stages: hemostasis, inflammation, proliferation, and remodeling [26]. Upon injury, the body promptly initiates hemostasis to halt bleeding. This intricate process entails a myriad of biological responses [27](Fig. 2). Initially, there is reactive constriction of small blood vessels and capillaries surrounding the wound, leading to reduced local blood flow, thereby preventing excessive bleeding [28]. Moreover, exposure of endothelial cells prompts the aggregation of nearby platelets, drawn by collagen fibers and tissue factors, culminating in the formation of a blood clot to avert further blood loss. The blood clot comprises cross-linked fibrin, activated factor XIII, and fibrinogen, with fibrin acting as a provisional extracellular matrix (ECM) [29]. Recruited platelets release adhesive proteins such as serotonin, thromboxane A2, fibrinogen, and fibrinogen-binding protein. Under the influence of adhesive proteins and local clotting factors, additional platelet aggregation occurs, leading to platelet plug formation [30]. Through the action of thrombin, fibrinogen gradually converts into fibrin, thereby forming a fibrin clot. This process not only promotes hemostasis but also prevents pathogen invasion, serving as a scaffold for the attachment of various cells in the later stages. Upon entering the inflammation stage of the wound healing process, local vasoconstriction induces tissue ischemia, prompting the release of histamine and various vasoactive substances, consequently facilitating the dilation of local blood vessels [31]. Provoked by necrotic tissue and invading microorganisms, the body instigates an inflammatory response, characterized by the migration of white blood cells and other pertinent cells toward the wound site. The initial cells to infiltrate the wound

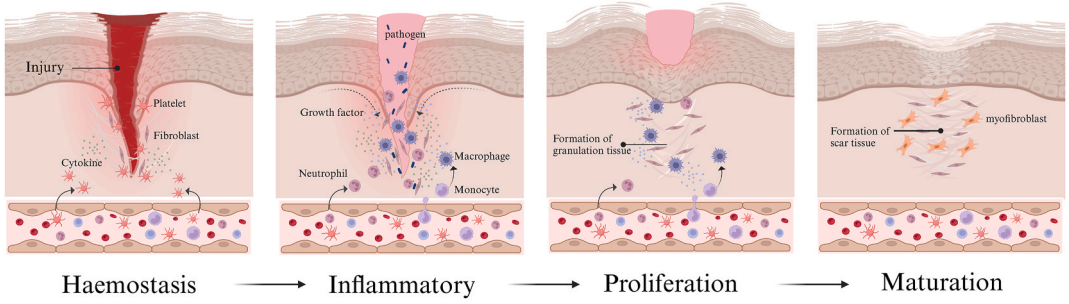

Fig. 2. The process of wound healing comprises four distinct stages: hemostasis, inflammation, proliferation, and remodeling.

are neutrophils, proficient in eradicating invading microorganisms while simultaneously engulfing necrotic debris and microbes. Neutrophils execute their bactericidal function by releasing proteases. Furthermore, neutrophils play a pivotal role in initiating the production of growth factors for macrophages, keratinocytes, and fibroblasts [32]. Following neutrophils, monocytes enter the wound and differentiate into macrophages, constituting indispensable cells in the wound healing cascade. Macrophages are primarily tasked with phagocytosing necrotic debris, microbes, and apoptotic neutrophils. Furthermore, during the inflammatory phase, macrophages secrete growth factors and other substances conducive to wound healing, which hold substantial importance in cell proliferation and migration [33]. Neutrophils and macrophages possess the capability to modify the provisional ECM, facilitating the progressive development of granulation tissue, which holds pivotal significance in the wound healing process [34]. The repair phase can be delineated into two stages: epithelial regeneration and granulation tissue formation. Wound repair commences from the wound edges, where residual basal cells proliferate and progressively migrate inward. Throughout this process, the proliferative activity of basal cells stimulates reactive proliferation of capillaries and connective tissue within the wound bed. Simultaneously, basal cell proliferation exerts a promotive effect on the growth of granulation tissue. Macrophages secrete a multitude of growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and transforming growth factor-alpha (TGF-α), among others, which play a pivotal role in fostering the conversion of the provisional ECM into granulation tissue [35]. The ECM acts as a scaffold for fibroblasts and endothelial cells, facilitating angiogenesis and fibroblast proliferation. Granulation tissue fills tissue defects while also safeguarding the wound site, thereby thwarting bacterial infection. The remodeling phase is typified by cellular apoptosis and collagen remodeling. Endothelial cells and myofibroblasts within granulation tissue undergo apoptosis, progressively transitioning into wound scars. Notably, the apoptosis of endothelial cells results in a decrease in blood vessel density, leading to a gradual lightning of scar color. In order to augment the tensile strength of the wound, collagen precursor fibers progressively align into bundles and undergo intermolecular cross-linking [27], culminating in scab formation at the wound site.

Following the aforementioned dynamic processes, the wound gradually undergoes healing. Nevertheless, diabetic patients present with anomalous wound characteristics: owing to the sustained hyperglycemic and inflammatory conditions within the body, the formation of granulation tissue is compromised, consequently hindering tissue remodeling [36]. Consequently, wound healing in patients stagnates or regresses to a certain stage, resulting in delayed wound healing.

4. Factors affecting DFU healing

In normal circumstances, the healing of skin wounds represents a transient, time-bound process of self-repair aimed at reinstating the damaged skin's anatomical structure and physiological function [37]. When patients are afflicted with diabetes, factors including high blood glucose stimulation, aberrant local wound microenvironment, and microvascular occlusion, among intrinsic factors, as well as external factors like wound infection, inadequate debridement, and the patient's unhealthy lifestyle habits [38], impede DFU wound healing(Fig. 3). The mechanism behind delayed wound healing is intricate and remains incompletely understood to date. Presently, several acknowledged factors influencing DFU healing comprise the following.

4.1. Inflammation caused by bacteria

The high glucose microenvironment provides an optimal environment for the growth and proliferation of bacteria and various pathogens, rendering bacteria less susceptible to clearance, leading to their accumulation around the wound site. Common bacteria

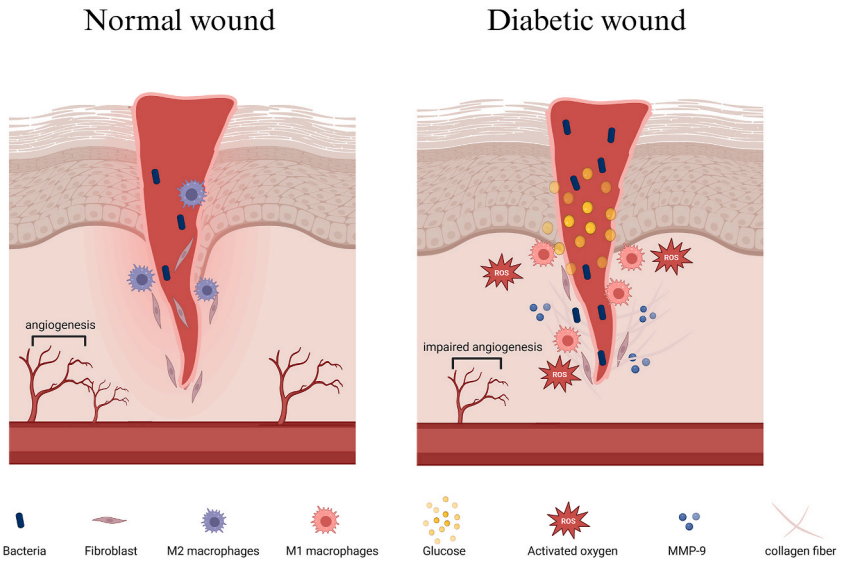

Fig. 3. Comparative analysis of wound healing in diabetic wounds versus normal wounds.

found in wound sites include Staphylococcus aureus, *Pseudomonas aeruginosa*, and β-hemolytic streptococcus. Apart from directly harming the host, these bacteria amplify inflammatory cytokines, proteases, and ROS to attract white blood cells, thus initiating and perpetuating inflammatory cascade reactions [39]. The proteases and ROS produced by bacteria degrade ECM and growth factors, thereby affecting cell migration and impeding the wound healing process.

4.2. Hypoxia at the site of localized injury

Elevated blood glucose levels in diabetic patients damage microvascular endothelial cells, resulting in microcirculatory abnormalities, erythrocyte membrane narrowing, local tissue ischemia, and circulatory disturbances. This ultimately leads to inadequate oxygen delivery to the local injured area, causing local tissue hypoxia, which is a primary contributing factor to diabetic wound damage. Furthermore, during the inflammatory phase, there is an increased oxygen demand by cells at the wound site, exacerbating wound hypoxia [40]. This ultimately results in inadequate energy supply for wound repair, impeding fibroblast proliferation, cell proliferation, and tissue self-repair mechanisms. Local tissue hypoxia in diabetic patients may be exacerbated by increasing ROS to amplify the inflammatory response.

4.3. Imbalance in matrix metalloproteinases (MMPs) expression

MMPs, as zinc-dependent endopeptidases, play a crucial role in the degradation of ECM components [41]. MMPs are involved in all stages of wound healing. During the inflammatory phase, MMPs remove damaged proteins and temporary ECM; in the proliferative phase, MMPs facilitate the degradation of capillary basement membrane, angiogenesis, and cell migration. In the remodeling phase, MMPs regulate tissue contraction and remodeling [42]. Based on the specificity of MMP substrates, they can be categorized into collagenases (MMP-1, MMP-8, MMP-13, MMP-18), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, MMP-11), membrane-type (MT) metalloproteinases (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, MMP-25), matrilysins (MMP-7, MMP-26), and others (MMP-12, MMP-19, MMP-20, MMP-21, MMP-23, MMP-27, MMP-28) [43,44]. Among these, gelatinases (MMP-2 and MMP-9) and collagenases (MMP-1 and MMP-8) play indispensable roles in the wound healing process. Tissue inhibitors of metalloproteinases (TIMPs) regulate MMPs by blocking their activity. Under normal physiological conditions, the interaction between MMPs, growth factors, and receptors ensures the equilibrium of the ECM. However, in the state of DFU, the expression of ECM proteins and their inhibitors becomes imbalanced, leading to matrix degradation [45]. This further results in decreased levels of growth factors and TIMPs, and increased expression of proteases and MMPs, with notable changes in MMP-1, MMP-2, MMP-8, and MMP-9. Concurrently, neutrophils and macrophages release pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α). Among the various upregulated MMPs, MMP-9 has been identified through research as a biochemical marker associated with the formation and persistence of chronic wounds [46]. In DFU, MMP-9 plays roles in regulating keratinocyte function and degrading collagen. Its overexpression impairs the migratory ability of keratinocytes, preventing their migration to the wound site, thereby hindering wound healing.

4.4. Abnormal macrophage phenotype

The dysregulation of macrophage polarization induced by high blood sugar and oxidative stress is one of the primary factors contributing to DFU. Excessive inflammation at the wound site prolongs the healing phase, causing it to remain in the inflammatory stage for an extended period, preventing a normal transition to the stages of cell proliferation and tissue remodeling, thus substantially delaying wound healing and significantly heightening the risk of wound infection. The excessive inflammatory response at the wound site is intimately linked to aberrant macrophage phenotypes. Macrophages are immune effector cells that play a crucial role in tissue damage response and the maintenance of homeostasis. Functionally, activated macrophages can be broadly classified into two types: classically activated macrophages (caMϕ or M1 type) and alternatively activated macrophages (aaMϕ or M2 type). M1 macrophages represent a pro-inflammatory activation state, whereas M2 macrophages are characterized by anti-inflammatory and tissue repair-promoting functions. M1 macrophages are activated by interferon-γ secreted by $CD4^+$ T helper cells (Th1), lipopolysaccharides (LPS) from Gram-negative bacteria, macrophage colony-stimulating factor (M-CSF), and tumor necrosis factor (TNF) [47]. The release of antimicrobial and anti-tumor inflammatory factors by M1 macrophages leads to tissue damage induced by ROS, which in turn affects tissue regeneration and wound healing. In contrast, M2 macrophages are predominantly induced by cytokines such as IL-4 and IL-13 and exhibit high levels of CD206 expression. These macrophages demonstrate enhanced phagocytic abilities and secrete anti-inflammatory cytokines, including IL-10 and TGF-β. M2 macrophages play a pivotal role in promoting tissue repair and angiogenesis while mitigating inflammatory responses in inflammatory diseases. In diabetic wounds with impaired healing, there is an accumulation of M1 macrophages and a reduction in M2 macrophages, leading to the retention of wound healing in the inflammatory phase and impeding the transition to the phases of cell proliferation and migration [48]. The impaired transition of macrophage phenotypes from M1 to M2 in DFU is associated with compromised wound closure, inadequate vascularization, and diminished collagen deposition [49].

4.5. Excessive deposition of collagen fibers

The excessive deposition of collagen fibers results in the formation of dense fibrotic tissue, which diminishes tissue elasticity and flexibility, restricts cell migration, and obstructs the transport of nutrients and oxygen. Furthermore, collagen fibers entrap and

sequester growth factors, reducing their availability for interaction with cell surface receptors, thus significantly impairing tissue repair and remodeling processes. This may be associated with reduced secretion of urinary plasminogen activator (uPA) and increased secretion of urinary plasminogen activator inhibitor (uPAR) at the site of diabetic wounds [50].

4.6. Impaired angiogenesis and neovascularization injury

Continuously elevated blood glucose levels inhibit the typical flow of blood to the extremities, resulting in localized tissue hypoxia and insufficient nutrient delivery, consequently limiting angiogenesis. Additionally, abnormalities in endothelial progenitor cell function induced by diabetes could further aggravate the impairment of neovascularization in DFU [51].

4.7. Reduced activity of functional factors

Elevated blood glucose levels impact the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1α (HIF-1α), concurrently promoting the non-enzymatic glycation of numerous vital proteins. Non-enzymatic glycation of proteins refers to a chemical reaction in which sugar molecules covalently bond with amino groups of proteins without the participation of enzymatic catalysts. This process disrupts protein structure and functionality, thereby impeding multiple physiological processes crucial for wound healing [52]. In individuals with diabetes, non-enzymatic glycation is a major factor contributing to delayed wound repair. Therefore, effective management of blood glucose levels and regulation of non-enzymatic glycation reactions are critical for enhancing wound healing.

5. Treatment methods

Presently, standard therapeutic modalities for DFU predominantly involve wound debridement, dressing changes, hyperbaric oxygen therapy, negative pressure wound therapy, and wound offloading. These prevalent foundational therapies contribute to the management and amelioration of symptoms. Moreover, MSC-based therapies have garnered significant attention due to their novel approach in enhancing the healing of DFUs (Refer to Table 1 for a comparative analysis of the advantages and limitations of various

Table 1
A comparative analysis of the advantages and limitations of various treatment modalities for DFU.

Treatment methods	Advantages	limitations
Regulate blood glucose levels	By addressing the underlying causes of DFUs, wound healing can be significantly promoted.	Indirect treatment methods for DFUs fail to directly target the wound itself.
Manage and control infections	By managing infections to eliminate the substantial presence of pathogenic microorganisms around the wound, the inflammatory response induced by bacteria can be effectively suppressed.	The sources of infection in DFU can be diverse and complex and conventional antibiotic therapy alone may not effectively address all infection-related issues. Furthermore, deep-seated infections commonly associated with DFU are often difficult to control with topical antibiotic therapy alone and may require more invasive treatment approaches.
Perform wound debridement	Debridement facilitates the healing of healthy granulation tissue by removing necrotic tissue and foreign debris from the wound bed, making it a fundamental approach in the treatment of DFUs.	Debridement is only one component of a comprehensive treatment strategy for DFUs. Relying solely on debridement may not achieve complete resolution of DFUs. To attain optimal outcomes, debridement must be integrated with other therapeutic modalities.
Apply wound dressings	Wound dressings not only serve as a protective barrier for DFUs but also play a role in antimicrobial activity and the promotion of vascular and tissue regeneration.	Wound types and conditions vary among patients, and universal dressings may not meet the needs of all individuals. Therefore, treatment plans must be tailored based on the specific wound characteristics and the overall health status of the patient.
Decompression of wounds	Implementing offloading therapy to reduce plantar pressure is crucial for enhancing the healing of DFUs.	Wound decompression therapy does not address the underlying causes of delayed wound healing. To achieve the best therapeutic outcomes, it is essential to combine offloading therapy with other treatment modalities.
Employ negative pressure wound therapy (NPWT)	NPWT not only maintains a clean and dry wound environment but also reduces the frequency of dressing changes.	The safety and efficacy of NPWT for DFU remain largely unclear. with ongoing debate and uncertainty regarding its application [95].
Administer hyperbaric oxygen therapy (HBOT)	HBOT not only promotes collagen synthesis, growth factor production, and neovascularization but also exhibits significant antimicrobial effects against anaerobic bacteria.	HBOT is currently limited to improving the short-term outcomes of DFUs, and there remains substantial debate regarding the overall efficacy of this treatment.
Employ skin substitutes	Skin substitutes can mimic the natural ECM and serve as carriers for cells and growth factors, thereby effectively enhancing wound healing.	As foreign materials, skin substitutes carry the risk of being rejected by the body.
Treatment utilizing MSCs	Unlike traditional therapies that merely protect the wound and rely on the body's inherent healing capacity, MSC therapy promotes wound healing through systematic regulation of multiple aspects related to DFUs. Therefore, it presents a more effective alternative to conventional treatments.	MSCs therapy is associated with limitations such as the risk of recipient cell rejection and potential for tumorigenesis.

treatment modalities.).

5.1. Regulate blood glucose levels

The sustained hyperglycemic state in diabetic individuals can result in complications such as Diabetic Neuropathy (DN), Peripheral Arterial Disease (PAD), and immune suppression, all of which exert substantial influence on the healing process of DFU. Hence, blood sugar control can profoundly facilitate wound healing from its root. Research indicates that a notable enhancement in wound healing occurs with decreased blood sugar level [53]. Currently, clinical practices typically employ biguanides, sulfonylureas, and insulin to regulate patients' abnormally elevated blood glucose levels. Additionally, maintaining dietary control, regular exercise, and healthy lifestyle habits play an indispensable role in regulating blood glucose levels.

5.2. Manage and control infections

Owing to the extended duration required for wound healing in diabetic patients, wounds are exposed for prolonged periods, resulting in a significant presence of pathogenic microorganisms surrounding the wound. Research suggests that the infection rate at the wound site of DFU patients is approximately 40 % [54]. To initiate targeted therapy, it is crucial to accurately identify the causative agent. Microbiological analysis of soft tissue or bone samples from the patient is commonly performed to ascertain the pathogen's identity. Antibiotics are commonly employed for infection control, with frequently used medications including penicillins, cephalosporins, fluoroquinolones, carbapenems, and others. In cases where antibiotic therapy fails to yield satisfactory outcomes, surgical intervention may be warranted.

5.3. Perform wound debridement

Debridement encompasses the elimination of infected tissue, aging and necrotic tissue, bacterial biofilm, and foreign bodies from the wound bed to promote the healing of surrounding healthy granulation tissue [55]. This method stands as the cornerstone in the treatment of DFU. Multiple approaches, such as surgical debridement, enzymatic debridement, mechanical debridement, ultrasound debridement, and bio-surgical debridement, can be employed. The objective is to stimulate wound healing through the targeted stimulation of the wound bed. Debridement stands as the most fundamental method for treating DFU.

5.4. Apply wound dressings

Dressings function as a protective barrier for DFU. Moreover, certain advanced bandages possess antimicrobial properties while concurrently stimulating vascular and tissue regeneration [56]. The materials for wound dressings are varied, and the choice of suitable dressings should be meticulously aligned with the wound type, severity, and specific characteristics, aiming to minimize treatment duration and facilitate DFU healing. Presently, commonly used materials for medical dressings encompass hydrogels, alginate, foam, and silver-containing dressings [57]. Among these, hydrogel dressings are the most prevalent. Studies have shown that hydrogels exhibit better therapeutic efficacy in treating DFU compared to conventional dressings [58]. Hydrogels, characterized by their high water content and three-dimensional porous network, are capable of loading therapeutic agents or cytokines that promote the healing of diabetic wounds. Current research has extensively utilized hydrogels for the delivery of various glycemic control medications [59–62] and antimicrobial agents [63–66] to enhance wound healing. Moreover, studies indicate that hydrogels can also incorporate ROS scavengers [67,68] and angiogenic compounds [69,70] to synergistically promote the healing of DFUs. Moreover, hydrogels can serve as carriers for cells or growth factors [49,50], which have been demonstrated to play a crucial role in facilitating DFU healing. In comparison to hydrogels, alginate demonstrates more favorable swelling properties, mechanical characteristics, and maximal adhesion to biological tissues [71]. Studies indicate that alginate sodium hydrogel loaded with vicenin-2 can suppress pro-inflammatory cytokines and facilitate diabetic wound healing under elevated blood glucose levels [72].

5.5. Decompression of wounds

DFU arise due to the presence of localized high-pressure areas on the foot, either short-term and concentrated or long-term and diffuse low-pressure regions. Additionally, the presence of peripheral arterial disease and diabetic neuropathy complicates diabetic ulceration further. As DFU occurrence has been linked to elevated plantar pressure, offloading assumes a pivotal role in DFU treatment. Common offloading techniques encompass the utilization of crutches, surgical shoes, and bandages [73]. Total contact casts (TCCs), made of special materials, are regarded as the gold standard for offloading in DFU treatment. Employing TCCs can notably reduce the healing time of DFUs compared to conventional dressing therapy [74]. Moreover, alternative offloading strategies encompass removable knee-high devices, removable ankle-high devices, or felted foam, which can be inserted into suitable footwear for cushioning purposes [75].

5.6. Employ negative pressure wound therapy (NPWT)

To clear fluid and infection from the wound site, negative pressure wound therapy (NPWT) is commonly used to apply negative pressure to the covered wound. This approach effectively reduces the frequency of dressing changes while ensuring wound cleanliness

and dryness [76]. Research findings suggest that NPWT can suppress the mRNA expression of pro-inflammatory cytokines IL1β and MMP-9, while concurrently enhancing the expression levels of VEGF and TGF-β1 [77].

5.7. Administer hyperbaric oxygen therapy (HBOT)

HBOT encompasses both local oxygenation of ulcers and systemic oxygenation. This approach stimulates collagen synthesis, promotes growth factor production, and facilitates neovascularization [78]. Furthermore, HBOT demonstrates notable inhibitory effects on anaerobic bacteria, leading to a reduction in antibiotic usage [79]. Nevertheless, HBOT is currently surrounded by considerable controversy. Current studies indicate that HBOT can only enhance short-term treatment outcomes for DFU [80].

5.8. Employ skin substitutes

With the successful therapeutic outcomes observed with skin substitutes, the application of skin substitutes in treating DFU has seen a steady rise. Skin substitutes mimic the natural ECM, boasting commendable biocompatibility and serving as carriers for cells and growth factors, thereby fostering effective wound healing. Nonetheless, the risk of rejection as an exogenous substance remains present. Presently, bioengineered membranes composed of materials such as collagen are frequently employed to mitigate this risk [81].

5.9. Utilizing the tissue regeneration-promoting properties of MSCs

In recent years, MSCs therapy has emerged as a promising avenue for enhancing tissue regeneration and wound healing [82–84], owing to the myriad characteristics and advantages of stem cells. Numerous studies have substantiated the role of MSCs in enhancing wound healing. Shi et al. employed intravenous transplantation of human umbilical cord mesenchymal stem cells (hUC-MSCs) in a diabetic rat model induced by streptozotocin. The findings indicate that intravenous infusion of hUC-MSCs can enhance epithelialization and granulation tissue formation in DFUs, thereby promoting tissue regeneration [85]. Additionally, hUC-MSCs play a significant role in angiogenesis, cellular proliferation, and apoptosis reduction [85–87] (Fig. 4). Moreover, the investigation by Han et al. underscored the regulatory influence of the Wnt signaling pathway on the proliferation and differentiation of hUC-MSCs. Activation of the Wnt signaling cascade facilitated the proliferation and differentiation of hUC-MSCs on the CCLDADM scaffold, thereby effectively fostering the resolution of diabetic cutaneous injuries [88]. Wei et al. discerned that hUC-MSCs exhibited the potential to enhance the healing process of cutaneous wounds in a murine model of DFU by inhibiting the expression of MMP-9 while concurrently fostering the expression of MMP-8 [89]. Gustavo et al.'s investigation demonstrates the efficacy of Allogeneic adipose-derived mesenchymal stromal cells (ASCs) in alleviating bleomycin-induced pulmonary and dermal fibrosis in elderly mice, concurrently accelerating wound healing [90]. Furthermore, Liu et al. elucidated that hUC-MSCs significantly augmented the expression levels of CD31 and VEGF, thereby fostering the formation of neovessels and consequently accelerating the regeneration of radiation-induced cutaneous ulcers [91]. Additionally, Wang et al. revealed that LPS-pretreated BM-MSCs facilitated the healing process of diabetic foot ulcers by enhancing angiogenesis and local epithelialization at the wound site [92]. Although MSCs exhibit significant advantages in promoting tissue regeneration and wound healing, as a novel cellular therapy, MSC treatment also has certain limitations. MSCs typically disappear within 24 h, preventing their long-term presence at injury sites and sustained action. Additionally, standardized protocols for MSC therapy in DFUs have yet to be established. For the clinical translation of MSC therapy for DFUs, numerous challenges must be addressed, including conditions for cell isolation and culture, cryopreservation methods, MSC infusion dosage, timing, frequency, and routes of administration, as well as donor MSC heterogeneity. On the other hand, MSCs exhibit a propensity for tumorigenesis. Studies have shown that under certain culture conditions, MSCs can bypass senescence and generate malignant cells [93]. Furthermore, despite their immune-privileged status, allogeneic MSCs can be recognized by the host immune system and induce donor-specific immune responses due to the presence of alloantigens on the cell surface [94]. The associated challenges and limitations of MSC therapy warrant further research and improvement.

6. The characteristics of MSCs

MSCs, originating from the mesoderm, are a multipotent non-hematopoietic adult stem cell population. They were initially identified in the 1960s during investigations into bone marrow, characterized by their fibroblast-like morphology [96]. MSCs are characterized by their adhesion and plasticity; they have the potential to differentiate into osteoblasts, adipocytes, and chondrocytes. Additionally, they express CD73, CD90, CD105, and lack the expression of CD14, CD34, CD45, CD11b, CD79a, CD19, HLA-DR. MSCs have a broad spectrum of sources [97]. Apart from bone marrow, they can be extracted from adipose tissue, placenta, umbilical cord, amniotic fluid, dental pulp, Wharton's jelly, and tissues including liver, spleen, kidney, lung, and brain [98]. Among these, bone marrow-derived mesenchymal stem cells (BM-MSCs), adipose tissue-derived mesenchymal stem cells (ADSCs), and umbilical cord-derived mesenchymal stem cells(UC-MSCs) are the most prevalent sources utilized for therapy. MSCs exhibit a plethora of characteristics including self-renewal, colony formation, and multi-lineage differentiation abilities. They have the capability to differentiate into diverse cell types including osteoblasts, chondrocytes, adipocytes, endothelial cells, cardiomyocytes, hepatocytes, and neurons [99]. The differentiation potential of MSCs is contingent upon factors such as cell source, cell culture environment, and expansion conditions. Apart from the properties mentioned above, MSCs secrete a plethora of growth factors, cytokines, and other bioactive substances. These bioactive molecules can recruit other cells to the site of tissue damage, promoting angiogenesis and

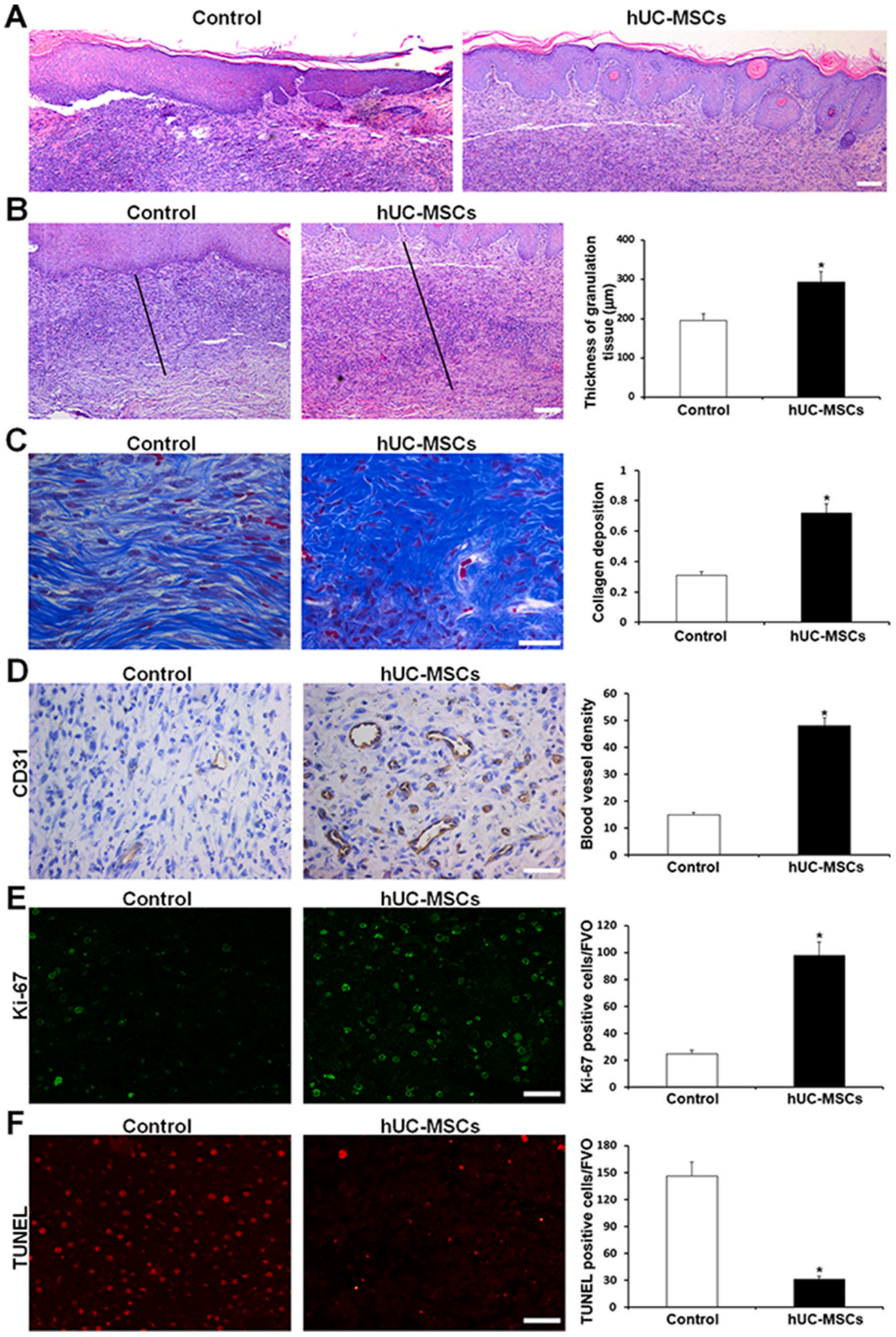

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Fig. 4. Samples of wound tissues were collected for analysis on the 16th day post-treatment. hUC-MSCs exert significant effects on enhancing epithelialization, promoting granulation tissue formation, facilitating tissue regeneration, stimulating angiogenesis, fostering cell proliferation, and mitigating apoptosis in DFUs.(a) Histological analysis using H&E staining revealed enhanced re-epithelialization effects on DFU wounds following hUC-MSCs treatment compared to the control group. (b) Remarkable thickening of granulation tissue was observed in the hUC-MSCs treatment group compared to the control group.(c) Masson's trichrome staining revealed that the collagen deposition effect was superior in the hUC-MSCs treatment group compared to the control group. (d) Immunohistochemical staining was employed to assess CD31 expression. In comparison to the control group, the hUC-MSCs group exhibited a greater abundance of blood vessel structures at the wound site. (e)Ki-67 expression was assessed through staining. The hUC-MSCs group displayed a substantially higher number of Ki-67-positive cells than the control group, indicating its superior capacity to promote cell proliferation. (f) The TUNEL assay was employed to evaluate cell apoptosis. Relative to the control group, the hUC-MSCs group demonstrated a marked reduction in apoptotic cells, underscoring its enhanced ability to suppress cell apoptosis.Data were presented as mean ± standard deviation for each group. *P < 0.05. Scale bar, 100 μm.Reproduced under the terms of the CC-BY license [85]. Copyright 2020, the authors.

facilitating tissue repair [100]. Additionally, MSCs play a role in immune modulation, thereby mitigating inflammatory responses [101]. Furthermore, MSCs can differentiate into keratinocytes [102,103], endothelial cells [104–106], and fibroblasts [107–109], which play crucial roles in wound healing by replacing damaged cells. This promotes wound healing, stimulates angiogenesis, reduces inflammation, regulates extracellular matrix remodeling, and minimizes scar formation.

Since most stem cells exhibit low expression levels of HLA class I and lack or have low expression of HLA class II molecules, along with the absence of co-stimulatory factors such as CD80, CD40, and CD86, as well as hematopoietic cell surface markers including CD14, CD34, and CD45 [110,111], they do not trigger immune rejection during allogeneic transplantation. Research indicates that the downregulation of HLA class I expression can mitigate the cytotoxic effects of natural killer (NK) cells on MSCs [112]. Moreover, MSCs can regulate the expression of interleukin-4 (IL-4) and interferon-gamma (IFN-γ) in effector T cells, facilitating the conversion of helper T cells (Th2) to Th1 cells and exerting inhibitory effects on disease responses. Furthermore, studies have reported that BM-MSCs, by expressing HLA-DR molecules, evade recognition by NK cells [113]. Consequently, transplanting allogeneic MSCs does not trigger the production of alloantibodies or the initiation of T cells [114]. As cytotoxic immune factors can trigger the dissolution of MSCs [115], IFN-γ serves as an antagonist to NK cells, safeguarding MSCs from NK cell attacks [116]. Additionally, IFN-γ enhances the differentiation potential of these cells through both nuclear factor kappa B (NF-κB)-dependent and -independent pathways [117]. MSCs, owing to these immunological attributes, emerge as a viable source for cell transplantation experiments.

7. Paracrine effects of MSCs

The paracrine actions of MSCs were first characterized by Haynesworth et al. [118]. Their research revealed that MSCs have the capability to produce and secrete diverse bioactive molecules including growth factors, cytokines, chemokines, proteins, and miRNAs. These bioactive factors exert regulatory effects on neighboring cells. Research has indicated that MSCs secrete a plethora of bioactive

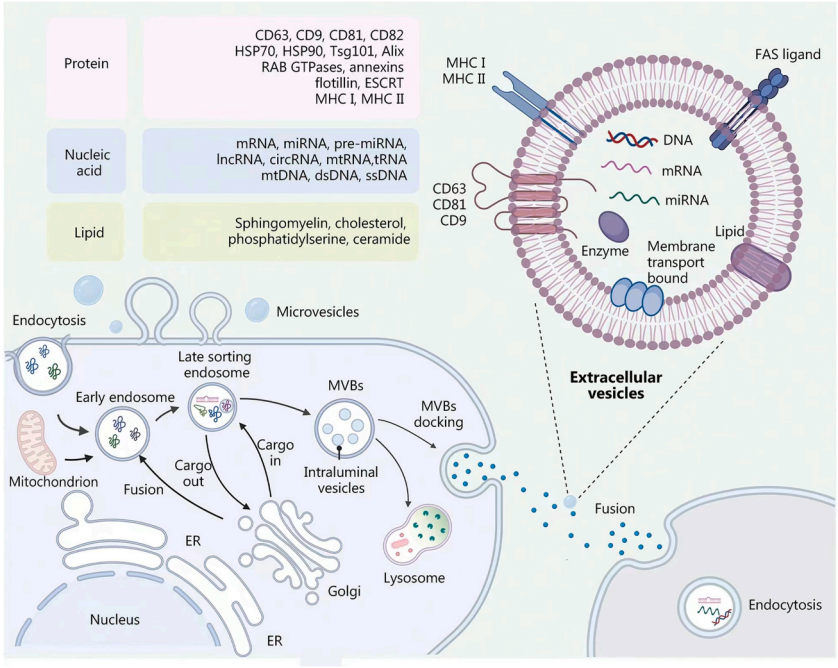

Fig. 5. The process of exosome generation and secretion. Reproduced under the terms of the CC-BY license [151]. Copyright 2023, the authors.

factors that facilitate angiogenesis, stimulate ECM remodeling, impede cellular fibrosis and apoptosis, dampen local inflammatory responses, and modulate immune reactions [119–121]. This evidence suggests that MSCs contribute to tissue regeneration by either direct intervention or paracrine signaling [96]. Presently, extensive research has investigated the therapeutic implications of bioactive factors secreted by MSCs across a spectrum of diseases, encompassing neurological disorders, cardiovascular ailments, bone and cartilage regeneration, immune-related conditions, liver injury, among others [122]. These investigations elucidate that the bioactive factors released by MSCs serve as mediators to activate target cells, while also stimulating neighboring cells to secrete pertinent bioactive factors [123]. Additionally, the paracrine effects of MSCs encompass the secretion of exosomes. Exosomes are nanoscale extracellular vesicles with a lipid bilayer membrane structure secreted by MSCs. By transmitting signaling molecules such as non-coding RNAs and proteins, they facilitate intercellular communication [124], promoting the survival, proliferation, migration, and gene expression of recipient cells while reprogramming the behavior of target cells, thereby regulating physiological and pathological processes. Multiple studies have confirmed that exosomes secreted by MSCs possess therapeutic effects similar to those of MSCs [125–128].

8. Exosomes released by MSCs

Exosomes, non-replicative lipid bilayer spherical structures, constitute a diverse array of double-layered lipid membrane-enclosed extracellular vesicles secreted by eukaryotic cells or prokaryotes. Their dimensions typically span from 30 nm to 200 nm. Moreover, they encompass two subtypes: microvesicles, ranging from 100 to 1000 nm, and apoptotic bodies, ranging from 500 to 2000 nm [129]. Exosomes were initially identified in investigations into iron uptake in reticulocytes, presenting as small vesicles suspended in the reticulocyte's supernatant [130]. Exosomes originate from cellular endocytosis, whereby components of the extracellular milieu, such as proteins, lipids, and diverse small molecules, undergo internalization to form early endosomes. These early endosomes gradually mature into late endosomes and eventually transform into multivesicular bodies (MVBs). The fusion of MVBs with the plasma membrane occurs through interactions with microtubules and the cellular cytoskeleton, culminating in exosome formation. Notably, a subset of MVBs engages in fusion with lysosomes for degradation purposes. Upon their formation, exosomes employ various mechanisms, including endocytosis, uptake by macrophages, phagocytosis, and direct fusion with the plasma membrane, to enter recipient cells [131](Fig. 5). Exosomes manifest distinct physiological attributes and functionalities contingent upon divergent physiological or pathological milieus. They are disseminated by an array of dynamic cellular entities within the organism, orchestrating an information relay via the payload they harbor. Inter-cellular communication assumes paramount significance in upholding the homeostasis of the microenvironment in multicellular organisms. Exosomes partake in intercellular communication, a function that transcends interactions solely among cells within the same organism, extending its relevance to cells of disparate organisms, irrespective of species affiliation. Initially, exosome communication was posited to entail the transfer of extracellular proteins and certain soluble factors. Nonetheless, as investigations progressed, the consensus recognizing exosomes' ability to disseminate non-coding RNA and proteins, alongside other signaling molecules, to mediate intercellular communication has gained widespread acceptance [132]. Exosomes, serving as conduits for intercellular information transfer, harbor a plethora of unparalleled advantages: their nano-sized dimensions facilitate unhindered intercellular transport, while their distinctive phospholipid bilayer structure safeguards bioactive cargo from degradation enroute. Moreover, the abundance of specific proteins on the exosome surface endows them with superior targeting prowess. Exploiting these distinctive merits, contemporary research endeavors concentrate on harnessing exosomes as vectors for delivering drugs, RNA, proteins, and various other payloads [133]. The RNA cargo transported by exosomes encompasses mRNA, miRNA, ribosomal RNA, circular RNA, DNA, lncRNA, and various others, pivotal in orchestrating intercellular regulation [134]. Aside from harboring proteins linked to membrane transport and fusion, such as annexins, ESCRT complexes, Rab-GTPases, actin, and β-tubulin, exosomes are enriched with membrane surface markers, including CD9, CD63, CD81, and CD82, alongside HSP70, HSP90, Tsg101, and Alix [135]. Accordingly, these are conventionally acknowledged as ubiquitous exosomal markers. Exosomes demonstrate heterogeneity, whereby exosomes sourced from distinct tissues and cells, or even from identical tissues but disparate cells, manifest notable disparities. This diversity stems from variances in gene expression across different cells, discrepancies in intercellular culture conditions, divergent concentrations of membrane-bound proteins, and disparities in the exosomes' cargo-loading capabilities [136]. The contemporary techniques employed for exosome isolation and purification encompass ultracentrifugation, ultrafiltration, size-exclusion chromatography, immunoseparation, and microfluidics, among a plethora of methodologies [137]. Subsequent to exosome retrieval, elucidation is conducted employing an array of methodologies, including transmission electron microscopy, nanoparticle tracking analysis, protein imprinting, enzyme-linked immunosorbent assay (ELISA), flow cytometry, scanning electron microscopy, atomic force microscopy, dynamic light scattering, fluorescence-activated cell sorting, and resistive pulse sensing, to name a few [138].

Research has demonstrated MSCs as the predominant origin of exosomes [139]. The concept of utilizing exosomes derived from MSCs for the treatment of myocardial ischemia/reperfusion injury was introduced early on, thereby shifting the research focus towards elucidating the role and therapeutic applications of exosomes in diseases [140]. The morphological attributes and isolation techniques of exosomes originating from MSCs closely resemble those from alternative sources. In addition to their identification via ubiquitous exosomal markers like CD9 and CD81, discernment can also be facilitated through the utilization of MSC surface markers including CD29, CD44, CD73, and CD90. Analogously, exosomes originating from MSCs encapsulate a diverse array of bioactive substances. Profiling of exosomes released by BM-MSCs unveiled the presence of 730 functional proteins, pivotal in governing cellular proliferation, migration, and assorted physiological processes [141]. Furthermore, exosomes derived from MSCs harbor a plethora of cytokines conducive to angiogenesis and immune regulation, including VEGF, transforming growth factor-β1 (TGF-β1), interleukin-6 (IL-6), interleukin-10 (IL-10), among others [142]. Exosomes sourced from MSCs convey a plethora of signaling molecules capable of

transmitting and exerting effects on endothelial cells, fibroblasts, immune cells, keratinocytes, among others, thereby fostering wound vascularization, enhancing wound circulation, modulating inflammatory responses, and thereby facilitating tissue repair [143]. Alongside functional proteins and cytokines, exosomes derived from MSCs encapsulate a myriad of RNA species. Presently, extensive research endeavors concentrate on elucidating the enrichment of miRNA within exosomes secreted by MSCs [144]. Contemporary investigations posit that the miRNA and mRNA encapsulated within exosomes wield pivotal roles in the intricate process of biological information transmission [145]. Owing to the advantageous attributes of exosomes secreted by MSCs, including low immunogenicity, small size, prolonged half-life, potent penetrative capacity, and elevated biocompatibility, they harbor immense therapeutic potential in disease management [146]. Reports indicate that exosomes sourced from MSCs exhibit analogous functionalities to stem cells in terms of anti-inflammatory responses, tissue regeneration, and immune modulation [147]. Moreover, unlike stem cells, exosomes have the added advantage of mitigating the adverse reactions associated with stem cell therapy [148]. Exosomes serve as a mirror of the functional capabilities of MSCs and constitute crucial mediators in the paracrine activity of MSCs. The existence of exosomes renders cell-free therapies for wound healing plausible [149]. Recent research endeavors have been focused on augmenting the reparative potential of exosomes for tissue injury by manipulating the expression profile of exosomal contents, preconditioning MSCs, and artificially modifying the receptors on the exosome surface [149].

In conclusion, exosomes secreted by MSCs manifest superior advantages in facilitating wound healing compared to MSCs themselves. Prevalent MSCs employed in contemporary wound healing investigations involving exosomes comprise BM-MSCs,UC-MSCs and ADSCs [150].

9. The role of MSCs and their derived exosomes in DFU

Due to their advantages in regenerative medicine, MSCs play a crucial role in facilitating the healing of DFU. MSCs exert their healing effects through a multifaceted and coordinated approach. As subcellular components of MSCs, exosomes inherit the regenerative and immunomodulatory properties of their parent MSCs. These exosomes possess a unique bilayer membrane structure that safeguards their contents from degradation or damage. Research indicates that exosomes can exert functions similar to those of MSCs, underscoring their crucial role in promoting tissue repair and regeneration [125](Table .2).

9.1. Regulation of keratinocyte function

Keratinocytes play a pivotal role in the wound healing process. MSCs possess the capability to regulate the functionality of keratinocytes, thereby augmenting their contribution to epidermal formation at the wound site. Research indicates that the administration of BM-MSCs significantly enhances the proliferative capacity of endothelial cells and keratinocytes in mouse wounds. Additionally, MSCs promote the migration of endothelial cells, keratinocytes, and macrophages to the site of injury, thereby fostering wound healing [152]. Administration of BM-MSCs to wounds in genetic diabetic db/db mice results in elevated levels of angiopoietin-1, VEGF, and keratinocyte-specific protein keratin at the wound site. BM-MSCs effectively stimulate the proliferation of keratinocytes at the wound site. Moreover, hUC-MSCs demonstrate robust targeting specificity; in diabetic foot ulcer animal models, hUC-MSCs exhibit precise targeting to the injured site, stimulating the growth of cellular keratin 19 and consequently augmenting the regenerative potential of the wound epithelium, thereby fostering wound healing [153]. Furthermore, studies have reported that culturing human keratinocytes in BM-MSCs conditioned medium containing high glucose levels results in elevated levels of phosphorylated focal adhesion kinase, matrix metalloproteinase-2, EGF, and insulin-like growth factor 1. These outcomes suggest that BM-MSCs can ameliorate keratinocyte functionality, thereby fostering wound healing in a diabetic foot rat model [154].

9.2. Promote angiogenesis

Through exploration of the difficulties in DFU healing, it becomes apparent that local vascular injury and pathology contribute to the development of diabetic foot. Interventions aimed at addressing this cause, thereby enhancing neovascularization at the site of the lesion, hold immense importance for ulcer healing and repair. Upon local tissue injury, MSCs exhibit migratory tendencies toward the injured site and undergo targeted differentiation into endothelial and smooth muscle cells. Upon differentiation, they integrate into the damaged vasculature, thereby facilitating neovascularization. Furthermore, MSCs promote vascular formation through their paracrine activity, secreting various cytokines including VEGF, basic fibroblast growth factor (bFGF), stromal cell-derived factor-1 (SDF-1), hypoxia-inducible factor-1 (HIF-1), keratinocyte growth factor-2 (KGF-2), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1). These factors facilitate the production of ECM while suppressing endothelial cell apoptosis, thereby fostering vascular formation and wound healing [24,28,155]. Among these cytokines, VEGF plays a pivotal role [156]. Research indicates that the localized administration of VEGF can mobilize bone marrow-derived cells while concurrently promoting growth factors conducive to tissue repair. By recruiting these cells to the site of local injury, wound healing at the local wound site can be facilitated. Consequently, VEGF can be utilized in the treatment of diabetic complications characterized by vascular impairment [157].

Extensive research has demonstrated the angiogenic potential of MSC-derived exosomes [158–160]. Zhang et al. developed a full-thickness skin injury model in diabetic mice to investigate the role of exosomes secreted by ADSCs in diabetic wound healing. The results confirmed that treatment with ADSC-derived exosomes led to a significant increase in the expression levels of Angiopoietin 1 (ANG1), Fetal Liver Kinase 1 (FILK1), and VEGF, while the expression levels of endogenous angiogenesis inhibitors Angiopoietin 1 (VASH1) and Thrombospondin 1 (TSP1) were decreased. This finding validates that ADSC-derived exosome treatment significantly enhances endothelial cell activity and angiogenic potential, thereby contributing to the accelerated healing of diabetic wounds in mice

Table 2
The role of MSCs and their derived exosomes in DFU.

	Source	Mechanisms	Researchers	References
Regulation of keratinocyte function	BM-MSCs	BM-MSCs enhance the levels of angiogenin-1, VEGF, and keratin, a keratinocyte-specific protein, at the wound site, thereby stimulating keratinocyte proliferation.	*W. Zhang* et al.	[152]
	hUC-MSCs	hUC-MSCs precisely target the site of injury, stimulating the growth of cytokeratin 19 and enhancing the regenerative potential of the epithelial tissue at the wound site.	*L. Chen* et al.	[153]
	BM-MSCs	The conditioned medium from BM-MSCs increases the levels of phosphorylated focal adhesion kinase, matrix metalloproteinase-2, epidermal growth factor, and insulin-like growth factor 1 in human keratinocytes.	*J. Kato* et al.	[154]
Promotes angiogenesis	ADSCs	Enhances the expression of ANG1, FILK1, and VEGF, while simultaneously inhibiting the expression of VASH1 and TSP1. Enhanced endothelial cell activity and vascular regenerative potential.	*Y. Zhang* et al.	[161]
	ADSCs	Overexpression of Nrf2-exosomes enhances endothelial progenitor cell proliferation and angiogenesis by promoting the phosphorylation levels of SMP30, VEGF, and VEGFR2.	*X. Li* et al.	[162]
	hUC-MSCs	Inhibition of high glucose-induced oxidative stress in HUVECs promotes their activity, proliferation, and angiogenesis.	*C. Yan* et al.	[163]
	hUC-MSCs	CircHIPK3 in exosomes inhibits miR-20b-5p, thereby promoting the expression of Nrf2 and VEGFA. Furthermore, circHIPK3 suppresses the expression of miR-124 in endothelial cells, protecting them from high glucose-induced damage. The protective role of circHIPK3 extends to mitigating cellular damage, significantly reducing apoptosis and inflammation, and thereby enhancing angiogenesis.	*Z. H. Liang* et al.	[165]
	hiPSC-MSCs	Exosomes are internalized by HUVECs, enhancing their proliferation and migratory capabilities. Furthermore, these exosomes activate the Erk-1/2 signaling pathway, thereby promoting angiogenesis.	*J. Zhang* et al.	[166]
Promotes collagen deposition	ADSCs	HSP90 within exosomes binds to the LRP1 receptor on the cell membrane, thereby activating the downstream AKT signaling pathway. This activation subsequently enhances the proliferation and migratory capabilities of keratinocytes and fibroblasts.	*S. Ren* et al.	[167]
	ADSCs	By stimulating monocytes or macrophages to secrete higher levels of TGF-β1, and subsequently activating the TGF-β/Smad3 signaling pathway, the proliferation of fibroblasts is enhanced, which in turn promotes the synthesis of type I collagen in diabetic wounds.	*H. H. Hsu* et al.	[169]
	ADSCs	hypADSCs-exo promote the proliferation, migration, and ECM secretion of fibroblasts by activating the PI3K/AKT signaling pathway.	*J. Wang* et al.	[170]
Promoting cell proliferation	BM-MSCs	Promotes re-epithelialization, epidermal junction formation, skin appendage regeneration, inflammatory infiltration, vascularization, granulation tissue formation, and the development of dense collagen fibers, while simultaneously concentrating regenerative factors and proteins.	*T. de Mayo* et al.	[171]
	ADSCs	Facilitates the proliferation and migration of fibroblasts while simultaneously enhancing collagen synthesis capacity.	*L. Hu* et al.	[172]
	ADSCs	Promotes the formation of neovascularization, protects ischemic/reperfusion flap grafts, and increases IL-6 levels.	*C. M. Pu* et al.	[173]
	hiPSC-MSCs	Enhances fibroblast proliferation and migration, increases the synthesis of collagen and elastin, and stimulates the formation of capillary networks in vitro.	*J. Zhang* et al.	[174]
Antiapoptotic effect	BM-MSCs	By activating APAF1, apoptosis in epithelial HaCaT cells is effectively inhibited.	*C. Shen* et al.	[170]
	hP-MSCs	Enhance proliferation and anti-inflammatory capabilities while suppressing apoptosis of corneal epithelial cells.	*H. Tao* et al.	[176]
	ADSCs	miR-10b within exosomes targets PEA15 to enhance the expression of CDK6, thereby promoting the proliferation and migration of H_2O_2-damaged HaCaT cells and inhibiting their apoptosis.	*X. Liao* et al.	[179]
	ADSCs	Through Wnt/β-catenin signaling pathway, the proliferation and migration of HaCaT cells are promoted while simultaneously inhibiting their apoptosis	*T. Ma* et al.	[180]
Anti-inflammatory effect	hUC-MSCs	In a hyperglycemic environment, inhibition of inflammatory factors such as IL-1β, IL-6, and TNF-α, as well as the expression	*C. Yan* et al.	[163]

(*continued on next page*)

Table 2 (*continued*)

	Source	Mechanisms	Researchers	References
		levels of NOX1 and NOX in HUVECs, effectively suppresses oxidative stress and promotes cellular proliferation.		
	ADSCs	Exosomes with high Nrf2 expression inhibit the levels of inflammatory and oxidative stress-related proteins in HaCaT cells, fibroblasts, and HUVECs. This inhibition facilitates the formation of granulation tissue, angiogenesis, and growth factor expression at the wound site.	*X. Li* et al.	[162]
	ADSCs	By modulating SIRT3/SOD2, oxidative stress levels induced by high glucose are suppressed, leading to reduced ROS accumulation, minimized mitochondrial dysfunction and inflammation, and activation of endothelial cells.	*Y. Zhang* et al.	[161]
	BMMSCs, JMMSCs	By transferring miR-223 to target human PKNOX1, macrophage polarization towards the M2 phenotype is induce.	*X. He* et al.	[184]
Regulation of macrophage polarization.	hUC-MSCs	Regulating macrophage polarization improves pancreatic dysfunction in type 2 diabetic mice.	*D. Philipp* et al./	[190]
	hUC-MSCs	Regulating macrophage polarization promotes the restoration of endothelial cell function. Secreting PGE2 modulates the expression levels of IL-10 and VEGF in the local microenvironment of endothelial cells.	*J. B. Tefft* et al./	[155]
	G-MSCs	Inducing macrophage polarization plays a crucial role in wound healing.	*X. Yu* et al./	[24]
	ADSCs	By delivering miR-21 to target the PI3K/Akt signaling pathway, this process promotes the polarization of macrophages from the M1 to the M2 phenotype, thereby facilitating angiogenesis.	*D. Zhu* et al.	[193]
	BMMSCs	By delivering miR-223, the polarization of macrophages towards the M2 phenotype is facilitated, which accelerates wound healing.	*X. He* et al.	[184]
	Immortalized E1-MYC 16.3 human embryonic stem cell-derived MSCs	Inhibiting the expression levels of M1 macrophages and inflammation-related cytokines, while promoting the infiltration of M2 macrophages.	*S. Zhang* et al.	[194]
	BM-MSCs	Promoting macrophage polarization towards an M2-like phenotype, thereby facilitating tissue repair and modulating the inflammatory response.	*C. S. Chamberlain* et al.	[195]
Inhibition of ROS generation	BM-MSCs	Inhibition of myeloperoxidase and induction of inducible nitric oxide synthase lead to reduced expression of inflammatory cytokines IL-1β, IL-4, IL-6, IL-9, TNF-α, andIFN-γ.	*K. F. Al-Massri* et al.	[198]
	ASCs	Inhibition of ROS and myeloperoxidase expression in stimulated monocytes and macrophages results in a reduction of M1 macrophage expression.	*M. Ortiz-Virumbrales* et al.	[199]
	BM-MSCs	mitochondrial ROS production in macrophages, inhibits the activation of intracellular NLRP3 and caspase-1, thereby affecting the transcription of TNF-α and IL-6, and modulating the expression of IL-1β.	*Y. Zhou* et al.	[200]
	BM-MSCs	Hypoxia-preconditioned MSC-derived exosomes regulate the accumulation of ROS in epithelial cells through HIF-1α.	*F. Zhu* et al.	[201]
	WJSCs	Modulates the GP91/ROS/inflammasome signaling pathway while simultaneously enhancing the expression of antioxidant proteins.	*W. S. Hu* et al.	[202]
	hUC-MSCs	The released exosomes inhibit glucocorticoid-induced osteoblast apoptosis by modulating the MAPK pathway and regulating ROS levels.	*H. Lu* et al.	[203]
	BM-MSCs	Modulating the innate immune response capability through the ROS/NLRP3 signaling pathway.	*A. Qin* et al.	[204]
Regulation of T cells	BM-MSCs	Inhibiting the differentiation and function of pro-inflammatory T cells while inducing a regulatory T cell phenotype, thereby effectively modulating inflammation at the site of injury.	*A. Li* et al.	[211]
Regulation of NETs formation	BM-MSCs	When used in conjunction with antibiotics, it significantly reduces bacterial load at the wound site, thereby promoting wound healing.	*L. Chow* et al.	[216]
	hAM-MSCs	By mediating immune regulation through PGE2, it effectively inhibits the release of NETs.	*G. A. Estúa-Acosta* et al.	[217]
	hAM-MSCs	Regulate the formation of NETs through miR-125a-3p contained within the exosomes.	*Y. Morishima* et al.	[218]
Inhibition of ferroptosis	hUC-MSCs	The miRNA-17-92, which is highly expressed and enriched in exosomes, promotes the proliferation and migration of HUVECs, thereby accelerating cellular proliferation, migration, angiogenesis, and resistance to ferroptosis.	*W. Nie* et al.	[231]

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Table 2 (*continued*)

	Source	Mechanisms	Researchers	References
	hUC-MSCs	Exosomes derived from MSCs inhibit the expression of NETs, which, in turn, regulate ferroptosis by suppressing the PI3K/AKT signaling pathway.	*W. Lu* et al.	[232]
	BM-MSCs	Exosomes derived from BM-MSCs carry miR-367-3p, which binds to EZH2 and inhibits its expression, resulting in the overexpression of SLC7A11. This overexpression of SLC7A11 subsequently leads to the activation of GPX4 and the suppression of ferroptosis.	*J. Fan* et al.	[233]
	hUC-MSCs	Exosomes derived from hUC-MSCs utilize miR-129-5p to target ACSL4, thereby inhibiting LPO and ferroptosis. This inhibition significantly alleviates inflammation and promotes tissue repair.	*Z. Wei* et al.	[234]
Regulation of signaling pathways	BM-MSCs	The secretion of various growth factors upregulates the expression levels of VEGF-1α, MMP-9, pAKT, and VEGF-r in HUVECs, thereby activating the VEGF/AKT signaling pathway. This activation enhances HUVEC proliferation, migration, and tube formation capabilities while inhibiting apoptosis.	*J. Liu* et al.	[235]
	BM-MSCs	MSC-VEGF-CM activates the PI3K/AKT/mTOR/eNOS and p38/MAPK signaling pathways, thereby enhancing the activity and migratory capacity of pancreatic microvascular endothelial cells.	*T. Xu* et al.	[236]
	BM-MSCs	Significantly enhances the migratory and proliferative capabilities of human umbilical vein endothelial cells through the activation of the AKT signaling pathway.	*C. Hou* et al.	[237]
	BM-MSCs	Mechanism by which the PI3K/Akt signaling pathway regulates cell activity, proliferation, migration capabilities, and vascularization functions.	*J. Chen* et al.	[238]
	hUC-MSCs	The hUC-MSCs-CCLDADM scaffold material promotes wound healing in diabetic rats through the Wnt signaling pathway.	*Y. Han* et al.	[88]
	AF-MSCs	Increasing endogenous vascular growth factor levels enhances the regenerative potential of epithelial cells. Under hypoxic conditions, AF-MSCs enhance the proliferation and migration abilities of dermal fibroblasts through the TGF-β/SMAD2 and PI3K/AKT signaling pathways, thereby promoting wound healing.	*E. K. Jun* et al.	[240]
	ADSCs	Combined with PRP therapy, modulation of the Notch signaling pathway promotes angiogenesis and the proliferation of epidermal stem cells, thereby significantly accelerating wound healing.	*N. Ebrahim* et al.	[241]
Recruitment of other cells	BM-MSCs	It reduces excessive ROS production mediated by HG and/or LPS. Additionally, it effectively regulates the phosphorylation of MEK1/2 and ERK1/2 induced by high glucose and/or LPS, thereby enhancing the proliferation and migration abilities of keratinocytes under hyperglycemic conditions.	*M. Li* et al.	[242]
	BM-MSCs	Activation of the β2-AR signaling pathway enhances the migration of keratinocytes around the wound site, thereby promoting ulcer healing.	*J. Huo* et al.	[243]
	BM-MSCs	Exosomes released by MSCs induce the expression of HGF, IGF1, NGF, and SDF1 while simultaneously activating the Akt, ERK, and STAT3 signaling pathways, thereby enhancing fibroblast proliferation and migration capabilities.	*A. Shabbir* et al.	[244]
Secretion of growth factors	ADSCs	Release of TGF-β1 and HGF promotes the differentiation of myofibroblasts and the synthesis of collagen types I and III.	*J. Ma* et al.	[245]
	USCs	The secretion of PGE2 inhibits the differentiation of $CD4^+$ T cells into Th17 cells.	*C. Zhou* et al.	[247]
	BM-MSCs, ADSCs	The secretion of anti-inflammatory cytokines promotes the polarization of macrophages from M1 to M2 type, thereby accelerating the wound healing process.	*M. El-Sayed* et al.	[248]
Production of antimicrobial peptides	BM-MSCs	The interaction between MSCs and AMP influences the proliferation, migration, and regeneration of MSCs.	*A. Silva-Carvalho* et al.	[249]
Release of apoptotic vesicles	BM-MSCs	The release of ABs from mesenchymal stem cells promotes macrophage polarization towards the M2 phenotype, thereby facilitating skin wound healing.	*J. Liu* et al.	[254]

[161]. Xue et al. demonstrated that exosomes derived from ADSCs overexpressing Nrf2 enhance endothelial progenitor cell proliferation and angiogenesis by promoting the phosphorylation of SMP30, VEGF, and VEGFR2, thereby accelerating the healing of diabetic wounds [162]. Yan et al. confirmed that exosomes secreted by hUC-MSCs significantly inhibit oxidative stress induced by high glucose in HUVECs, thereby promoting HUVEC activity, proliferation, and angiogenesis. This finding offers a novel approach for the repair of chronic diabetic wounds [163]. Circular RNA (CircRNA) represents a novel class of non-coding RNA characterized by the

covalent linkage between the 5′ and 3′ ends. This unique circular structure imparts greater stability compared to linear RNA, and CircRNA effectively regulates the pathogenesis of various diseases [164]. Liang et al. demonstrated that circHIPK3, present in exosomes secreted by hUC-MSCs, upregulates the expression levels of Nrf2 and VEGFA while inhibiting miR-20b-5p [165]. Furthermore, overexpression of circHIPK3 significantly suppresses the expression levels of miR-124 in endothelial cells, thereby protecting these cells from high glucose-induced damage. Additionally, overexpression of circHIPK3 confers protective effects against cellular damage, significantly reducing apoptosis and inflammation while promoting angiogenesis [165]. Moreover, studies have shown that exosomes can be internalized by HUVECs in vitro, significantly enhancing their proliferation and migration capabilities. Building upon this, exosomes promote angiogenesis by activating the extracellular signal-regulated kinase 1/2 (Erk-1/2) signaling pathway, thereby effectively facilitating the healing process of skin wounds in diabetic rats [166].

9.3. Promotes collagen deposition

MSCs and their derived exosomes exhibit significant capacity to promote re-epithelialization and collagen deposition. The heat shock protein (HSP) family comprises a group of highly conserved proteins that protect the organism from various stress-induced damages. Ren et al. demonstrated that HSP90 in exosomes derived from ADSCs binds to the low-density lipoprotein receptor-related protein 1 (LRP1) on the recipient cell membrane, thereby activating the downstream AKT signaling pathway. This interaction enhances the proliferation and migration abilities of keratinocytes and fibroblasts, thereby accelerating the healing of diabetic wounds [167]. The TGF-β/Smad3 pathway is a well-established signaling pathway closely associated with collagen production and tissue fibrosis. TGF-β activates the Smad3 complex by binding to receptors on fibroblasts, which in turn promotes nuclear transcription and facilitates the synthesis of collagen and α-smooth muscle actin (α-SMA) [168]. Hsu et al. demonstrated that exosomes derived from ADSCs stimulate monocytes or macrophages to secrete increased levels of TGF-β1, which subsequently activates the TGF-β/Smad3 pathway, thereby promoting fibroblast proliferation and enhancing the production of type I collagen in diabetic wounds. This application provides a novel cell-free therapeutic approach for accelerating the healing of diabetic wounds [169]. Wang et al. demonstrated that ADSCs secrete a higher amount of exosomes (hypADSCs-exo) under hypoxic conditions, which activate the PI3K/AKT signaling pathway to enhance fibroblast proliferation, migration, and ECM secretion [170].

9.4. Promoting cell proliferation

Studies have demonstrated that BM-MSC-derived products promote wound closure in diabetic mice by facilitating re-epithelialization, dermal-epidermal junction restoration, skin appendage regeneration, inflammatory infiltration, vascularization, granulation tissue formation, and dense collagen deposition. Simultaneously, these products concentrate regenerative factors and proteins that further enhance wound healing. This effect surpasses that achieved by BM-MSCs alone in terms of therapeutic efficacy [171]. Moreover, the proliferative effects of ADSCs have been extensively validated. Exosomes derived from ADSCs facilitate the proliferation and migration of fibroblasts while enhancing collagen synthesis, thereby significantly accelerating the healing process of skin wounds in mice [172]. Additionally, ADSCs can promote wound repair by facilitating the formation of new blood vessels, protecting ischemic/reperfusion flaps, and enhancing IL-6 levels at the injury site [173]. Furthermore, exosomes derived from MSCs obtained from induced pluripotent stem cells (iPSCs) promote wound healing in rats by enhancing fibroblast proliferation and migration, increasing the synthesis of collagen and elastin, and stimulating the formation of capillary networks in vitro [174].

9.5. Antiapoptotic effect

The anti-apoptotic effects of MSCs and their derived exosomes have been confirmed by numerous studies. Exosomes derived from BM-MSCs inhibit apoptosis in epithelial HaCaT cells by activating apoptotic protease-activating factor 1 (APAF1), which facilitates wound healing [175]. Exosomes secreted by placenta-derived MSCs (hP-MSCs) not only enhance proliferation and anti-inflammatory capabilities but also inhibit apoptosis of corneal epithelial cells, thereby improving corneal wound healing in mice [176]. Cyclin-dependent kinase 6 (CDK6) is an oncogenic kinase that functions as a cell cycle regulator. Research indicates that CDK6 may be closely associated with wound or injury healing [177,178]. Exosomes derived from ADSCs contain miR-10b, which targets PEA15 to enhance the expression of CDK6. This, in turn, promotes the proliferation and migration of $H_2O_2^-$ damaged HaCaT cells and inhibits cell apoptosis, thereby facilitating skin wound healing [179]. Exosomes secreted by ADSCs modulate Wnt/β-catenin signaling, thereby enhancing HaCaT cell proliferation and migration while simultaneously inhibiting apoptosis. This mechanism plays a crucial role in skin wound healing [180].

9.6. Anti-inflammatory effect

Numerous studies have shown that MSCs and their secreted exosomes significantly suppress systemic inflammation, making them a focal point in wound repair research. The study conducted by Yan et al. revealed that exosomes derived from hUC-MSCs significantly suppress the expression levels of inflammatory cytokines such as IL-1β, IL-6, and TNF-α, as well as NOX1 and NOX in HUVECs under hyperglycemic conditions. This suppression, in turn, reduces oxidative stress and promotes cellular proliferation [163]. The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) plays a pivotal role in mitigating oxidative stress and additionally exerts its effects by modulating the activity of antioxidant enzymes mediated through the antioxidant response element (ARE) [181]. Diabetic wound sites frequently exhibit diminished Nrf2/ARE activity coupled with elevated oxidative stress levels [182]. Xue et al.

have demonstrated that exosomes derived from ADSCs, which exhibit high expression of Nrf2, effectively suppress the expression levels of inflammation- and oxidative stress-related proteins in HaCaT cells, fibroblasts, and HUVECs. This suppression, in turn, promotes the formation of granulation tissue at the wound site, enhances angiogenesis, and increases the expression levels of growth factors, thereby significantly accelerating wound healing in diabetic rats [162]. Superoxide dismutase (SOD) is an essential antioxidant enzyme that scavenges harmful superoxide anion radicals, playing a pivotal role in maintaining cellular redox balance [183]. Zhang et al. have shown that exosomes secreted by ADSCs can modulate the SIRT3/SOD2 axis to inhibit hyperglycemia-induced oxidative stress, reduce ROS accumulation, mitigate mitochondrial dysfunction and inflammatory responses, and activate endothelial cells, thereby accelerating wound healing in diabetic models [161]. Furthermore, exosomes secreted by MSCs can exert anti-inflammatory effects by modulating macrophage polarization. Exosomes derived from BM-MSCs can accelerate wound healing by transferring miR-223, which targets human PBX/knotted 1 homeobox 1 (PKNOX1) to induce M2 macrophage polarization [184].

9.7. Regulation of macrophage polarization

As widely recognized, macrophages are pivotal in orchestrating the body's immune response. Induced by specific external cues, macrophages undergo polarization. This polarization aids in modulating the organism's inflammatory response, safeguarding against pathogen incursion, and orchestrating tissue repair mechanisms. Macrophage polarization encompasses classical M1 activation and alternative M2 activation. M1 macrophages are conventionally regarded as exerting pro-inflammatory effects during the early stages of the inflammatory response [185]. M1 macrophages are known to release pro-inflammatory cytokines like IL-1b, IL-12, iNOS, TNF, among others [186]. In contrast, M2 macrophages are associated with anti-inflammatory and wound healing properties. They secrete molecules such as IL-1, IL-10, TGF-β, arginase-1, CD206, chitinase 3-like protein 3 (also known as YM1), and resistin-like molecule-alpha (also known as Fizz1) [187]. These molecules play pivotal roles in combatting parasitic infections, facilitating tissue repair, promoting angiogenesis, and more. M1 and M2 macrophages do not exhibit complete antagonism; they can undergo mutual transformation under specific conditions. During the healing process of diabetic ulcers, macrophages secrete a plethora of pro-inflammatory cytokines under sustained high-level glucose stimulation, resulting in the sustained M1 phenotype of macrophages at the ulcer site [188]. Hence, promoting the polarization of M1 macrophages to M2 macrophages at the site of injury might potentially serve as an effective therapeutic approach for treating diabetic ulcers.

As reported, hUC-MSCs have been reported to exert a modulatory effect on macrophage polarization, thereby ameliorating pancreatic dysfunction in type 2 diabetic mice [189,190]. Numerous studies have indicated that intravenous infusion of BM-MSCs and jaw bone-derived mesenchymal stem cells (JM-MSCs) can selectively migrate to the injured site, while also promoting the polarization of M1 macrophages into M2 macrophages, thus facilitating wound healing. In vitro experiments have also demonstrated that co-culturing MSCs with macrophages can promote their polarization into M2 macrophages [191]. UC-MSCs possess the ability to modulate macrophage polarization, thereby facilitating the recovery of endothelial cell function in diabetic mice and expediting wound healing processes [155]. Mesenchymal stem cells derived from human gingiva have the capability to induce macrophage polarization, thus playing a crucial role in the wound healing process [24]. Prostaglandin E2 (PGE2) secreted by hUC-MSCs has the capacity to modulate the expression levels of IL-10 and VEGF in the local microenvironment of endothelial cells. This induction of M2 polarization in macrophages infiltrating local diabetic defects fosters wound healing processes [155].

At the site of injury, skin cells are exposed to various danger signals, including damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). These signals are recognized by the immune system, triggering the initiation of the inflammatory process [192]. Recent studies have demonstrated that exosomes derived from ADSCs promote macrophage polarization from the M1 to M2 phenotype by delivering miR-21, which targets the PI3K/Akt pathway, thereby directly enhancing the pro-angiogenic effects of exosomes [193]. Similarly, He et al. have also shown that exosomes secreted by BM-MSCs promote macrophage polarization towards the M2 phenotype through the delivery of miR-223, a mechanism that accelerates wound healing [184]. Zhang et al. demonstrated that MSC-derived exosomes significantly enhance the infiltration of M2 macrophages and sustain this effect within cartilage, thereby contributing to the overall therapeutic impact. Following MSC-derived treatment, the expression levels of M1 macrophages and inflammation-related cytokines were significantly reduced [194]. These studies collectively demonstrate that microRNAs in MSC-derived exosomes may serve as critical therapeutic targets in tissue repair processes. Furthermore, exosomes secreted by BM-MSCs promote the polarization of macrophages to an M2-like phenotype, effectively modulating tissue repair and inflammatory responses in a tendon injury model, thereby facilitating tendon healing [195].

9.8. Inhibition of ROS generation

In a state of low ROS content, the body facilitates cell proliferation and differentiation, whereas elevated ROS levels stimulate the immune response, resulting in cell damage and dysfunction [196]. When tissues are damaged by various external stimuli, phagocytic cells in the body ingest foreign pathogens, cellular debris, and apoptotic inflammatory cells to eliminate pathogens. During this process, long-lived neutrophils produce abundant ROS after phagocytosis, initiating a respiratory burst, ultimately leading to tissue damage. Recent studies have demonstrated that MSCs alleviate protein oxidation and lipid peroxidation through their paracrine secretion of exosomes [197]. Moreover, MSCs display notable mitigating effects on various inflammatory conditions and pathological oxidative stress states. MSCs suppress myeloperoxidase, induce inducible nitric oxide synthase, and concurrently decrease the expression of inflammatory cytokines, including IL-1b, IL-4, IL-6, IL-9, TNF-α, and IFN-g [198]. Moreover, MSCs suppress the expression of ROS and myeloperoxidase elicited by monocytes and macrophages upon stimulation, consequently mitigating the M1 macrophage expression [199]. Additionally, MSCs augment the expression of stanniocalcin (STC), which diminishes ROS generation in

macrophage mitochondria and restrains the activation of nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) and caspase-1 in these cells, thereby influencing the transcription of TNF-α and IL-6, and the expression of IL-1β [200].

Studies demonstrate that exosomes released by MSCs post hypoxic preconditioning exert regulatory effects on the accumulation of ROS in epithelial cells via HIF-1α. This process concurrently prevents DNA damage, preserving immune homeostasis, and ultimately mitigates the damage induced by ulcerative colitis [201]. Furthermore, the transplantation of MSCs improves the GP91/ROS/inflammasome signaling in diabetic cardiomyopathy while enhancing the expression of antioxidant proteins [202]. Studies have documented that exosomes released by human MSCs exert a significant inhibitory effect on glucocorticoid-induced apoptosis of osteoblasts through the modulation of the MAPK pathway and regulation of ROS levels [203]. Additionally, it has been demonstrated that human MSCs possess the capability to modulate the innate immune response through the ROS/NLRP3 signaling pathway, thereby mitigating the severity of MHV-68 pneumonia [204]. Considering the antioxidative and immunomodulatory properties of MSCs, their application in clinical therapy for DFU offers a plethora of possibilities, thereby expanding the horizons for DFU treatment.

9.9. Suppression of pro-inflammatory T cells and regulatory Treg cells

Certain chemokines and their corresponding receptors within the organism serve to regulate wound healing processes, orchestrating functions such as epithelialization, angiogenesis induction, and tissue remodeling [205]. The chemokine receptor CCR4, accompanied by its ligands CCL17 (TARC/Thymus and Activation Regulated Chemokine) and CCL22 (MDC/Macrophage-Derived Chemokine), intricately modulates the recruitment process of T cells (Tregs) [206]. Tregs, constitutively expressing the transcription factor Foxp3-, within the $CD4^+$ T cell subset, are deemed pivotal in immune surveillance [207]. Treg cells constitute a substantial fraction of T cells within human skin and play a suppressive role in the body's autoimmune responses [208]. In instances of local inflammatory reactions, Treg cells migrate to the inflamed site, where they enact suppression upon both innate and adaptive immune responses [209]. Research has indicated that the chemokine receptor CCR4 inhibits the wound healing process, potentially attributed to the recruitment of Tregs to the wound site [207]. Moreover, the Th1 and Th17 subsets of helper T cells orchestrate inflammation in the pathogenesis of inflammatory diseases [210]. MSCs possess the capacity to suppress the differentiation and functionality of pro-inflammatory T cells, including Th17 cells, while inducing a regulatory T cell phenotype [211], thus efficiently modulating inflammation at the injury site. This property of MSCs introduces a novel therapeutic approach for the healing of DFU.

9.10. Regulation of neutrophil extracellular trapping networks (NETs) formation

Neutrophils, the initial innate immune cells recruited to the injury site during wound healing, eliminate pathogens and necrotic material via phagocytosis, degranulation, and the release of Neutrophil Extracellular Traps (NETs). NETs, composed of mesh-like DNA, histones, and various granular proteins, are released by activated neutrophils to limit bacterial spread and kill pathogens, a process considered a protective mechanism [212]. However, accumulating evidence indicates that excessive NET formation may contribute to tissue damage [213]. The release of NETs is stringently dependent on glucose and partially reliant on glycolysis [214]. In 2015, Wong et al. were the first to propose that diabetes markedly enhances NET formation in wounds, consequently delaying wound healing in diabetic patients [215].

Current research has confirmed that MSCs and their derived exosomes have a significant regulatory effect on the release of NETs. Upon activation, MSCs engage with the host's innate immune response, which encompasses the induction of NET formation and the augmentation of bacterial phagocytosis. When MSCs are used in conjunction with antibiotic therapy, the administration of activated MSCs into mice with established Staphylococcus aureus biofilm infections results in a marked reduction in bacterial load at the wound site, thus facilitating wound healing [216]. hAM-MSCs mediate their immunomodulatory effects via prostaglandin E2 (PGE2), effectively inhibiting NET release. This discovery unveils a novel mechanism through which hAM-MSCs modulate innate immune responses and suppress NET release [217]. Intravenous administration of mesenchymal stem cell-derived exosomes can significantly mitigate spinal cord injury, a process mediated through the regulation of NET formation by miR-125a-3p contained within the exosomes [218]. Luo et al.'s research was the first to observe that the terminal complement activation complex C5b-9 can initiate NET release and stimulate interleukin-17 production. Conversely, exosomes secreted by MSCs can inhibit the assembly of C5b-9 and subsequently suppress neutrophil-induced NET and IL-17 production [219].

9.11. Inhibition of ferroptosis

Ferroptosis has been closely implicated in the pathogenesis of various diseases. Evidence suggests a strong correlation between the progression of type 2 diabetes and iron overload [220]. As previously discussed, keratinocytes play a critical role in promoting epithelial regeneration during wound healing. Research indicates that the inhibition of ferroptosis in keratinocytes can markedly reduce the expression of inflammatory cytokines such as TNF-α and IL-6, thereby effectively mitigating inflammation in psoriasis [221]. During the resolution phase of inflammation, M1 macrophages gradually transition into M2 macrophages, thereby facilitating wound healing. Zhou et al.'s research revealed that iron overload markedly upregulates the expression of M1 macrophage markers, including IL-1β, TGF-α, and CD86, whereas the expression levels of M2 macrophage markers such as arginase-1 (Arg-1) and CD206 remain largely unaffected. This observation underscores the strong link between iron overload and macrophage polarization. Further mechanistic insights suggest that iron overload substantially promotes ROS release and concurrently activates p53 acetylation, thereby inducing M1 macrophage polarization [222]. High-mobility group box 1 (HMGB1) is a non-histone chromatin-binding protein present in the nuclei of eukaryotic cells, playing a pivotal role in DNA damage repair and the maintenance of genomic stability [223].

Ferroptotic cells can trigger the release of HMGB1, thereby promoting the polarization of pro-inflammatory macrophages [224]. Vascular complications represent another critical factor contributing to delayed wound healing in diabetes. Evidence suggests that iron overload in endothelial cells leads to increased ROS production, which is accompanied by a marked release of the chemokine monocyte chemoattractant protein-1 (MCP-1). Elevated MCP-1 levels enhance the recruitment of monocytes. These monocytes subsequently differentiate into macrophages, which engulf oxidized low-density lipoprotein (Ox-LDL), ultimately transforming into foam cells and exacerbating atherosclerosis [225]. Ferrostatin-1 (Fer-1), a well-known ferroptosis inhibitor, prevents ferroptosis by mitigating lipid peroxidation. Bai et al. discovered that endothelial nitric oxide synthase (eNOS) expression levels were significantly elevated in Fer-1-treated mice. eNOS plays a pivotal role in NO synthesis, thereby promoting vasodilation, inhibiting platelet activation, and reducing immune cell adhesion, which collectively mitigate systemic inflammation [226]. Furthermore, investigations on endothelial cells and fibroblasts cultured under high glucose conditions reveal a reduction in VEGF expression levels in endothelial cells, inhibition of fibroblast migration, and a decrease in activity in both cell types. The diminished ability of endothelial cells to support fibroblast synthesis under high glucose conditions can be restored by Fer-1 [227]. Moreover, Fer-1 facilitates a decrease in ferroptosis-related markers GXP4 and SLC7A11 in endothelial cells and fibroblasts under high glucose conditions, while enhancing TFR1 expression at both the transcriptional and translational levels [227]. Administration of Fer-1 significantly accelerates wound healing in diabetic rats [228]. Deferoxamine (DFO) is a clinically utilized iron chelator for treating iron overload, demonstrated to reduce oxidative stress and stimulate hypoxia-inducible factor-1 alpha (HIF-1α) activation, thereby promoting angiogenesis and improving chronic wound healing [229]. Duscher et al.'s study employing an advanced drug delivery system (TDDS) for delivering DFO to the skin demonstrated significant improvements in wound healing rates in diabetic mice, accompanied by increased neovascularization and enhanced dermal thickness [230].

Research has shown that miRNA-17-92 is highly expressed and enriched in exosomes secreted by MSCs. This miRNA promotes proliferation and migration of HUVECs in vitro, accelerating cell proliferation, migration, angiogenesis, and resistance to ferroptosis. Knockdown of miR-17-92 significantly diminishes its effect on wound healing. These findings indicate that miR-17-92 in MSC-derived exosomes plays a crucial role in protecting HUVECs from ferroptosis [231]. Additionally, exosomes derived from MSCs significantly inhibit the expression of NETs. NETs, in turn, regulate ferroptosis by inhibiting the PI3K/AKT pathway, thereby significantly enhancing diabetic wound healing [232]. Additionally, in vitro studies have shown that BMSC-derived exosomes carrying miR-367-3p significantly inhibit ferroptosis in microglia. Mechanistic analysis reveals that miR-367-3p binds to enhancer of zeste homolog 2 (EZH2) and inhibits its expression, leading to the overexpression of solute carrier family 7 member 11 (SLC7A11). Moreover, the overexpression of SLC7A11 leads to the activation of glutathione peroxidase 4 (GPX4) and suppression of ferroptosis [233]. Exosomes derived from hUC-MSCs inhibit lipid peroxidation (LPO) and ferroptosis through miR-129-5p targeting of acyl-CoA synthetase long-chain family member 4 (ACSL4), thereby significantly reducing inflammation and promoting tissue repair [234].

9.12. Regulation of signaling pathways

Various signaling pathways have been reported to facilitate the pathophysiological mechanisms regulating DFU, thereby promoting its healing. BM-MSCs secrete a variety of growth factors including VEGF-1α, ANG, HIF-1α, and MMP-9. These growth factors elevate the expression levels of VEGF-1α, MMP-9, pAKT, and VEGF-R in endothelial cells (HUVECs), thus activating the VEGF/AKT signaling pathway. This activation leads to enhanced proliferation, migration, and tube formation capabilities of HUVECs, while concurrently suppressing their apoptosis [235]. Treatment with MSC-conditioned medium engineered to overexpress VEGF (MSC-VEGF-CM) activates the PI-3K/AKT/mTOR/eNOS and p38/MAPK signaling pathways. This activation leads to enhanced activity and migratory capacity of pancreatic islet microvascular endothelial cells [236]. The utilization of conditioned medium derived from BM-MSCs markedly enhances the migratory and proliferative capabilities of human umbilical vein endothelial cells. Importantly, this process has been experimentally verified to operate independently of the extracellular signal-regulated kinase (ERK) signaling pathway, with the AKT signaling pathway playing a pivotal role [237]. The protein-serine-threonine kinase (AKT) stands out as a ubiquitous serine/threonine kinase, assuming a central role as a signaling hub across diverse cellular functions in the organism. The Pi3-dependent activation of AKT emerges as a regulatory mechanism governing the activity, proliferation, migratory prowess, and vascularization functionality of MSCs, underscoring the pivotal role this pathway plays in the modulation of MSCs behavior [238]. Given its remarkable evolutionary conservation, the Wnt signaling pathway assumes a pivotal role in embryonic development and the intricate processes of stem cell differentiation. Indeed, mounting evidence underscores the correlation between dysregulated Wnt signaling and the pathogenesis of diverse diseases [239]. The investigation implemented hUC-MSCs-CCLDADM scaffold materials on skin ulcer wounds in both normal and diabetic rats, accompanied by the administration of a Wnt signaling agonist or antagonist at the scaffold periphery. The findings demonstrated a notable enhancement in the healing speed of diabetic rat skin wounds upon activation of the Wnt signaling pathway, whereas the suppression of this pathway resulted in a substantial deceleration in wound healing [88]. Amniotic fluid-derived MSCs (AF-MSCs) notably elevate the levels of endogenous vascular growth factors, thereby fostering wound healing, while also substantially augmenting the regenerative potential of epidermal cells. Under hypoxic conditions, AF-MSCs bolster the proliferation and migration capacities of dermal fibroblasts via the transforming growth factor-β (TGF-β)/SMAD2 and PI3K/AKT signaling pathways, thereby fostering the process of wound healing [240]. Studies have demonstrated that the combined application of PRP and rat ADSCs in treating diabetic wounds in rats significantly accelerates wound healing by modulating the Notch signaling pathway to enhance angiogenesis and the proliferation of epidermal stem cells [241].

9.13. Recruitment of other cells

MSCs facilitate the recruitment of other cells to the site of injury, thereby fostering wound healing, a crucial hallmark of their function. There is ample evidence from various studies supporting this pivotal role of MSCs. Treatment of rat keratinocytes with mesenchymal stem cell-conditioned medium leads to a significant reduction in ROS overproduction mediated by HG and/or LPS. Furthermore, it demonstrates effective regulation of the HG and/or LPS-induced phosphorylation of MEK1/2 and Erk/2, consequently enhancing the proliferation and migratory capacities of keratinocytes under high-glucose environments [242]. The in vitro application of MSCs also facilitates ulcer healing by activating the β2-AR signaling pathway to enhance the migration of keratinocytes around the wound site [243]. Moreover, exosomes released by MSCs activate the Akt, ERK, and STAT3 pathways while inducing the expression of HGF, IGF1, NGF, and SDF1, thereby promoting the proliferation and migratory abilities of fibroblasts. Furthermore, it stimulates angiogenesis, ultimately contributing to the facilitation of chronic wound healing [244].

9.14. Secretion of growth factors

The process of wound healing is intricately woven, requiring the orchestrated interplay of diverse entities such as cells, proteins, growth factors, cytokines, and chemokines. MSCs possess the unique capability to regulate the microenvironment in damaged tissues, consequently fostering the restoration of injured tissues. In the intricate ballet of wound healing, growth factors, in addition to their direct roles in promoting the healing process, function as conductors harmonizing a symphony of cellular activities. The growth factors secreted by MSCs exert their effects through direct secretion or encapsulation within substances such as exosomes. MSCs have the capability to release factors including TGF-β1 and hepatocyte growth factor (HGF). TGF-β1 plays a pivotal role in promoting the differentiation of myofibroblasts and the synthesis of type I and type III collagens [245]. HGF has the ability to augment the expression of MMPs in fibroblasts, leading to the breakdown of ECM molecules such as collagen, laminin, and proteoglycans [246]. Additionally, MSCs have the capacity to secrete prostaglandin E2 (PGE2), which plays a role in inhibiting the differentiation of $CD4^+$ T cells into Th17 cells [247]. MSCs secrete anti-inflammatory cytokines, thereby contributing to the process of wound healing. Moreover, MSCs release the anti-inflammatory factor IL-10, facilitating the transition of macrophages from the M1 phenotype to the M2 phenotype [248], thereby expediting the process of wound healing.

Table 3
The role of bioactive molecules in MSC-derived exosomes in preclinical studies of DFU.

		Source		Researchers	References
miRNA	miR-21	UC-MSCs	miR-21 enhances the proliferation and migration capabilities of corneal epithelial cells by inhibiting PTEN, subsequently upregulating the PI3K/Akt signaling pathway, thereby effectively facilitating corneal injury repair and regeneration./miR-21-5p enhances angiogenesis by upregulating VEGFR and activating the AKT and MAPK signaling pathways.	*Liu, X.* et al./*Huang, C.* et al.	[263,265]
	miR-23	UC-MSCs	miR-23 suppresses the excessive deposition of α-smooth muscle actin and collagen by inhibiting the TGF-β/SMAD2 signaling pathway.	*S. Fang* et al.	[267]
	miR-126	SMSCs	miR-126 enhances the proliferation of human dermal fibroblasts and HMEC-1, as well as the migration of HMEC-1, collagen and tubule formation.	*Tao, S.C.* et al.	[269]
	miR-146	BM-MSCs	miR-146a downregulates the target genes TRAF6 and IRAK1, leading to reduced expression levels of NF-kB, IL-6, and MIP-2, which in turn suppresses the local inflammatory response in the body.	*Xu, J.* et al.	[270]
	miR-210-3p	BM-MSCs	miRNA-210-3p enhances the expression of the VEGF gene and activates the expression of pro-angiogenic proteins ERK and AKT, thereby exerting a role in improving microcirculation and promoting angiogenesis.	*Gangadaran, P.* et al.	[271]
	miR-221-3p	BM-MSCs	miRNA-221-3p modulates the AKT/eNOS pathway, thereby enhancing the functional activity of endothelial cells.	*Yu, M.* et al.	[272]
	miR-223	BM-MSCs, JMMSCs	miR-223 induces M2 polarization of macrophages by targeting pknox1.	*He, X.* et al.	[273]
lncRNA	lncRNA H19	BM-MSCs, HF-MSCs	lncRNA H19 orchestrates the healing process of DFU by modulating the PI3K/AKT signaling pathway and fostering a synergistic interplay between miR-152-3p and PTEN./lncRNA H19 inhibits the activation of the NLRP3 inflammasome, thus facilitating HaCaT proliferation, migration, and suppressing cell pyroptosis.	*Li, B.* et al./*Yang, H.* et al.	[274,275]
	MALAT1	BM-MSCs	MALAT1 induces a reduction in the expression level of miR-205-5p and enhances the translation level of VEGF protein.	*Zhu, L.* et al.	[276]
circRNA	mmu_circ_0000250	ADSCs	mmu_circ_0000250 upregulates SIRT1 expression level while absorbing miR-128-3p.	*Shi, R.* et al.	[277]

9.15. Production of antimicrobial peptides

MSCs possess the capacity to generate antimicrobial peptides (AMPs), exerting regulatory effects on immune responses and suppressing infections caused by pathogens. MSCs can secrete AMPs with anti-inflammatory activity against a spectrum of microorganisms, including bacteria, fungi, and viruses, comprising hepcidin, cathelicidin LL-37, and β-defensin-2 [249]. Hepcidin mainly functions in antimicrobial activity by reducing extracellular iron availability. This effect has been proven effective against various clinically relevant bacteria. LL-37 is an antimicrobial peptide known as "host defense peptide". It exhibits potent antimicrobial activity against both susceptible and antibiotic-resistant gram-positive and gram-negative bacteria [250]. Furthermore, LL-37 can exert antimicrobial effects by inhibiting the formation of biofilms and eradicating pre-existing biofilms, representing a principal antimicrobial mechanism of LL-37 [251]. LL-37 has the ability to facilitate the migration of immune cells to the site of infection and modulate the activity of immune cells, thus achieving pathogen clearance. β-defensin-2 can induce the substantial production of the classical anti-inflammatory cytokine IL-10 [252]. As reported, defensins can induce the conversion of $CD4^{+}$T cells into Tregs, thereby exerting immunomodulatory effects [253]. In addition to the functions mentioned above, these AMPs also engage in interactions among MSCs, thus affecting the proliferation, migration, and regeneration of MSCs. These attributes of AMPs have made them a focal point of research in recent years.

9.16. Release of apoptotic vesicles

Research has demonstrated that apoptotic bodies (ABs) released by MSCs facilitate macrophage polarization towards the M2 phenotype, consequently enhancing skin wound healing [254].

10. Bioactive substances in MSCs-exo

Exosomes released by MSCs are capable of transporting a diverse array of bioactive substances over long distances within the body, thereby playing a pivotal role in cellular communication [255]. Exosomes contain a plethora of bioactive substances, including miRNA, lncRNA, circRNA, and proteins. These exosomes released by MSCs aid in the wound healing process of DFU by transporting these active factors(Table .3).

10.1. miRNA

Within the spectrum of diabetes complications, miR-21 has garnered considerable attention [256]. Its involvement in the diabetic wound healing process spans across diverse mechanisms. Research indicates that miR-21, while reducing the expression levels of phosphatase and tensin homolog (PTEN), stimulates the PI3K/AKT signaling pathway, consequently enhancing dendritic cells (DCs) and facilitating full-thickness wound healing in rat models [257]. Moreover, under high-glucose conditions, cultured fibroblasts exhibit a direct interaction between the NF-κB p65 subunit and the miR-21 promoter upon TGF-β stimulation, facilitating fibroblast migration [258]. This discovery unveils novel therapeutic avenues for DFU management. Through RNA sequencing, studies have identified miRNA-21-5p, known for its capability to promote angiogenesis. Consequently, miRNA-21-5p emerges as a novel mechanism facilitated by MSC-exo to foster angiogenesis and repair ischemic tissue in diabetic foot [259]. Furthermore, the miR-23 family (miR-23a/b/c) targets Sprouty2 and Sema6A, consequently activating angiogenic signals and fostering vascular development within the organism [260]. Exosomes derived from UC-MSCs carrying miR-23 play an active role in suppressing the TGF-β/SMAD2 signaling pathway during the process of wound healing, illustrating a dynamic interplay between cellular components in tissue repair. This inhibition mitigates the excessive deposition of α-SMA and collagen, thereby contributing to the prevention of scar formation during the wound healing process [261]. miR-126 is pivotal in physiological blood vessel formation and inflammation regulation [262,263]. Exosomes derived from synovial mesenchymal stem cells (SMSCs), which overexpress miR-126, not only stimulate the proliferation of human dermal fibroblasts and human microvascular endothelial cells (HMEC-1) but also facilitate HMEC-1 migration, collagen synthesis, and lumen formation. Consequently, they promote the healing of full-thickness skin wounds in diabetic rats [264]. Furthermore, in the exosomes derived from BM-MSCs, the downregulation of miR-146a selectively targets TRAF6 and IRAK1, leading to a decrease in NF-κB, IL-6, and MIP-2 expression levels. This suppression effectively inhibits local inflammatory responses, thereby fostering diabetic wound repair [265]. Within exosomes originating from BM-MSCs, miRNA-210-3p amplifies the expression of the VEGF gene, consequently activating the expression of pro-angiogenic proteins ERK and AKT. Consequently, it facilitates the enhancement of microcirculation and the promotion of angiogenesis [266]. BM-MSCs have the capability to modulate the AKT/eNOS pathway via miRNA-221-3p contained within their exosomes, resulting in heightened functional activity of endothelial cells and contributing to the facilitation of wound healing in individuals with diabetes [267]. miR-223 within exosomes derived from BM-MSCs and JM-MSCs targets pknox1, leading to the polarization of macrophages toward the M2 phenotype, consequently enhancing the healing process of skin wounds [184]. The aforementioned research findings indicate that miRNAs originating from MSCs-exosomes play a pivotal role in facilitating wound healing throughout the DFU healing process.

10.2. lncRNA

Studies suggest that the long non-coding RNA H19 (lncRNA H19) intricately regulates hepatic glucose production and insulin resistance within a high-glucose microenvironment [268]. lncRNA H19 derived from MSCs-exosomes facilitates the healing of DFU by

modulating the PI3K/AKT signaling pathway and orchestrating the reciprocal interplay between miR-152-3p and PTEN [269]. Moreover, exosomes derived from hair follicle mesenchymal stem cells (HF-MSCs-Exo) with elevated levels of lncRNA H19 exhibit the capability to dampen the stimulation of NLRP3 inflammasome, consequently fostering the proliferation, migration, and restraining the pyroptosis of HaCaT cells [270]. According to reports, the therapeutic efficacy of VEGF in treating DFU is improved upon the deletion

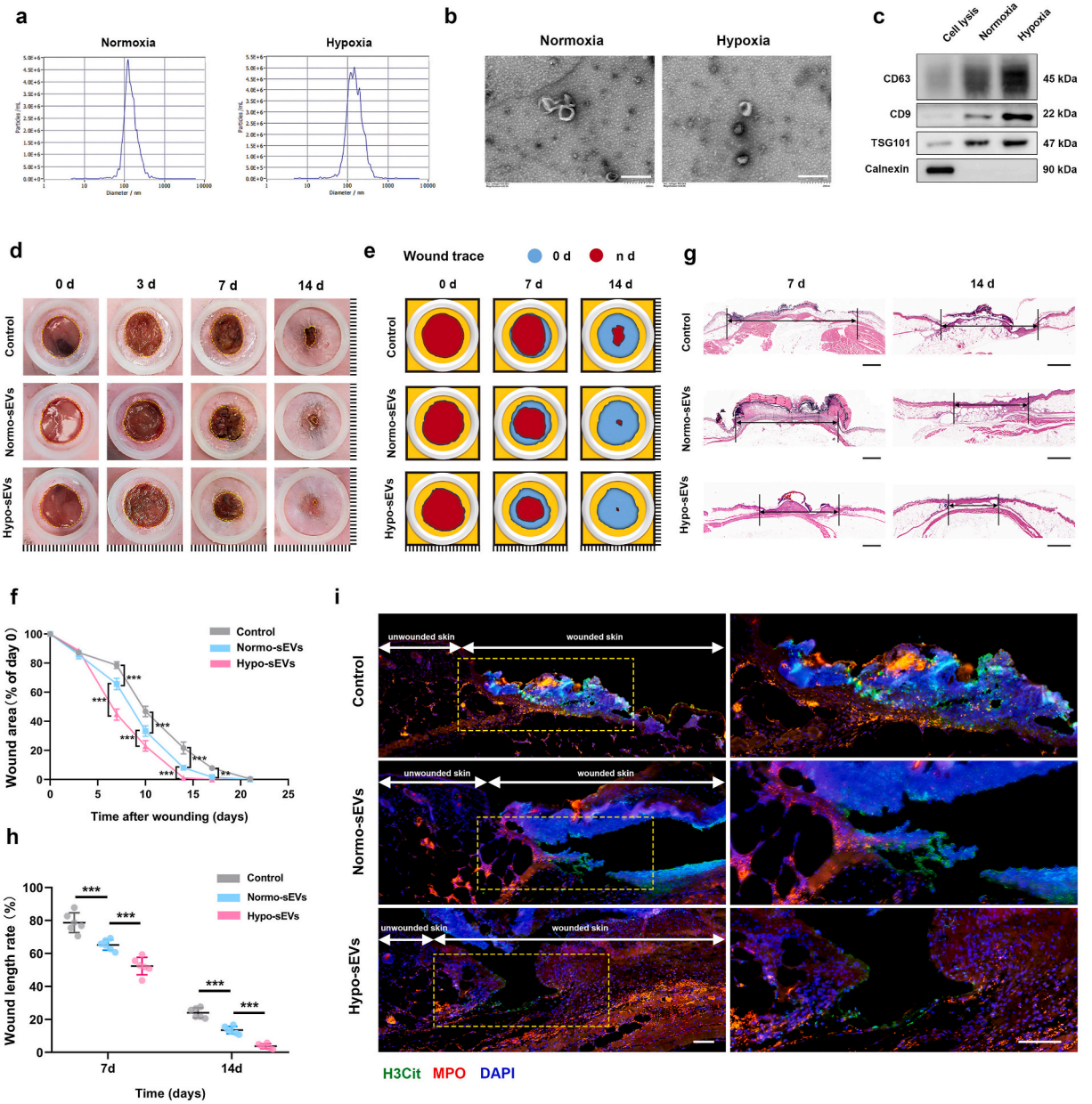

Fig. 6. Low-oxygen pre-treated exosomes expedite the healing of diabetic wounds by attenuating the excessive formation of neutrophil extracellular traps (NETs) in vivo. (a) Nanoparticle tracking analysis (NTA) was employed to evaluate the size and concentration distribution of Normo-sEVs and Hypo-sEVs derived from MSCs. (b) Representative micrographs of Normo-sEVs and Hypo-sEVs were obtained via transmission electron microscopy (TEM). (c) Western blot analysis was performed to evaluate the expression levels of sEV markers, including CD63, CD9, TSG101, and Calnexin. (d) Animals were intravenously injected with PBS, Normo-sEVs, or Hypo-sEVs, and representative images depicting wound closure were captured at days 0, 3, 7, and 14 post-injection, respectively. (e) Illustrative diagrams depicting the wound healing process. (f) Wound closure rates were quantitatively assessed; N = 6. (g) Representative images illustrating H&E staining at various time points of the wound sites were captured. The scale bar represents 2 mm. (h) Quantitative analysis was conducted to assess wound length; N = 6. (i) Immunofluorescence staining was employed to evaluate the NETs area in the wound sites of each group on the third day post-injection in the animal model (H3Cit: green; MPO: red; DAPI: blue); N = 6. The scale bar indicates 100 μm. Significance levels are denoted as $^{**}p < 0.01$ and $^{***}p < 0.001$. Reproduced under the terms of the CC-BY license [274]. Copyright 2023, the authors.

of miR-205-5p within human mesenchymal stem cells. Additionally, lncRNA MALAT1 acts as a competitive endogenous RNA (ceRNA) for miR-205-5p. Consequently, the upregulation of MALAT1 results in a reduction in the expression levels of miR-205-5p and an elevation in the translation levels of VEGF protein, thereby enhancing wound healing in diabetic mice [271].

10.3. circRNA

Circular RNAs (circRNAs) play a pivotal role in modulating the microenvironment during wound healing. The vascular regeneration capacity is hindered under high-glucose conditions, resulting in delayed wound healing. Exosomes derived from ADSCs modified by mmu_circ_0000250 exhibit the ability to counteract this inhibitory effect. Exosomes derived from ADSCs, enriched with mmu_circ_0000250, efficiently sequester miR-128-3p and concomitantly elevate the expression level of SIRT1, consequently manifesting a therapeutic impact on enhancing the healing of diabetic wounds [272].

11. Pretreatment of exosomes

Recent investigations have demonstrated that pretreating MSCs with diverse approaches, including pharmacological agents, cytokines, and physical stimuli, can augment their biological activity and functionality in the context of tissue regeneration [273,278]. According to reports, excessive neutrophil extracellular trap (NET) formation was identified in diabetic wounds, highlighting the pivotal role of NETs in delayed wound healing. Notably, hypoxia-preconditioned MSC-derived exosomes demonstrated enhanced suppression of NETs compared to those secreted by normal MSCs, underscoring the superior advantage of hypoxia-preconditioned MSC-secreted exosomes in the field of regenerative medicine [274](Fig. 6). Furthermore, MSCs pre-treated with red ginseng augment diabetic wound healing by amplifying their paracrine activity [275]. Exosomes derived from pioglitazone-pretreated MSCs expedite diabetic wound healing by stimulating angiogenesis [276]. Exosomes secreted by melatonin-primed MSCs regulate macrophage polarization through the PTEN/AKT pathway, thereby enhancing diabetic wound healing [277]. Exosomes derived from BM-MSCs pre-treated with DMOG facilitate bone regeneration by targeting the AKT/mTOR pathway [279]. Pretreatment with fluoxetine enhances the effectiveness of MSCs in diabetic neuropathy [280]. Modulating the secretion profile of proteins from MSCs through pre-treatment represents a potential new avenue in the study of MSCs and their exosomes.

12. Summary and outlook

In comparison to conventional therapeutic approaches, MSCs along with their secreted exosomes offer considerable advantages in the treatment of DFU. MSCs can facilitate DFU wound healing through diverse mechanisms, such as modulating macrophage polarization, optimizing the local microenvironment, and augmenting angiogenesis. These processes constitute a complex cascade involving multiple signaling pathways. Furthermore, the paracrine activity of MSCs, resulting in the secretion of a myriad of bioactive substances, also exerts a pivotal role in the wound healing process. Within the spectrum of bioactive substances secreted by MSCs, exosomes have been empirically shown to transport a broad array of materials, encompassing miRNA, lncRNA, circRNA, proteins, among others, across long distances within the body. This attribute also bestows considerable advantages in fostering DFU healing. Present conclusions from studies on MSCs and their secreted exosomes indicate substantial therapeutic promise in addressing DFU. Nonetheless, the utilization of MSCs and their exosomes in practice presents specific constraints and hurdles.

The differentiation potential, proliferation capacity, and reparative ability of MSCs diminish gradually with both increasing passage number and age. Hence, to optimize the advantageous application of MSCs, it is imperative for us to standardize their treatment in future research endeavors. Various clinical studies have assessed the safety and efficacy of MSCs in treating DFUs. Standardizing the therapeutic outcomes of MSCs prior to commencing clinical trials is paramount. Despite the preliminary confirmation of the efficacy and safety of MSCs therapy for DFU, there is a need for further investigation into treatment mechanisms, efficacy evaluation, individualized selection of MSCs, and application protocols. In conclusion, MSCs therapy presents a promising, relatively safe, and effective treatment modality for DFUs.

In comparison to the solitary use of MSCs-exo, the utilization of engineered exosomes, achieved through co-administration with biomaterial scaffolds or additional modifications to their surface and interior, exhibits enhanced efficacy in wound healing. This innovation also offers a novel avenue for ongoing investigations into MSCs-exo's role in promoting DFU recovery. Nonetheless, limitations exist in the application of MSCs-exo due to their susceptibility to expansion and inactivation at room temperature, as well as the low yields obtained during extraction. Presently, investigations into the efficacy of MSCs-exo for DFU primarily revolve around preclinical studies, including cell experiments and animal models. Notably, clinical trials validating the therapeutic potential of exosomes remain absent. To surmount the transition from preclinical to clinical application, future research endeavors should prioritize understanding the mechanistic underpinnings of MSCs-exo and instituting standardized production protocols. Although facing myriad challenges in the clinical application of MSCs-exo, the undeniable potential of MSCs and their exosomes in DFU therapy underscores the necessity for deeper exploration.

CRediT authorship contribution statement

ShuHui Wu: Writing – original draft, Conceptualization. **ZhongSheng Zhou:** Writing – original draft, Conceptualization. **Yang Li:** Investigation. **Jinlan Jiang:** Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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