



REVIEW ARTICLE

Research Advances in the Application of Adipose-Derived Stem Cells Derived Exosomes in Cutaneous Wound Healing

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Cutaneous wound healing has always been an intractable medical problem for both clinicians and researchers, with an urgent need for more efficacious methods to achieve optimal outcomes morphologically and functionally. Stem cells, the body's rapid response 'road repair crew,' being on standby to combat tissue injuries, are an essential part of regenerative medicine. Currently, the use of adipose-derived stem cells (ADSCs), a kind of mesenchymal stem cells with multipotent differentiation and self-renewal capacity, is surging in the field of cutaneous wound healing. ADSCs may exert influences either by releasing paracrine signalling factors or differentiating into mature adipose cells to provide the 'building blocks' for engineered tissue. As an important paracrine substance released from ADSCs, exosomes are a kind of extracellular vesicles and carrying various bioactive molecules mediating adjacent or distant intercellular communication. Previous studies have indicated that ADSCs derived exosomes (ADSCs-Exos) promoted skin wound healing by affecting all stages of wound healing, including regulating inflammatory response, promoting proliferation and migration of fibroblasts or keratinocytes, facilitating angiogenesis, and regulating remodeling of extracellular matrix, which have

provided new opportunities for understanding how ADSCs-Exos mediate intercellular communication in pathological processes of the skin and therapeutic strategies for cutaneous wound repair. In this review, we focus on elucidating the role of ADSCs-Exos at various stages of cutaneous wound healing, detailing the latest developments, and presenting some challenges necessary to be addressed in this field, with the expectation of providing a new perspective on how to best utilize this powerful cell-free therapy in the future. (**Ann Dermatol 33(4) 309 ~ 317, 2021**)

-Keywords-

Adipose-derived mesenchymal stem cells, Exosomes, Wound healing

INTRODUCTION

The skin protecting various tissues and organs of the body from the external environment as a barrier is the largest organ of the human body¹. A skin wound caused by trauma, thermal or radiation injury consists of an area of disrupted tissue integrity, architecture and homeostasis. Skin wound healing is a complex and orderly dynamic physiological response process made by body after injury, usually including three stages: inflammation, cell proliferation and migration, tissue remodeling². A poor wound healing usually means hyperpigmentation, prolonged healing, scar formation, long-lasting ulceration and other morphological and functional abnormalities. Due to the importance and complexity of wound healing, the research on this has great theoretical and practical significance. Regenerative medicine is a new discipline paying attention to promoting post-traumatic tissue regeneration and functional re-

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construction³. At present, the use of stem cells and related derivatives to promote skin wound healing has been widely studied in the field of tissue or organ repair⁴. Main stem cell types involved include epidermal stem cells, endothelial progenitor cells, hematopoietic stem cells, bone marrow mesenchymal stem cells (BMSCs), adipose-derived stem cells (ADSCs), and embryonic stem cell (ESCs) (Fig. 1)⁵. Although the application of stem cells in the treatment of wound has been widely recognized, there are still some shortcomings, such as uncertain differentiation, potential tumorigenicity, low long-term survival rate, difficult storage and transportation, limiting the further application and propagation of stem cells in clinical wound repair^{6,7}. ADSCs are a kind of mesenchymal stem cells (MSCs) with multipotent differentiation and self-renewal capacity, for the past few years, having gained increasing attention in regenerative medicine due to the advantages of easy acquisition, sufficient source and low immune rejection⁸. With the in-depth study of ADSCs, more and more evidences show that the paracrine mechanism is the main way for them to exert influences. ADSCs can secrete sub-cellular particles of lipid bilayer membrane with diameter 50 to 150 nanometers (nm), which are called adipose-derived stem cells derived exosomes (ADSCs-Exos)^{9,10}. ADSCs-Exos can regulate biological behavior by specifically binding to target cells to release their active substances for ma-

terial transportation and signal transmission, including specific proteins, lipids, mRNA, microRNA (miRNA), and other signaling molecules^{11,12}. Compared with ADSCs, ADSCs-Exos are stabler in property, lower in antigenicity, easier to preserve and transport; therefore, at present, extensive studies have been carried out on their compositions, formations, functions and acting mechanisms¹³. Relevant studies have shown that ADSCs-Exos can promote wound healing through different mechanisms, including regulation of inflammatory response, accelerating migration and proliferation of fibroblasts or keratinocytes, promotion of angiogenesis, and regulation of extracellular matrix (ECM) remodeling¹¹. Here, the research progress in the application of ADSCs-Exos in skin wound healing is reviewed.

ADIPOSE-DERIVED STEM CELLS

ADSCs existing in adipose tissues as pre-adipocytes, are a kind of MSCs with self-renewal ability and multidirectional differentiation potential, which are widely used in ADSCs-enhanced fat grafting and ADSCs therapy. In 2001, Zuk et al.¹⁴ first cultured pluripotent stem cells from adipose tissue suspension by liposuction and named them processed lipoaspirate cells. In 2004, these cells were unanimously named ADSCs at the second annual meeting of the International Fat Applied Technology Society¹⁵. As is

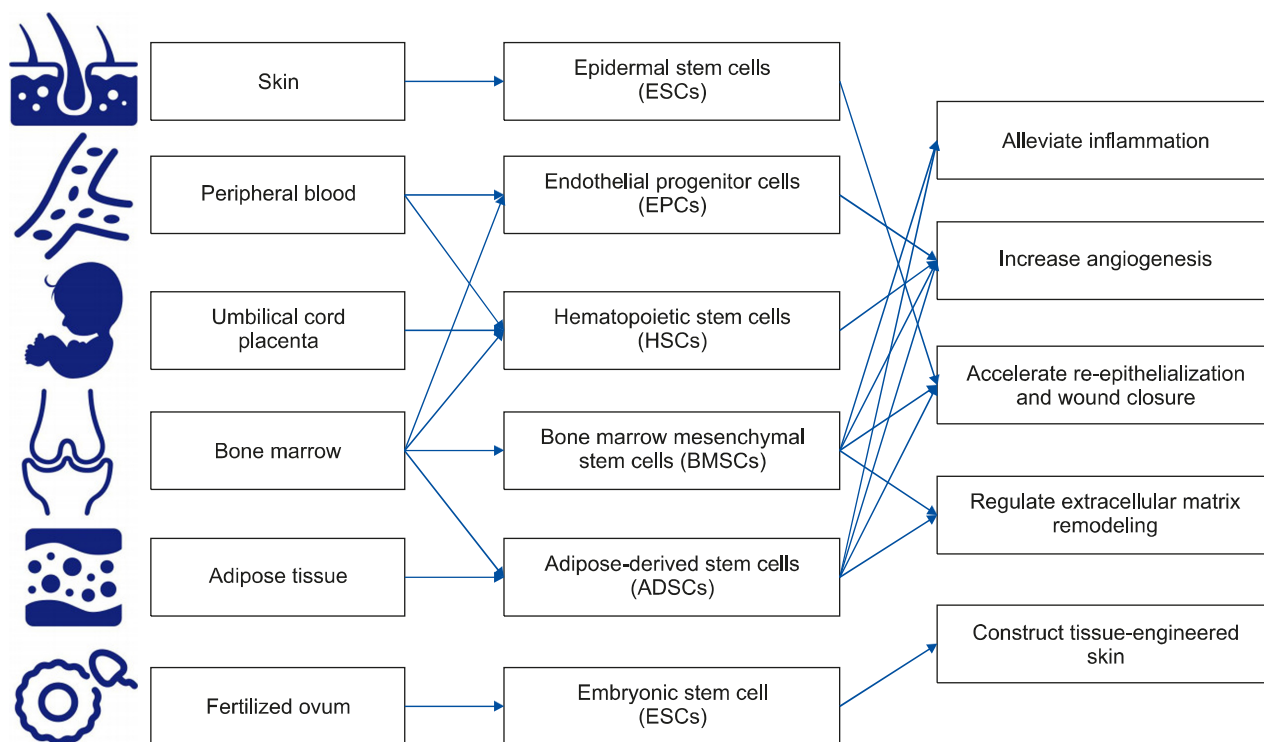


Fig. 1. The roles of main stem cell types involved in cutaneous wound healing.

the case in BMSCs, ADSCs can differentiate into fat, muscle, cartilage, bone, and skin under specific conditions with a particular set of induction factors. ADSCs have similar surface markers, gene expression profile and biological function to BMSCs, however, compared with BMSCs, ADSCs has the advantages of easy or less invasive to harvest and sufficient source, which makes it a hot topic of stem cell research¹⁶. In addition, some scholars suggested that ADSCs had lower metabolic demands and were more resistant to the mechanical trauma of fat grafting, thus being more robust compared to adipocytes¹⁷. Other studies also supported the theory that adding ADSCs may augment fat graft survival by bolstering adipogenesis, supporting vasculature and diminishing cell apoptosis, which are key features of the regenerative properties of fat graft^{18,19}. For the cellular secretory profile, ADSCs produce a more extensive range of chemokines, cytokines and protein growth factors, therefore, in contrast to previously held theories that ADSCs would differentiate to actually replace damaged cells, providing the 'building block' or 'host replacement,' the paracrine effects of the secretome are now considered as more likely to orchestrate the events needed tissue regeneration^{12,20-22}.

CHARACTERISTICS AND FUNCTIONS OF EXOSOMES

Extracellular vesicles (EVs) are membranous vesicles released from the inside of the cell to the outside, having a phospholipid bilayer structure, with a diameter ranging from 50 nm to 5 micrometers (μm)²³. EVs deliver its contents to the recipient cells, thus changing the biological behaviors of that, which not only involves normal physiological processes, also the pathological one²⁴. The contents of EVs depend on the source of the cell types, mainly including protein, lipids, DNA, mRNA, miRNA^{25,26}. According to the source and size, EVs can be divided into three subtypes: apoptotic bodies (diameter, 100 nm~5 μm), microvesicles (diameter, 50 nm~1 μm), and exosomes (diameter, 50~150 nm)²⁷. Apoptotic bodies are released during apoptosis and usually contain organelles and DNA fragments of maternal cells. Microvesicles are falling off from the cell membrane and released to the outside of the cell mainly through the 'budding' way.

Exosomes are the smallest EVs with diameters ranging from 50 to 150 nm, showing a saucer shape under an electron projection microscope²⁸. In 1983, Pan and Johnstone²⁹ firstly discovered the vesicles in mammalian reticulocytes from mature sheep and named it 'exosomes'. Although the underlying mechanism of exosomes formation is not fully understood, it is generally believed that

exosomes originate as intraluminal vesicles which are released by multivesicular bodies (MVBs) as exosomes into the extracellular space via the endosomal maturation pathway³⁰. There are some specific proteins on the exosomes membrane, such as membrane transport and fusion proteins (GTPases, annexins and flotillin), proteins involved in MVBs biogenesis (Alix and tumor susceptibility gene 101 proteins), tetramolecular transmembrane proteins (CD9, CD63, CD81), heat shock proteins (Hsps; heat shock cognate 70 and Hsp90), which could be used as surface markers to identify them³¹⁻³³. At present, various kinds of cells secreting exosomes have been found, such as T cell, B cell, dendritic cell, Schwann cell, platelets, endothelial cell, cardiomyocyte³⁴. In addition, exosomes are also found in the majority of, if not all, biological fluids, such as blood, urine, saliva, bile, cerebrospinal fluid, breast milk, epididymal fluid, semen, ascites, amniotic fluid^{35,36}. The contents of exosomes are a variety of specific biological small molecules, mainly including functional proteins, lipids, mRNA, miRNA, cytokines and transcription factors, which transmit biological information to all parts of the body through the exosomes-mediated adjacent or distant intercellular communication^{37,38}. Studies have shown that the contents of exosomes from different tissues and cells are different, and also different even the same source^{39,40}. It is generally believed that there are three kinds of mechanisms for exosomes to mediate intercellular communication: exosomes release 'messenger substances' into the cytoplasm after the phagocytosis of exosomes and form vesicles again in receptor cells; after the exosomes fuses with the plasma membrane, the 'messenger substance' are released into the cytoplasm; the receptors on the target cells membrane bind to the ligands on the exosomes^{11,41,42}.

THE ROLE OF ADIPOSE-DERIVED STEM CELLS DERIVED EXOSOMES IN SKIN WOUND HEALING

Wound healing is an essential process that can restore the structure and function of injured skin or tissue, requiring multiple cells from several different lineages to coordinate and produce a series of signals at different time⁴³. The wound healing process involves a series of overlapping stages, including inflammatory, cell proliferation and migration, tissue remodeling⁴⁴. None of conventional therapeutic approaches to improve healing, such as therapeutic dressings, laser, hyperbaric oxygen, electrical stimulation, is completely satisfactory, also not widely used in clinical practice⁴⁵⁻⁴⁸. Although the underlying mechanisms of cutaneous wound healing have not been fully clarified to date,

the safety and efficacy of ADSCs-Exos in skin wound repair is increasingly being elucidated through some studies *in vivo* and *in vitro*^{49,50}. It has been confirmed that exosomes are widely involved the above three phrases of wound repair processes to achieve the quicker, better, less-scar healing (Fig. 2)⁵¹. Here, we present an overview of the role of ADSCs-Exos at various stages of cutaneous wound healing and summarize latest research advances in this field.

Inflammatory phase

Inflammation is the body self-defense mechanism in response to harmful stimuli; and varying degrees of inflammation, as the earliest response of wound healing, usually occurring in 24 to 48 hours within a state of ischemia, is characterized by redness, swelling, hyperemia, exudation, leukocyte infiltration⁵². A moderate inflammatory response helps to accelerate wound repair by removing inflammatory factors and cell debris, fighting infection,

while, a chronic or excessive one leads to poor wound healing, including fibrosis, excessive scarring or inhibition of re-epithelialization. Chen et al.⁵³ found that the ADSCs-Exos demonstrated effects comparable to those of their source cells in achieving improved graft retention by up-regulating early inflammation. Zhang et al.⁵⁴ illustrated that a single-exon circular RNA_0075932 in ADSCs-Exos induced inflammation and apoptosis in dermal keratinocytes of burned skin of obese persons by directly binding with Pumilio homolog 2 (PUM2) and promoting PUM2-mediated activation of NF- κ B pathway (nuclear factor kappa-light-chain-enhancer of activated B cells), of which the results could help us understand why obese persons displayed a slower wound healing rate than the normal. Similarly, with a focusing on obese persons, in a study exploring the efficacy of adipose-derived stem cells conditioned media (ADSCs-CM) to counteract persistent inflammation, Kruger et al.⁵⁵ described the therapeutic potential of ADSCs-CM to restore the inflammatory balance

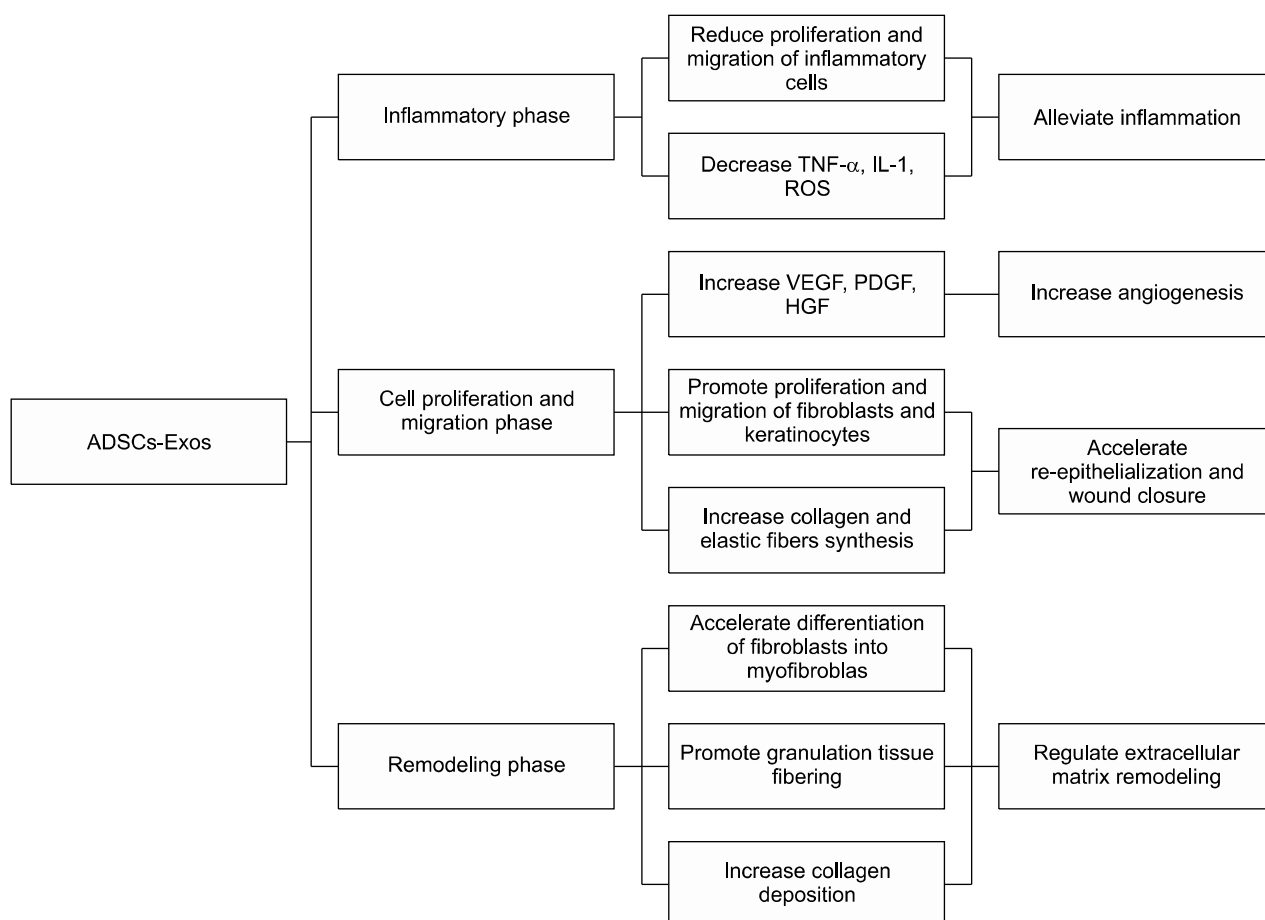


Fig. 2. The influence of ADSCs-Exos on different phases of cutaneous wound healing. ADSCs-Exos: adipose-derived stem cells derived exosomes, TNF- α : tumor necrosis factor- α , IL-1: interleukin 1, ROS: reactive oxygen species, VEGF: vascular endothelial growth factor, PDGF: platelet-derived growth factor, HGF: hepatocyte growth factor.

in immune-compromised obese individuals. Li et al.⁵⁶ confirmed that ADSCs-Exos overexpressing Nrf2 (nuclear factor erythroid2-related factor2) could significantly stimulate the healing of foot wounds in diabetic rats by inhibiting the inflammatory proteins expression and reactive oxygen species production, which was associated with Nrf2-induced oxidative stress reduction and apoptosis during wound healing.

Cell proliferation and migration phase

In the second stage of wound healing, various growth factors in the traumatic microenvironment regulate angiogenesis, cell proliferation and migration. Simultaneously, fibroblasts greatly proliferate and synthesize ECM, with the newly formed thin-walled capillary buds, thus forming granulation tissue to provide new attachment scaffolds for keratinocytes to settle down at later stages. Several studies demonstrated the essential role of ADSCs in angiogenesis which is necessary for newly formed granulation tissue to get adequate blood supply providing nutrients and oxygen. Hoang et al.⁵⁷ found that ADSCs-Exos could secrete crucial growth factors mediating wound healing, like vascular endothelial growth factor A (VEGF-A) and platelet-derived growth factor BB (PDGF-BB), which were indispensable in driving angiogenesis. Shi et al.⁵⁸ found that exosomes derived from circular RNA_0000250-modified ADSCs increased angiopoiesis and suppressed apoptosis in wound skin of diabetic mice through inducing autophagy mediated by miRNA-128-3p/sirtuin 1 (SIRT1). In another mice experiment, ADSCs-Exos in combination with hyaluronic acid accelerated mouse wound healing through enhancing reepithelialization and vascularization, which was more obvious than the group using exosomes alone⁵⁹. As the representative content of exosomes, miRNA makes a substantial contribution to intercellular information transmission. Liang et al.⁶⁰ observed that the ADSCs-Exos will transfer the carried miRNA-125a into vascular endothelial cells (VECs) to down-regulate the expression of Delta-like ligand 4 (DLL4), a angiogenesis inhibitor, thus promoting VECs proliferation and angiogenesis. Kang et al.⁶¹ observed that exosomes were released from ADSCs and able to increase the migration and tube formation of human umbilical vein endothelial cells (HUVECs), with the better effects of ADSCs under preconditioning by endothelial differentiation medium. Further studies exhibited that miRNA-31 in exosomes contributed to the migration and tube formation of HUVECs, and FIH-1 (factor-inhibiting hypoxia inducible factor-1), an antiangiogenic gene, was identified as the target of miRNA-31 in HUVECs⁶¹. In order to avoid the easy degradation of miRNA in wound microenvironment, some researchers tried to load miRNA into engineered ADSCs-Exos by electro-

poration, taking advantage of natural availability and biocompatibility of exosomes as extracellular miRNA transporting particles. Results showed that the engineered exosomes exhibited excellent effects on accelerating diabetic wound healing by increasing re-epithelialization, angiogenesis, and vessel maturation *in vivo*, which would provide a new idea for applying ADSCs-Exos to deliver future drug substances and developing cell-free therapy for wound-healing treatments⁶².

After being activated by trauma, fibroblasts accelerate wound healing by proliferating extensively and synthesizing large amounts of ECM components such as collagen and elastic fibers, which is the fundamental features of wound proliferation. Hu et al.⁶³ found that ADSCs-Exos were internalized by fibroblasts to influence cell migration, proliferation and collagen synthesis through promoting the gene expression of N-cadherin, cyclin 1, proliferating cell nuclear antigen, type I and III collagen. Zhang et al.⁶⁴ proved by *in vitro* and *in vivo* experiments that ADSCs-Exos promoted the proliferation and migration of fibroblast stem cells, synthesis and secretion of collagen or other cell growth factors, where PI3K/Akt signal pathway played a vital role. Previous studies proved that the ADSCs-Exos could induce fibroblasts proliferation and migration by secreting fibroblast growth factor 2 (FGF-2); and, the induction of cell migration was a dependent manner with the higher dose of exosomes was used, the faster migration rate was observed⁵⁷. Similar to the role of miRNA in driving angiogenesis, it also promotes the proliferation of fibroblasts. Choi et al.⁶⁵ found that ADSCs-Exos treatment of human dermal fibroblasts seemed to induce enrichment of the miRNA within the fibroblasts that contribute to healing. At the same time, through miRNA chip analysis, it was found that 292 kinds of miRNA changed during the process of ADSCs-Exos promoting the proliferation and differentiation of dermal fibroblasts, of which 199 were up-regulated and 93 were down-regulated.

Remodeling phase

In the stage of tissue reconstruction, more fibroblasts differentiate into myofibroblasts; granulation tissue gradually fiberizes; collagen increases gradually; and the wound begins to contract, eventually forming scar tissue. Through animal experiments, Hu et al.⁶³ found that injection of ADSCs-Exos in the early stage of wound healing could increase the secretion of collagen I and III and promote wound healing, while the secretion caused by injection in the late stage decreased, which inhibited scar formation. Pelizzo et al.⁶⁶ described the effects of intradermal injection of ADSCs-Exos in an experimental cutaneous wound repair model. After ADSCs-Exos inoculation, well-regen-

erated tissue with the presence of a complete epithelial layer, dermal papillae and cutaneous annexes and restored connective matrix were obtained. Some cytokines and proteins are core elements in this stage, such as matrix metalloproteinase (MMP), transforming growth factor (TGF)- β . Wang et al.⁶⁷ found that ADSCs-Exos, in the late stage of wound healing, prevented fibroblasts from differentiating into myofibroblasts and inhibited granulation tissue formation to reduce scar through increasing both the ratios of collagen III to collagen I as well as TGF- β 3 to TGF- β 1 in a study of intravenous injection of ADSCs-Exos on full-thickness dorsal wounds of mice. In addition, they confirmed that exosomes regulated ECM remodeling by activating the ERK/MAPK pathway in skin dermal fibroblasts and increasing the ratio of MMP3 to TIMP1, leading to a scarless wound healing. Yang et al.⁶⁸ also found that high expression levels of miRNA-21 in ADSCs-Exo promoted wound healing by enhancing MMP-9 expression and inhibiting TIMP2 expression, where the PI3K/AKT pathway worked. Moreover, high miR-21 expression levels could downregulate TGF- β 1 protein levels, thereby reducing the formation of scars in the wound. In addition to fibroblasts, keratinocytes are also the main source of synthesis and secretion of ECM, therefore, there is a consensus that keratinocytes play a key regulatory role in wound healing, scar formation and tissue remodeling. Ma et al.⁶⁹ established a skin injury model by treating human immortalized keratinocytes line (HaCaT) with hydrogen peroxide and proposed that ADSCs-Exos promoted the migration of damaged keratinocytes to regulate ECM remodeling by activating Wnt/ β -catenin signaling pathway. Similarly, by constructing a HaCaT cells model and a mouse wound healing model, Zhang et al.⁷⁰ found that ADSCs-Exos could accelerate the remodeling of wound healing through accelerated keratinocyte migration and proliferation by activating the AKT/HIF-1 α axis.

CONCLUSIONS

A considerable number of patients are still at a high risk of chronic wounds development with associated co-morbidities of infection, sepsis, and osteomyelitis, although the majority of lesions repair quickly and completely using current standard-of-care approaches, thus remaining a considerable burden on medical systems around the world¹. Despite a status that the research about specific mechanism underlying the ability of ADSCs-Exos to promote wound healing is still in its infancy and no related drugs have been approved yet, it is undeniable that the significance of ADSCs-Exos effects has presented a new opportunity to study wound healing and clinic trails about its

therapeutics has been performing for many years, showing good therapeutic outcomes on the whole^{45,71-73}. There are lots of advantages in the applications of ADSCs-Exos, such as easy acquisition, sufficient source, convenient storage or transportation, high long-term survival rate, quantitative usage, avoiding immune rejection, no ethical problems. Compared with the single cytokine, it also has greater tissue regeneration potential. Related studies have shown that the microenvironment has a certain impact on the efficiency and activity of ADSCs-Exos, where hypoxia, inflammation, potential of hydrogen, and other intervention conditions can regulate the capacity to repair tissue^{74,75}. Exploring appropriate microenvironmental conditions and the specific mechanism of their effects on the function of Exos will greatly improve the therapeutic potential of ADSCs-Exos in wound healing. In short, with the in-depth study of the mechanism underlying the capacity of ADSCs-Exos to promote wound healing, its mysterious veil will be gradually unveiled, which is helpful to develop the approach into a clinical reality as an alternative to cell-based therapy. There is no doubt that it will have a broad clinical application prospect in the field of skin wound repair.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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