

# The Emerging Role of Immune Cells and Targeted Therapeutic Strategies in Diabetic Wounds Healing

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**Abstract:** Poor wound healing in individuals with diabetes has long plagued clinicians, and immune cells play key roles in the inflammation, proliferation and remodeling that occur in wound healing. When skin integrity is damaged, immune cells migrate to the wound bed through the actions of chemokines and jointly restore tissue homeostasis and barrier function by exerting their respective biological functions. An imbalance of immune cells often leads to ineffective and disordered inflammatory responses. Due to the maladjusted microenvironment, the wound is unable to smoothly transition to the proliferation and remodeling stage, causing it to develop into a chronic refractory wound. However, chronic refractory wounds consistently lead to negative outcomes, such as long treatment cycles, high hospitalization rates, high medical costs, high disability rates, high mortality rates, and many adverse consequences. Therefore, strategies that promote the rational distribution and coordinated development of immune cells during wound healing are very important for the treatment of diabetic wounds (DW). Here, we explored the following aspects by performing a literature review: 1) the current situation of DW and an introduction to the biological functions of immune cells; 2) the role of immune cells in DW; and 3) existing (or undeveloped) therapies targeting immune cells to promote wound healing to provide new ideas for basic research, clinical treatment and nursing of DW.

**Keywords:** diabetic wounds, wound healing, immune cells, inflammation, targeted therapy

## Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia.<sup>1</sup> In 2019, 463 million people had diabetes worldwide.<sup>2,3</sup> Projections estimate an increase in the number of individuals with diabetes to 578 million by 2030 and as high as 700 million by 2045.<sup>3</sup> DW is the main complication in patients with diabetes.<sup>4</sup> The increasing number of patients with diabetes has caused a daily increase in the incidence of DW, substantially increasing the medical burden of wound repair worldwide.<sup>5</sup> The global advanced wound care market is projected to reach \$18.7 billion by 2027, increasing at a compound annual growth rate of 6.6% over the analysis period from 2020–2027.<sup>6</sup> Studies have shown that the key link in difficult-to-heal DW is abnormal inflammation.<sup>7–9</sup> Immune cells are an indispensable component regulating inflammation and maintaining body homeostasis. They are extremely sensitive to changes in signaling molecules in the body and respond quickly. The mechanism by which immune cells affect wound repair, especially



difficult-to-heal wounds such as DW, has always been a hot and difficult topic in clinical research. At present, the main methods for the treatment of DW are mainly pressure offloading and ulcer protection, restoration of tissue perfusion, treatment of infection, and local ulcer care,<sup>10</sup> but the desired results have not been achieved. Approaches targeting immune cells may provide new and efficient treatment strategies for DW repair. Therefore, in this article, we will use “immune cells” as the key point to discuss in detail the specific mechanisms of immune cells and DW repair. In addition, we also discuss methods to target the regulation of immune cells, promote the smooth transition of DW from inflammation to proliferation and remodeling, and finally promote the healing of DW. Our hope is to provide a new direction for DW treatment strategies.

## Epidemiology, Adverse Consequences and Treatment of DW

DW occur in a specific population of patients with diabetes presenting impaired skin integrity due to various factors, such as abnormal blood glucose levels, an imbalanced immune response, peripheral neuropathy, and vascular damage. In recent years, the number of patients suffering from DW has increased. Between 19% and 34% of persons with diabetes are likely to be affected by diabetic foot ulcers (DFUs).<sup>11,12</sup> The occurrence of DFUs substantially increases the risk of amputation.<sup>12</sup> Worldwide, a person with major lower-extremity amputations (above the ankle) is estimated to have a lower 5-year survival rate (65.01%), followed by those with minor lower-extremity amputations (72.24%) and those without lower-extremity amputations (81.61%).<sup>13</sup> The unpredictability of wound healing and the threat of amputation often leave patients in a state of guilt, depression, disappointment, worry and sadness. Moreover, the mean length of inpatient stay for ulcer-only, minor amputation, and major amputation was 13.3, 20.5, and 59.6 days, respectively.<sup>14</sup> The average annual cost per patient was US \$3368 (ulcer-only), US \$10,468 (minor amputation), and US \$30,131 (major amputation).<sup>14</sup> DW has a long treatment cycle, high disability and fatality rates, high hospitalization rate and high medical expenses, which not only seriously affect the patient's quality of life and physical and mental health but also impose a substantial economic burden on society.

The main treatment strategies for DW currently used in the clinic are described below.<sup>10,15</sup> First, pressure offloading and ulcer protection should be considered. Second, tissue perfusion is restored through revascularization surgery. Third, necrotic tissue is debrided to remove dead tissue and prevent deterioration of the wound. Antibacterial and anti-inflammatory agents are applied through topical or systemic application of antibiotics. Fourth, glycemic control should be optimized, such as using metformin or insulin. Fifth, local care is employed to restore the normal immune microenvironment of the wound and accelerate angiogenesis and collagen deposition in the wound, eg, by choosing dressings according to the location, size, depth, exudate, presence of infection or necrosis, and the condition of the surrounding tissues. Although these measures consider both local and systemic treatment, the incidence, disability, and mortality rates of DW are still high. Therefore, more effective treatments must be explored. The mechanism of refractory DW is complex and includes the abnormal inflammatory response theory,<sup>9,16</sup> microvascular injury theory<sup>17</sup> and neurotrophic factor starvation theory.<sup>18</sup> However, an abnormal inflammatory response is the most important mechanism of refractory chronic DW.<sup>8</sup> As mentioned above, immune cells are an indispensable and important component regulating inflammation. Therefore, an in-depth understanding of the specific relationship between DW and immune cells is necessary for treating DW.

## Brief Primer on Immune Cell Biology

### Neutrophils

Neutrophils, also known as polymorphonuclear neutrophils, are derived from bone myeloid lineage progenitor cells and enter the blood or tissues after the differentiation and development of the bone marrow. Neutrophils account for 50–70% of the total pool of peripheral blood leukocytes in humans (only 10–25% in mice).<sup>19,20</sup> Neutrophils respond to and resist microbial invasion or tissue damage through phagocytosis, degranulation and the release of neutrophil extracellular traps (NETs). They are the first cells in the immune defense of the body.<sup>21</sup> Abnormal neutrophils often lead to acute and suppurative infections, tissue damage, autoimmune diseases and the development of malignant tumors.

## Macrophages

Macrophages are a key component of the immune system. They are traditionally considered a homogeneous population of phagocytic cells derived from circulating monocytes.<sup>22</sup> The classification of macrophages is overly complicated. Different classifications have been developed based on activation cues, cell surface molecules or released proteins.<sup>23</sup> The most basic and common classification method is to divide macrophages into M1 and M2 subtypes based on phenotypes. M1 macrophages are classically activated macrophages that attract neutrophils, immature dendritic cells, natural killer cells and activated T cells to the wound surface by secreting proinflammatory factors, proteolytic enzymes and a variety of chemokines to promote inflammation, remove foreign bodies and strengthen the body's defense function. M2 macrophages mainly secrete anti-inflammatory and proangiogenic mediators, such as arginase-1 (Arg-1), IL-10, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and vascular endothelial growth factor (VEGF), to stop the inflammatory response in a timely manner and promote angiogenesis. The dynamic balance of M1 and M2 macrophages is an indispensable condition for bodily health. However, researchers have not clearly determined whether this classification method is completely applicable to the description of macrophages in vivo.<sup>24</sup> Expanded use of single-cell sequencing, transcriptomics and other techniques to analyze clinical samples is needed to mine and validate data.

## Mast Cells

Mast cells (MCs) originate from hematopoietic stem cells in the bone marrow and are present in the connective and mucosal tissues throughout the body after transport through the blood circulation. Then, they differentiate and develop into mature MCs rich in heparin, 5-hydroxytryptamine, histamine, protease and other bioactive compounds.<sup>25</sup> Based on their proteinase content, MCs have been divided into two subtypes: MC<sub>TC</sub> are rich in both tryptase and chymase, and MC<sub>T</sub> only express tryptase.<sup>26</sup> High-affinity immunoglobulin E (IgE) receptors (also called Fc $\epsilon$ RI) on the MC surface are activated when the body is invaded by pathogens, triggering a series of cascade reactions that play an important role in many physiological and pathological processes, including allergic reactions, inflammation, wound healing, immune defense, angiogenesis, tumor occurrence and development.

## Dendritic Cells

Dendritic cells (DCs), which develop from bone marrow-derived hematopoietic stem cells,<sup>27,28</sup> are a subset of antigen-presenting cells. They use pattern recognition receptors to initiate and regulate adaptive immune responses.<sup>29</sup> Although DCs are less abundant in humans than other immune cells, such as neutrophils or macrophages, they control infections by activating cytotoxic T lymphocytes or helper T lymphocytes. Langerhans cells (LCs) are unique DCs in the skin that are mainly distributed in the middle and upper epidermis, namely, between epidermal spinous cells, and they are also found in the dermis. They form a sophisticated monitoring system on the epidermis, constantly measuring the possible dangerous situations around the epidermis. Abnormal DCs often disrupt the function of the immune system, leading to pathological changes such as inflammation, fibrosis, and tumor formation and deterioration.

## T Lymphocytes

T lymphocytes are abbreviated as T cells, which are the main components of lymphocytes. They are derived from multipotent blood progenitors and reach the thymus through blood circulation. Early pro-T cells lack the expression of both CD4 and CD8 and are known as double-negative cells (DNs). DNs subsequently express CD4 and CD8 upon the induction of the thymus microenvironment to become double-positive cells (DPs).<sup>30</sup> Then, DPs develop into single-positive T cells expressing the surface markers CD4 or CD8 through the action of a series of regulatory signals, such as protein arginine methyltransferase 5<sup>31</sup> and T cell factor 1<sup>32</sup> to coregulate the immune response. Therefore, T cells are often divided into CD4<sup>+</sup> T cells or CD8<sup>+</sup> T cells.<sup>33</sup> The classification and naming principles of T cells are confusing due to their complex structures and constant differentiation and renewal. According to the expression of the T cell receptor, T cells have also been divided into two subgroups:  $\alpha\beta$ T and  $\gamma\delta$ T cells.  $\gamma\delta$ T cells are widely present in epithelial tissue cells.  $\gamma\delta$ T cells have invariant T cell receptors, and are known as dendritic epidermal T cells (DETCs). DETCs are important barrier cells in the skin that participate in skin homeostasis, regulating immunity, resisting pathogen invasion,

wound healing, protecting tissues and other activities.<sup>34</sup> DCs play an important role in maintaining the balance of the internal environment and promoting wound repair.

An introduction to the biology of these immune cells is provided in Table 1.

**Table 1** Introduction to the Biology of Immune Cells

Cell Type	Origin	Key Markers	Clinical Subtypes	Production	General Functions	References
Neutrophils	Myeloid lineage progenitor cells	Human: CD66b and CD16 Mouse: CD11b and LY6G	N1 N2	Antimicrobial peptides (AMPs, including cathelicidins and $\beta$ -defensins), ROS, and cytotoxic enzymes such as elastase and myeloperoxidase	Resisting microbial invasion and promoting wound healing via phagocytosis, degranulation and the release of NETs	[35–37]
Macrophages	Monocytes	Human: CD11b, CD36, CD206, CD204 or CD68 Mouse: F4/80, CD11c, CD86, MHC II, CD206, or CD163	M1 M2	IL-1, IL-6, IL-8, TNF- $\alpha$ , IL-12, IL-23, Arg-1, IL-4, IL-10, IL-13, Ym1, and TGF- $\beta$ 1	Promotion and inhibition of inflammation	[22,38–40]
MCs	Hematopoietic stem cells in the bone marrow	Tryptase	MC <sub>TC</sub> MC <sub>T</sub>	TNF- $\alpha$ , CXCL2, IL-1, IL-8, VEGF, CXCL16, Endothelin-1, GM-CSF, IL-8, and MCP-1	Regulating inflammation, tissue remodeling, allergic reactions, wound healing, heart function, immune defense and angiogenesis, among other processes	[25,26,41–45]
Dendritic cells	Hematopoietic stem cells	Human: Langerin, CD11c, CD141, CLEC9A (C-type lectin domain family 9 member A) or MHC II Mouse: Langerin, CD11c, MHC-II, CD86, CD8 $\alpha$ , CD11b, CD26, interferon-regulatory factor 8 (IRF8), CLEC9A, XCR1, CD103 or CX3CR1	cDC1s cDC2s PDCs	IFN-I and IL-33	Participation in the immune response via antigen presentation and IFN-I production	[27,46–48]
T lymphocyte	Multipotent blood progenitors	CD8, CD4, FoxP3, CD3 or $\zeta$ -chain	CD4 <sup>+</sup> T helper cells, CD8 <sup>+</sup> cytotoxic T lymphocytes, $\alpha\beta$ T cells, and $\gamma\delta$ T cells	Histamine, heparin and other proteoglycans, granule-associated proteases, leukotrienes and prostaglandins	Destruction of infected cells; synergizes with other types of immune cells to enhance or amplify immune responses	[33,49]

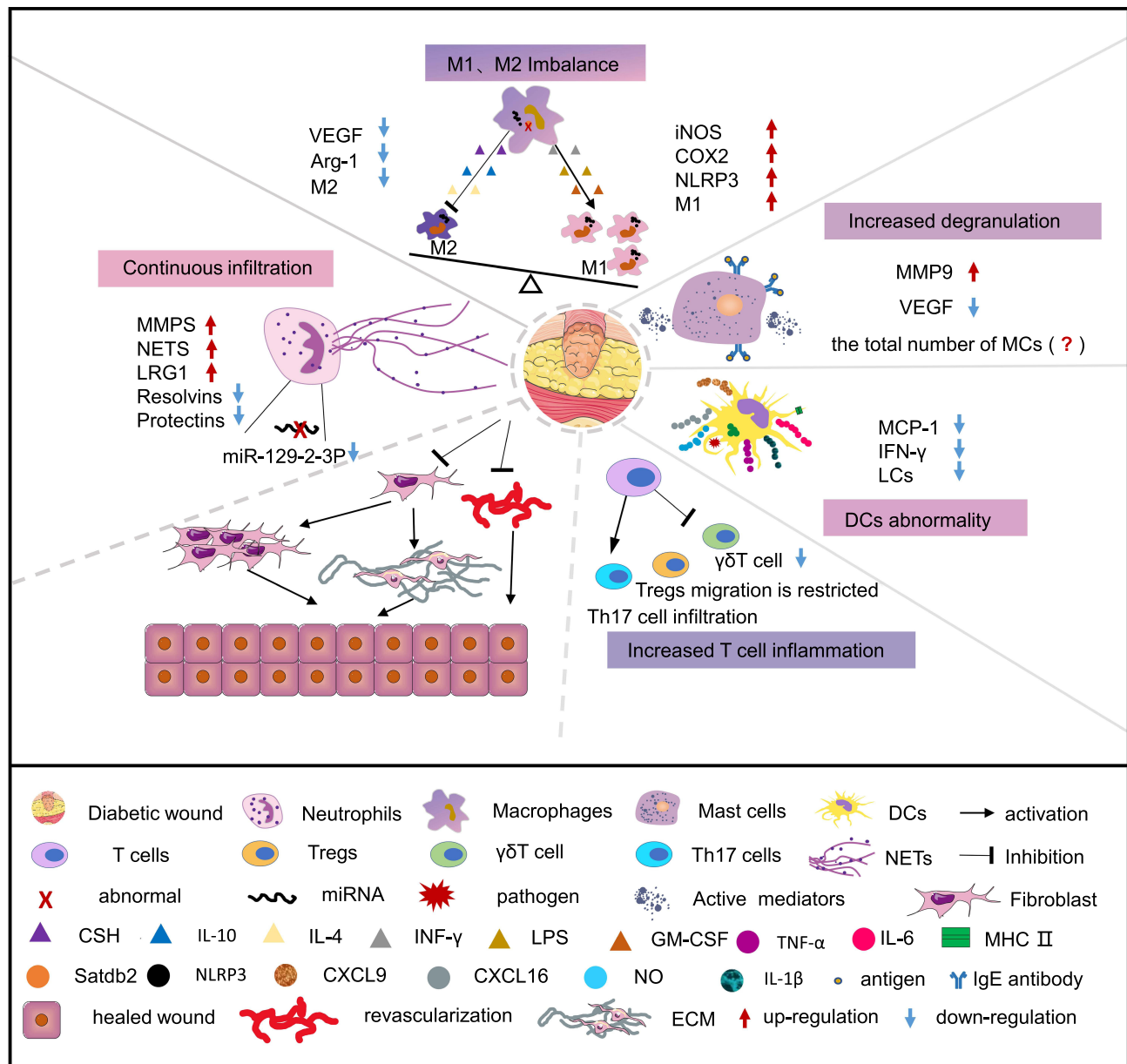
**Abbreviations:** AMPs, antimicrobial peptides; NETs, neutrophil extracellular traps; MCs, mast cells; CLEC9A, C-type lectin domain family 9 member A; IRF8, interferon-regulatory factor 8.

## Immune Cell Imbalance in the Process of DW

For wound healing, it is critical to coordinate the activities of immune cells accurately. The imbalance of immune cells will lead to the deterioration of immune microenvironment, and the wound will be stagnant in the inflammatory stage, which will further hinder the transition of wound healing from inflammation to proliferation and remodeling, and impair wound repair (Figure 1).

## Persistent Neutrophil Infiltration Exacerbates the Abnormal Inflammatory Cascade in DW

Neutrophils are the first leukocytes to infiltrate the injured tissue.<sup>21</sup> They are rarely present in healthy skin, but they populate the skin in inflammatory conditions and after wounding.<sup>50</sup> When the integrity of the body is damaged for 4 to 6 hours,



**Figure 1** Deregulated immune cells impair DW healing. Immune cells play an important role in the difficult-to-heal pathological process of DW. In DW, continuous infiltration of neutrophils, imbalance of M1 and M2 macrophages, increased degranulation of MCs, and dysregulation of DCs and T cells jointly lead to the continuous release of inflammatory factors, triggering inflammatory cascades, vascular maturation disorders, and decreased collagen deposition, which in turn cause DW to end up in a difficult or even non-healing ending.

neutrophils are recruited to wounded tissues through the actions of bacterial products and chemokines, among other factors, and reach a peak number after 18 to 24 hours.<sup>35,51,52</sup> Neutrophils, which are rapidly recruited to wounded tissues, form phagosomes by phagocytosing bacteria and tissue fragments. Then, a large amount of degranulation occurs, releasing matrix metalloproteinases (MMPs) and promoting the formation of an early proinflammatory microenvironment. Concurrently, NETs, which are composed of globulin/enzymes and nuclear material, destroy pathogens by trapping and removing the bacteria. In addition, neutrophil-derived cytokines and chemokines recruit monocytes to the wound site and then promote their differentiation into macrophages to jointly maintain the internal environmental balance of the body.<sup>53–55</sup> After performing their functions, the production of specific proinflammatory defibrating mediators, such as resolvins and protectins, increases, which limits neutrophil infiltration. Apoptotic neutrophils or tissue fragments are continuously removed by macrophages. Only the timely formation of the early proinflammatory microenvironment and termination of the inflammatory reaction promote the smooth transition of the wound to the proliferative stage and finally accelerate wound healing.

However, in DW with a dysregulated microenvironment, RNA abnormalities in neutrophils and their function are impaired. For example, microRNAs (miRNAs), especially miR-129 family members, are abnormally expressed. The expression of miR-129-2-3p in DW tissues is significantly reduced, while miR-129-2-3p target genes are closely related to inflammation and apoptosis.<sup>56</sup> However, researchers have not clearly determined whether this abnormality is related to the difficulty in healing DW or whether it is a key link in the difficult healing of DW, and further verification is needed. But there is no doubt that following the inflammatory phase, an intensive reduction in cellularity occurs by increased apoptotic cell death.<sup>57</sup> The smooth progress of inflammation and apoptosis is conducive to the smooth start of the proliferation phase, which is very important for wound repair. In addition, the release of NETs in DW increases. In contrast to stable or healed wounds, neutrophil elastase, the prototypical NET marker, is present at 59% higher levels in worsening wounds.<sup>58</sup> Lee YS and colleagues<sup>59</sup> used immunohistochemistry, polymerase chain reaction and other methods to verify that the expression of NET proteins and mRNAs in DW specimens was also significantly increased. NETs overproduced in DW trigger NLRP3 inflammasome activation,<sup>60</sup> which subsequently promotes the polarization of macrophages to the M1 subtype and intensifies the release of proinflammatory mediators, causing long-term abnormal inflammation. Therefore, in response to the overly inflammatory response, in addition to the targeted elimination of excessive inflammatory mediators by exogenous treatments such as drugs, timely termination of neutrophil infiltration, reduction of the generation and release of NETs, and elimination of overexpressed NETs are also very important for DW healing. Moreover, reactive oxygen species in patients with diabetes continue to increase in an uncontrolled manner because AGE (advanced glycation end product) formation increases when the body is continuously exposed to a high-glucose environment, activating the polyol pathway and PKC signaling.<sup>61,62</sup> Abnormal activation of the PKC signaling pathway and increased production of nitric oxide increase the expression of endothelial cell adhesion molecules, thereby augmenting the ability of neutrophils to adhere to the wound.<sup>63,64</sup> This process further increases the already excessive number of neutrophils in DW. As mentioned above, neutrophils are removed immediately after performing their functions to prevent excessive infiltration and inflammation. However, in DW, the synthesis of resolvins and protectins, which resolve inflammation by decreasing neutrophil infiltration and transmigration, increasing the phagocytic activity of macrophages and decreasing adipose tissue macrophages, is reduced.<sup>65,66</sup> Increased production of leucine-rich-alpha-2-glycoprotein-1 (LRG1) also exacerbates inflammation.<sup>67</sup> Therefore, proper regulation of the expression of resolvins, protectins and LRG1 in DW and termination of neutrophil infiltration in a timely manner are important for DW repair. However, the specific mechanisms underlying the effects of resolvins, protectins, and LRG1 on DW healing remain unclear, and further exploration is needed.

Neutrophils, the first group of cells arriving at the wound bed, release various inflammatory mediators and chemokines, which are prerequisites for guiding other immune cells to reach the wound bed to perform their respective biological functions. Neutrophil abnormalities often initiate and exacerbate a series of inflammatory cascades. As described above, neutrophils continuously infiltrate the wound bed. The excessive neutrophil-derived MMPs, neutrophil elastase, cathepsins and NETs also promote ECM cleavage by elastase and matrix metalloproteinase-9 (MMP-9), which destroys the tight junctions between cells.<sup>68</sup> The abnormal neutrophils will shift DW repair into a vicious cycle.



## The Abnormal Polarization of Macrophages Arrests DW Healing in the Inflammatory Stage

Macrophages are the core cells involved in the wound healing stage. In normal wound healing, M1 macrophages dominate the wound healing site from the time of injury to the third day and then begin to transition to M2 macrophages, with fewer M1 macrophages present. Most of the M1 and M2 macrophages disappear shortly after Day 10. In DW, M1 macrophages continue to be present in the wound bed, with few to no M2 macrophages present.<sup>69</sup> At the early stage of wound repair, M1 macrophages eliminate pathogenic bacteria, cell debris and damaged matrix by promoting inflammation. When most pathogenic microorganisms are removed from the wound, apoptotic neutrophils release a large number of cytokines to promote macrophage phagocytosis and clearance, thus promoting macrophage polarization into M2 macrophages. M2 macrophages exert an anti-inflammatory effect to terminate the inflammatory response by releasing IL-4 and IL-10, among other proteins. In addition, M2 macrophages release VEGF and TGF $\beta$ 1, among other proteins, to promote angiogenesis, collagen deposition and wound contraction. However, if a large number of foreign bodies or pathogens are present at the wound site, the number of M1 macrophages will increase continuously, these cells are not polarized to M2 macrophages, and the wound will stall at the inflammatory stage. During this prolonged period of inflammation, macrophages fuse and evolve into multinucleated giant cells,<sup>70</sup> which limits the application of biomaterials in wound repair and makes wound healing more difficult.

The impaired phenotype and function of macrophages are important factors that make DW difficult to heal. Studies have reported differences in the expression of the chromatin-modifying enzyme Setdb2 in macrophages isolated from diabetic and nondiabetic wounds. The histone methyltransferase Setdb2 is an important regulator promoting the resolution of early-stage inflammatory wound macrophages into a reparative phenotype.<sup>71,72</sup> In addition, the abnormal blood glucose level in individuals with DW will cause the activation of NLRP3 inflammasomes, and macrophages will subsequently polarize to the M1 subtype and secrete a large amount of proinflammatory mediators such as TNF- $\alpha$ , IL-6, and IL-1 $\beta$ .<sup>69</sup> The persistent inflammation and increased numbers of M1 macrophages in these wounds lead to reduced proliferation and migration of the keratinocytes, fibroblasts, and endothelial cells necessary for wound repair.<sup>73</sup> The polarization of M2 macrophages in DW is inhibited, which is also unfavorable for wound repair. And diabetes further disrupts macrophage function by impairing monocyte recruitment to the wound, reducing phagocytosis, and prohibiting macrophage polarization from the M1 to M2 phenotype.<sup>74</sup> What's more, S100A9, a Ca<sup>2+</sup>-binding protein of the S100 family, is overexpressed in DW and disturbs M2 macrophages differentiation via TLR4-NF $\kappa$ B signaling.<sup>75</sup> Moreover, the level of insulin in patients with diabetes is lower than that in normal people, and insulin deficiency will also lead to an increase in nitric oxide production,<sup>76</sup> which will aggravate the occurrence and development of oxidative stress. The release of a large number of proinflammatory mediators in DW, the activation of NLRP3 inflammasomes and the occurrence of oxidative stress lead to the imbalance of M1 and M2 macrophages, and thus DW exhibit a continuous inflammatory response, and the wound eventually becomes difficult to heal. Local use of insulin on diabetic foot wounds might accelerate wound healing, mainly because insulin promotes neutrophil apoptosis and subsequently triggers macrophage phenotypic polarization.<sup>77</sup> At present, a main goal is to induce macrophage polarization from the M1 to M2 phenotype in DW to facilitate the smooth transition of the wound surface from inflammation to proliferation. Many therapeutic methods have begun to be developed. Exosomes derived from epidermal stem cells,<sup>78</sup> the bioinspired design of mannose-decorated globular lysine dendrimers,<sup>79</sup> and a pH-responsive hyaluronic acid hydrogel stem<sup>80</sup> are good examples. However, because of the complexity of the immune system, the unpredictability of clinical trials, and ethical issues, these strategies have been validated only at the animal and cellular levels. Clinical research is particularly important.

## The Ongoing Degranulation of MCs Hinders DW Healing

MCs, the inflammatory sentinels, are some of the earliest cells in the body that are activated to resolve trauma. They participate in all stages of wound healing, including hemostasis, inflammation, proliferation, and remodeling.<sup>81</sup> After injury, MCs are recruited by the anaphylatoxins C3a and C5a that are produced through the effects of Hageman factor (XII) and stem cell factor secreted by keratinocytes.<sup>82</sup> Activated MCs, likely through secretion of TNF- $\alpha$ , increase the

expression of XIIIa, a potent fibrin-stabilizing factor that catalyzes the covalent cross-binding of fibrin fibrils in dermal dendrocytes to promote hemostasis and clot formation.<sup>83</sup> MC-derived mediators, including CXCL2, TNF- $\alpha$ , MIP-2, and IL-8, recruit neutrophils to the wound bed and activate macrophages, accelerating the elimination of foreign pathogens.<sup>83</sup> During the proliferation phase, shorter forms of perlecan are produced by MCs via proteolytic processing and alternative splicing to regulate angiogenesis and matrix conversion.<sup>84</sup> MC-derived VEGF, CXCL16, endothelin-1, GM-CSF, and IL-8, and keratinocyte-derived monocyte chemotactic protein-1 (MCP-1) also jointly promote wound angiogenesis and accelerate wound healing.<sup>85</sup> Weiskirchen R and colleagues documented that MCs proteases 4, 5, and 6 are mediators of the critical role of MCs in the proliferative phase of healing by promoting angiogenesis, fibrocyte proliferation, and collagen synthesis.<sup>86</sup> Pistacia atlantica hull ointment increases the MCs distribution and infiltration, which in turn promotes neovascularization and ultimately promotes wound healing.<sup>87</sup> During the remodeling phase, MC-derived mediators, such as histamine, proteases, SCF, TGF- $\beta$ , VEGF and FGF-2, promote the proliferation and differentiation of fibroblasts.<sup>88</sup> Some fibroblasts differentiate into myofibroblasts to promote wound contraction. Estevao LR et al increased the MCs concentration and promoted skin wound contraction in rats by applying an ointment containing 5% Brazilian pepper tree oil.<sup>89</sup> MCs, which are important sensors and effector cells of the immune system,<sup>90</sup> play a very important role in the process of wound healing. However, due to the significant difference in the contents of cytokines and proteolytic enzymes in mature MCs, the MCs phenotype is not fixed. Therefore, methods to promote the reasonable and stable expression of MCs conducive to wound healing are also directions that should be explored in the future.

A lack of MCs always promotes the development of severe insulinitis and accelerates hyperglycemia, which in turn accelerates the occurrence and development of DM.<sup>91</sup> However, when MCs persist, MC-derived mediators constantly recruit white blood cells to the wound bed, and inflammation continues to develop, leading to the failure of wound healing. At present, controversy exists regarding whether the total number of MCs in DW has changed. Some studies have reported no difference in the total number of MCs between DW and normal wounds,<sup>82</sup> but others have reported an increase in the number of MCs in DW.<sup>92</sup> This discrepancy may be due to the difference in modeling methods or the monitoring time of MCs in DW. Undoubtedly, in DW characterized by a high-glucose microenvironment, the degranulation of MCs increases, and the release of MC-derived VEGF is reduced. Georgios Theodoridis et al integrated skin transcriptomics and serum multiplex assays and verified this fact.<sup>16</sup> The increase in MMP-9 levels accelerates the degradation of the extracellular matrix, making DW more difficult to heal. An indole-carboxamide-type MC stabilizer, MCS-01, was reported to be an effective inhibitor of MC degranulation in vitro. MCS-01 promotes DW healing by activating the NF- $\kappa$ B and STAT3 signaling pathways, inhibiting the production of MMP-9, and promoting the production of IFN- $\beta$ , IFN- $\gamma$  and VEGF- $\alpha$ .<sup>93</sup> Targeted therapy of MCs that inhibits their degranulation and maintains stable functions has become a reliable and emerging method to promote DW healing. However, does the total number of MCs differ between chronic DW and normal wounds? If a difference exists, what role does this difference play in the healing process of DW? Clinical DW specimens should be collected ethically and with patient consent to better understand the role of MCs in DW. Then, more authoritative data can be obtained by combining emerging transcriptomics and single-cell sequencing to provide scientific evidence for DW treatment.

## Abnormal Numbers and Impaired Migration of LCs Aggravate the Difficulty in DW Healing

In wound healing, the dendritic cells that play a major role are LCs. An increase in the number of LCs at the wound bed is related to the rapid healing of human DW. On the one hand, these cells homeostatically migrate to lymph nodes and present antigens to antigen-specific T cells<sup>94</sup> to improve immune function and promote the “normalization” of the wound microenvironment. On the other hand, LCs contribute to the stability of the epidermal environment and promote keratinocyte proliferation.<sup>95</sup> After the occurrence of trauma, many LCs are present on the epidermis around the wound,<sup>96</sup> which facilitate keratinocyte proliferation. MCP-1 exerts a positive feedback effect on inducing LC recruitment to the skin.<sup>97</sup> Keratinocytes and LCs work together to facilitate wound healing.

Studies have shown that healing DFUs contain a higher number of LCs than nonhealing DFUs.<sup>96</sup> MCP-1, a chemokine produced by keratinocytes, functions to recruit LCs to the wound periphery. MCP-1<sup>-/-</sup> mice display



significantly delayed wound re-epithelialization, and mice with diabetic chronic ulcers that eventually heal show normalization of keratinocyte production of MCP-1, whereas unhealed diabetic ulcers do not.<sup>98</sup> The production of IFN- $\gamma$  also exerts a regulatory effect on the migration of dendritic cells, but IL-13 and IFN- $\gamma$  are inhibited in myeloid cells of patients with DM and DFUs, and the production of IFN- $\gamma$  is deregulated. Dysregulated dendritic cell migration in DW also contributes to impaired healing.<sup>16</sup> These results suggest that the reduced number of LCs and impaired migration together contribute to impaired healing of DW.

## Imbalanced T Lymphocyte Homeostasis Worsens DW

When skin integrity is impaired, T cells migrate to the wound following the secretion of chemokines, such as IFN- $\gamma$ . In the inflammatory phase, CD8<sup>+</sup> T cells specifically recognize the MHC protein in abnormal cells, secrete perforin and granzyme, and directly kill infected or mutated cells, thus preventing the further deterioration of early wounds. CD4<sup>+</sup> T cells are activated and differentiate into effector subsets, such as Th1, Th2, Th22 and Treg cells.<sup>99</sup> Tregs modulate macrophage polarization through arginase signaling pathways and allow them to respond to different immune microenvironments.<sup>100</sup> After the impaired cells are killed, some T cells transform into memory T cells, which renew in an antigen-independent manner. When T cells are exposed to the same antigen again, they immediately induce an immune response, effectively killing abnormal cells such as those carrying “bacteria or viruses”. In the proliferation stage, activated  $\gamma\delta$  T cells produce effective cytokines or growth factors, such as KGF-1 (also called fibroblast growth factor-7, FGF-7), KGF-2 (FGF-10), and IGF-1, to promote the proliferation and differentiation of keratinocytes.<sup>101,102</sup> In the remodeling stage, activated  $\gamma\delta$  T cells stimulate the proliferation of skin hair follicle stem cells to promote epidermal repair, ultimately accelerating wound healing.<sup>103</sup>

T cell abnormalities are an important pathogenic mechanism of diabetes. Type 1 diabetes mellitus (T1DM) is attributed to the death of insulin-producing  $\beta$  cells induced by CD8<sup>+</sup> T cells.<sup>104</sup> A tyrosine phosphatase PTPN2 deficiency in CD8<sup>+</sup> T cells alone is sufficient to drive the destruction of pancreatic  $\beta$  cells and the onset of diabetes. T cell-specific PTPN2 deficiency is also accompanied by increased CD4<sup>+</sup> T-helper type 1 differentiation and T follicular helper cell polarization and increases the abundance of  $\beta$  cells in pancreatic islets, as observed in humans with T1DM.<sup>105</sup> In addition, the function and diversity of T cells in the islets of patients with diabetes are also significantly impaired.<sup>106</sup> The systemic inflammation observed in patients with diabetes limits the migration of Tregs and increases the infiltration of Th17 cells in DW and thus represents one of the mechanisms underlying increased neutrophilic inflammation and a prolonged inflammatory phase.<sup>107</sup> Topical application of retinoic acid induces plasticity of T cells and differentiation of Th17 cells toward Tregs, thereby promoting DW healing.<sup>107,108</sup> T cell activation is increased in patients with type 2 diabetes mellitus (T2DM),<sup>109</sup> and the frequency of one variable gene, TRBV7-8, is higher in individuals with T2DM.<sup>110</sup> Defects in the functions of Tregs have been observed in patients with gestational DM, as evidenced by significantly decreased effector T cell production of interferon gamma and TNF- $\alpha$  through IL-10-mediated mechanisms.<sup>111</sup> Structural abnormalities, dysfunctions and subgroup imbalances of T lymphocytes lead to the occurrence and development of diabetes.

At present, research on T cells and DM mainly focuses on the occurrence and development of DM, and further research is needed to determine whether the expression, structure, function, and subpopulations of T cells in DW are abnormal. High glucose and advanced glycation end products significantly upregulate T cell expression and induce T mediated cell inflammation by upregulating ICOS/ICOSL.<sup>112</sup> The high-glucose environment increases the production of the peptide hormone islet amyloid polypeptide, activating the NLRP3 inflammasome.<sup>113</sup> Activation of the NLRP3 inflammasome prolongs inflammatory infiltration in DW. The activation of  $\gamma\delta$  T cells, which are an important component of human epidermal tissue, requires IL-1 $\alpha$  and IL-7 secreted by keratinocytes.<sup>114</sup> However, directional keratinocyte migration is defective and delayed in DW.<sup>115,116</sup> The number of activated  $\gamma\delta$  T cells is reduced, which further hinders the transition of wound healing from inflammation to proliferation. Xu Peng et al also confirmed that patients with chronic DW have a lower abundance of skin-resident  $\gamma\delta$  T cells than patients with nondiabetic wounds.<sup>101</sup> The single-cell transcriptomic landscape of DFUs also confirmed that compared with nonhealing DFUs, healing DFUs contain higher proportions of naive and early differentiated progenitor T lymphocytes, which activate various T cell subsets.<sup>117</sup> In DW, the activation and IGF-1 production of DETCs are impaired. IL-15, a very important growth factor for the efficient

activation of peripheral T cells, improves DW healing by enhancing the activation and IGF-1 production of DETCs.<sup>118</sup> Therefore, an in-depth analysis of the specific mechanism of T cells in DW healing is needed to accelerate DW healing.

## Targeted Immunotherapy for DW

### Therapeutic Strategies Targeting Immune Cells Based on Drugs

Due to the disorder of immune cells in DW, wounds often fall into a vicious cycle of abnormal inflammation. According to the severity of inflammation, patients are often prescribed anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs, to normalize the prolonged inflammation of DW. However, these drugs have many side effects. Therefore, a greater desire for effective targeted drugs has been noted. With the further study of immune cells in DW, several treatments targeting immune cells using drugs have been considered for wound healing. For example, clarithromycin restores the antibacterial activity of neutrophils and improves the wound healing capacity of fibroblasts through the upregulation of LL-37 on NET structures.<sup>119</sup> Quercetin,<sup>120</sup> astragaloside IV<sup>121</sup> and docosahexaenoic acid<sup>122</sup> also promote DW healing by restoring the balance of M1/M2 macrophages. Monika Vinish et al topically administered Fms-like tyrosine kinase-3 ligand to increase the number of dendritic cells in burn wounds, which in turn increased cellular proliferation and accelerated wound healing.<sup>123</sup> However, the most common studies of drugs based on immune cells to promote DW healing are limited to single immune cells, potentially because the specific mechanism of immune cell cooperation in DW is not well understood. Alternately, when discussing the mechanism of drug action, only the pathway of interest was examined. Insulin promotes neutrophil apoptosis and subsequently triggers macrophage phenotypic polarization.<sup>77</sup> However, we do not know whether the local application of insulin exerts an effect on other immune cells. In addition, these drugs act by targeting the immune system, and their toxicity and side effects are unclear. For patients with other immune system diseases, the selection of targeted drugs and delivery methods must be continuously verified.

### Therapeutic Strategies Targeting Immune Cells Based on Natural Compounds

Natural compounds are a very rich drug library due to their advantages, such as a wide range of sources and low toxicity and side effects. Researchers have posited that up to 40% of all new molecular entities submitted to the Food and Drug Administration for approval are natural products, natural product-derived, or natural product-inspired (containing a natural product pharmacophore) compounds.<sup>124,125</sup> As a global medical problem, DW has also been the subject of many studies examining natural products targeting immune cells to promote DW repair. However, most of them target neutrophils and macrophages, such as the yi medicine “yi bu a jie” extract,<sup>126</sup> fermented papaya preparation,<sup>127</sup> *Quercus infectoria*<sup>128</sup> and *Hsian-tsao*.<sup>129</sup> The focus on these cells is mainly because neutrophils and macrophages are the core cells involved in wound repair during inflammation, and the inhibition of their abnormal infiltration or the improvement of their functions is an important strategy to promote the normalization of the immune microenvironment. Among them, the yi medicine “yi bu a jie” extract promotes DW repair by inhibiting neutrophil infiltration.<sup>126</sup> Fermented papaya preparation,<sup>127</sup> *Q. infectoria*<sup>128</sup> and *Hsian-tsao*<sup>129</sup> all promote DW repair by inhibiting the abnormal infiltration of macrophages or improving macrophage function. Moreover, propolis not only inhibits the abnormal infiltration of neutrophils but also targets the regulation of macrophages to finally achieve gratifying results in the treatment of DW.<sup>130</sup> Compared with studies on neutrophils and macrophages, studies on MCs, T cells and LCs in DW are relatively limited, and few studies have assessed natural products targeting MCs, T cells and LCs to promote DW healing. However, *Gynura procumbens*, an herb found in Southeast Asia, has been reported to accelerate wound healing by targeting the increase in MC accumulation or migration and inducing the expression of fibroblast growth factor, angiogenin, epidermal growth factor, transforming growth factor and VEGF.<sup>131</sup> This result has emerged with the in-depth study of the pathophysiological mechanism of MCs in the process of DW healing. Therefore, with the identification of the pathophysiological mechanism of immune cells in DW, such as the study by Georgios Theodoridis et al using clinical specimens and single-cell RNA sequencing analysis to provide deep insights into cell function and disease pathophysiology, will facilitate the profiling of the transcriptome landscape of individual cells in DFU tissues.<sup>117</sup> In the future, an increasing number of studies will be conducted on natural compounds targeting immune cells to promote DW repair.

## Therapeutic Strategies Targeting Immune Cells Based on Physical Therapy

Physical therapy, an important auxiliary method, promotes wound repair by changing the physical environment of chronic wounds. As a form of validation, clinical treatment with hyperbaric oxygen promotes DW repair by inducing a significant reduction in the integrin-mediated adhesive properties of neutrophils through the engagement of both  $\alpha 4$  and  $\beta 2$  integrins.<sup>132</sup> Photobiomodulation significantly decreases the total number of MCs, which improves the healing process in diabetic animals.<sup>92</sup> Different electrical stimulations also promote wound healing by regulating different immune cells.<sup>133</sup> Holsapple JS<sup>134</sup> et al recruited patients with treatment-resistant venous ulceration for two weeks of extracorporeal shockwave therapy (ESWT), and the results showed that ESWT significantly increased the uptake of apoptotic cells, healing-associated cytokine and growth factor gene expression and modulated the macrophage morphology to one suggestive of macrophage activation, all of which contributed to wound resolution. Moreover, ESWT decreased the number of total and degranulated MCs and alleviated pelvic pain in a rat model of prostatitis.<sup>135</sup> Therefore, does ESWT promote DW repair by regulating macrophages and MCs in wound tissue? Although these physical therapies do not solve the root problem, they are safe, low cost, and stable when they are combined with other treatment strategies, they might promote wound repair more efficiently.

## Therapeutic Strategies Targeting Immune Cells Based on Biomaterials

With the continuous improvement of medical technology and in-depth research on the immune system, many studies have explored the combination of biological materials and immune cell-targeted therapy to jointly promote DW repair. Although the application of biomaterials, such as alginate and foam dressings, has partially corrected the DW microenvironment, methods that better use increasingly sophisticated and diverse biomaterials to promote DW healing are still an important issue. To date, biomaterial research has mainly included hydrogels, intelligent dressings, and nanoparticles, among others. Effective bioadaptability and biocompatibility are essential for excellent biomaterials. Hydrogels have been widely used in the clinic due to their good biocompatibility, hydrophilicity, controlled drug release and smart drug delivery. HA-JK1 hydrogels are an ideal delivery scaffold for JK1 with a pH-dependent prolonged H<sub>2</sub>S release profile. At the stage of inflammation in wound healing, the acidic microenvironment accelerates H<sub>2</sub>S release, resulting in the polarization of macrophages from the M1 type to the M2 type, which terminates the abnormal inflammatory response over time. As the wound heals, the pH value gradually changes to a neutral or even slightly basic, leading to a gentle release of H<sub>2</sub>S from the HA-JK1 hydrogel to promote wound regeneration.<sup>136</sup> The emergence of multifunctional injectable hydrogels makes full use of their advantages, such as better biodegradability and self-healing properties. Through a continuous supply of O<sub>2</sub> and timed release of ExosM2@miR-223 and FGF-2, the HA@MnO<sub>2</sub>/FGF-2/Exos hydrogel creates an appropriate immune microenvironment to improve the healing of DW in vivo.<sup>137</sup> Although the application of traditional biomaterials and wound dressings contributes to an effective wound healing outcome, DW repair is a dynamic and complex process. Excessive inhibition of wound inflammation is harmful. Fortunately, the emergence of intelligent dressings has provided a new solution to this problem. Fontenot KR<sup>138</sup> et al used fluorescent elastase tripeptide or tetrapeptide biomolecules to prepare intelligent dressings, which have selectivity and affinity for human neutrophil elastase present in DW wound fluid. Intelligent dressings not only perform biological functions but also monitor changes in the DW microenvironment in real time, which provides instructions for the treatment of DW. In addition, nanoparticles also play a very important role in wound repair due to their characteristics, including a low cost. For example, KGM-modified SiO<sub>2</sub> nanoparticles (KSiNPs), which are synthetic nanoparticles, do not contain any drugs and promote wound healing in individuals with diabetes by reprogramming mannose receptors on macrophages.<sup>7</sup> Many types of these biomaterials have been developed, each with its own strengths. The combination of degradable polymer materials may facilitate the design of composite materials with more desirable properties to meet the needs of the complex DW microenvironment and different healing stages.

## Therapeutic Strategies Targeting Immune Cells Based on RNA Interference

RNA interference (RNAi) is based on the interaction of a short RNA strand with messenger RNA due to sequence homology, resulting in translational inhibition.<sup>139</sup> Gene expression is specifically knocked out or silenced by RNAi.

Wound healing is promoted by regulating RNA expression, such as targeting Keap1,<sup>140</sup> tumor protein p53,<sup>141</sup> HIF-prolyl hydroxylase domain (PHD),<sup>142</sup> and miR-31-5p.<sup>143</sup> Localized and sustained delivery of an MMP-9 small interfering RNA (siRNA) promotes wound healing through effective silencing of the MMP-9 gene.<sup>144,145</sup> However, because RNA is easily degraded and difficult to store, coupled with the complexity of the immune system and techniques such as single-cell sequencing and the high cost of transcriptomics, studies exploring the differences in immune cell-specific RNA expression in DW are still in their infancy. Undoubtedly, an in-depth study of immune cell-specific RNA expression in DW will be an important direction of future research. According to previous reports, miRNAs are abnormally expressed in neutrophils present in DW, particularly members of the miR-129 family.<sup>56</sup> Moreover, the emergence of new gene carriers, such as bacterial cellulose-hyperbranched cationic polysaccharide derivatives,<sup>146</sup>  $\beta$ -CD-(D3)7,<sup>147</sup> and cationic star-shaped polymers,<sup>144</sup> protects siRNAs from degradation by various enzymes. The development of new gene carriers and research progress in immune cell-specific abnormalities in RNA expression in DW provide new therapeutic insights into wound healing through targeted immune cell therapy based on RNAi.

## Therapeutic Strategies Targeting Immune Cells Based on Stem Cells

Stem cells play very important roles in controlling tissue homeostasis and wound repair and are currently some of the most important, popular and cutting-edge directions in the world. For wound healing, the most common treatment strategy involves transplantation of cells and growth factors into the wound area.<sup>148</sup> When transplanted into a wound, stem cells act in a direct and paracrine manner to promote cell recruitment, immunomodulation, extracellular matrix remodeling, and angiogenesis by secreting cytokines and growth factors.<sup>149,150</sup> However, due to the harsh microenvironment in DW, treatment strategies often fail. Therefore, by focusing on the DW microenvironment, the medical community is investigating treatments targeting immune cells to promote the “normalization” of the trauma microenvironment and ultimately promote DW healing. Recent research has identified that a C–C motif chemokine receptor 2-engineered mesenchymal (CCR2-MSC) stromal cell infusion accelerates tissue repair in DW and reshapes local microenvironments by inhibiting monocyte infiltration, remodeling the inflammatory properties of macrophages, and promoting Treg cell accumulation.<sup>151</sup> Mesenchymal stem cell (MSC)-derived exosomes also promote the polarization of macrophages to the M2 type via the PTEN/AKT signaling pathway.<sup>152</sup> In addition, Guohu Di et al<sup>153</sup> found that MSCs not only accelerate M2 macrophage polarization but also induce the activation of corneal epithelial stem/progenitor cells by secreting TSG-6 and further promote the healing of corneal epithelial wounds in diabetic mice. MSCs have provided new hope to accelerate the healing of DW and have laid the foundation for the discovery and application of other stem cells. Dental follicles,<sup>154</sup> human dental pulp,<sup>155</sup> adipose<sup>156</sup> and umbilical cord-matrix stem cells<sup>157</sup> also exert an anti-inflammatory effect by accelerating M2 macrophage polarization, but researchers have not determined whether they promote DW healing. However, exogenous stem cells are expensive and prone to immune rejection. Certain safety problems are encountered in their clinical applications. The emergence of induced pluripotent stem cells (iPSCs) solves the aforementioned problems to a comparable extent. iPSCs are derived from adult cells through the in vitro induction of pluripotency, obviating the ethical dilemmas surrounding the use of embryonic stem cells; they are harvested noninvasively and can be transplanted autologously, reducing immune rejection. Additionally, iPSCs are the only cell type capable of differentiating into all cell types present in healthy skin.<sup>149</sup> iPSCs have also been explored in the microenvironment of immune cell disorders in DW. iPSC-derived fibroblasts are a good example.<sup>158</sup> Treatments based on stem cells that target immune cells and are designed to restore the homeostasis of the internal environment and ultimately promote the healing of DW undoubtedly have great development prospects and application value. However, the immune rejection of stem cells is still one of the main obstacles of regenerative medicine.<sup>159</sup> Although good progress has been achieved in diabetic stem cell intervention therapy to date, its long-term safety remains unclear.<sup>160</sup> Researchers have not yet determined whether stem cells have the potential to differentiate into other cell lines or increase the risk of cancer in patients with long-term use of stem cell interventions.<sup>159</sup> Coupled with issues such as ethics and economic costs, stem cell technology is still mainly in the basic research stage, and its clinical application is not yet mature.

Continuous development of targeted immunotherapy in DW has emerged and has achieved beneficial effects. Some examples are shown in Table 2.

**Table 2** Therapies Targeting Immune Cells in Wound Healing

Treatment Strategies	Examples	Targeted Cells or Genes	Effect on Skin Wound Healing	Wound Healing Outcomes/ Results	References
Drug	Clarithromycin	Neutrophils	Upregulation of LL-37 on NET structures, enhancement of antibacterial defenses and improvement of the wound healing capacity of fibroblasts	Wound closure is accelerated	[119]
	Quercetin, Astragaloside IV and Docosahexaenoic acid	Macrophages	Restoration of the M1/M2 balance of macrophages	Wound closure is accelerated	[120–122]
	Fms-like tyrosine kinase-3 ligand	Dendritic cells	Increases the number of dendritic cells	Wound closure is accelerated	[123]
	Insulin	Neutrophils and macrophages	Reestablishment of damaged neutrophil phagocytic function and induction of macrophage polarization from the M1 to M2 phenotype	Wound closure is accelerated	[77]
Natural compounds	yi medicine “yi bu a jie” extract	Neutrophils	Inhibits inflammatory neutrophil exudation and promotes the growth of granulation tissue and proliferation of fibroblasts	Wound closure is accelerated	[126]
	Fermented papaya preparation	Macrophages	Promotes the effective aggregation of monocytes and improve the function of macrophages	Wound closure is accelerated	[127]
	Q. infectoria	Macrophages	Inhibits the Set7/NF- $\kappa$ B pathway in macrophages to promote the normalization of the inflammatory microenvironment in DW	Wound closure is accelerated	[128]
	Hsian-tsao	Macrophages	Enhances macrophage-mediated phagocytosis and inhibits the release of inflammatory mediators	Wound closure is accelerated	[129]
	Propolis	Macrophages	Inhibits neutrophil and macrophage infiltration	Wound closure is accelerated	[130]
	G. procumbens	MCs	Increases the accumulation and migration of MCs	Wound closure is accelerated	[131]
Physical therapy	Hyperbaric oxygen therapy	Neutrophils	Reduces the cell adhesion function of the neutrophilic integrins $\alpha 4\beta 1$ and $\beta 2$	Wound closure is accelerated	[132]
	Photobiomodulation	MCs	Reduces the total numbers of MCs	Wound closure is accelerated	[92]
	Different types of electrical stimulation	Neutrophils, macrophages, MCs, and lymphocytes	Reduces the number of immunocytes and cytokine levels	Shortens the inflammation phase	[133]
	ESWT	Macrophages	Increases the uptake of apoptotic cells and healing-associated cytokine and growth factor gene expression, and modulates macrophage morphology to that suggestive of macrophage activation	Wound closure is accelerated	[134]

(Continued)



Table 2 (Continued).

Treatment Strategies	Examples	Targeted Cells or Genes	Effect on Skin Wound Healing	Wound Healing Outcomes/ Results	References
Biomaterials	HA-JKI hydrogels	Macrophages	Remodels the macrophage phenotype	Wound closure is accelerated	[136]
	HA@MnO <sub>2</sub> /FGF-2/ Exos hydrogel	Macrophages	Release of M2-derived exosomes and FGF-2 growth factor stimulates angiogenesis and epithelization	Wound closure is accelerated	[137]
	Intelligent dressings	Neutrophils	Binds specifically to human neutrophil elastase present in wound fluid	Provides indications for clinical treatment	[138]
	KSINPs	Macrophages	Reprogramming of macrophages with particle-induced clustering of mannose receptors	Wound closure is accelerated	[7]
RNAi	Silencing of Keap1 expression by topically delivered siRNA	Keap1	Inhibiting oxidative stress by inhibiting the combination of Keap1 and Nrf2	Wound closure is accelerated	[140]
	Silencing of p53 expression through the topical delivery of siRNAs	Tumor protein p53	Inhibition of apoptosis and stimulation of the HIF1 $\alpha$ -dependent pathways	Wound closure is accelerated	[141]
	Silencing of PHD expression through the topical delivery of siRNAs	PHD	Increased expression of HIF1 $\alpha$ and VEGF $\alpha$ , improving fibroblast proliferation	Wound closure is accelerated	[142]
	Engineered miR-31 exosomes	miR-31-5p	Enhanced angiogenesis, fibrogenesis and re-epithelization	Wound closure is accelerated	[143]
Stem cells	CCR2-MSCs	Macrophages, T cells	Inhibition of monocyte infiltration, remodeling the inflammatory properties of macrophages, and promotion of Treg cell accumulation	Wound closure is accelerated	[151]
	MSC-derived exosomes	Macrophages	Increased ratio of M2 polarization to M1 polarization through the upregulation of PTEN expression and inhibition of AKT phosphorylation	Wound closure is accelerated	[152]
	MSCs	Macrophages	Activation of corneal epithelial stem/progenitor cells and acceleration of M2 macrophage polarization	Wound closure is accelerated	[153]
	ASCs	Macrophages	Inhibition of inflammation and macrophage infiltration	Promotion of the proliferation and migration of skin fibroblasts, and wound closure is accelerated	[163]

**Abbreviations:** NET, neutrophil extracellular trap; DW, diabetic wounds; MCs, mast cells; ESWT, extracorporeal shockwave therapy; siRNA, small interfering RNA; KSINPs, KGM-modified SiO<sub>2</sub> nanoparticles; PHD, HIF-prolyl hydroxylase domain; CCR2-MSCs, C–C motif chemokine receptor 2-engineered mesenchymal; MSCs, mesenchymal stem cell; ASCs, adipose stem cells.

## Conclusion and Perspective

Immune cells not only resist the invasion of foreign pathogens but also indirectly promote tissue repair through their bactericidal properties and by regulating the behavior of other cells and jointly promoting wound repair. However, DW stagnate in the inflammatory stage and are unable to smoothly transition to the stage of proliferation and remodeling, which is the key link in DW refractoriness. Moreover, the abnormal expression and distribution of immune cells in DW and the synergy between the cells are also an important basis for the development of wounds that tend to be difficult to heal or nonhealing wounds. With in-depth research on the pathophysiological mechanism of immune cells in DW repair, awareness of the importance of immune cell regulation for DW repair has increased. Therefore, targeted immune cell therapy based on drugs, natural compounds, physical therapy, biological materials, iRNA and stem cells is also increasing. However, the current research is mainly focused on a single treatment plan. The combined application of multiple methods may obtain better curative effects. For instance, local delivery of antimicrobial peptides using nanotechnologies shows great therapeutic potential due to their lower toxicity, prolonged stability, greater targeting ability, higher bioavailability and sustained activity.<sup>161</sup> A flexible electrical stimulation device with a Chitosan-Vaseline<sup>®</sup> dressing is a combination of drugs (chitosan-Vaseline), biological materials (dressing) and physical therapy (high voltage monophasic pulsed current, HVMPC) designed to more effectively improve DW healing.<sup>162</sup> In addition, we currently know little about the mechanism underlying the coordination between immune cells and their subgroups in DW. How are the major immune cell subgroups, such as proinflammatory, anti-inflammatory, and proangiogenic subgroups, distributed in DW? The regulation of environmental signals and the generation of specific signals suggesting what measures are needed for targeted intervention have not reached a consensus, necessitating further exploration.

## Abbreviation

DM, diabetes mellitus; DW, diabetic wounds; DFUs, diabetic foot ulcers; NETs, neutrophil extracellular traps; Arg-1, arginase-1; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; VEGF, vascular endothelial growth factor; MCs, mast cells; IgE, immunoglobulin E; DN, double-negative cells; DP, double-positive cells; DETCs, dendritic epidermal T cells; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; MMPs, matrix metalloproteinases; RNAi, RNA interference; siRNA, small interfering RNA; PHD, HIF-prolyl hydroxylase domain; CCR2-MSCs, C–C motif chemokine receptor 2-engineered mesenchymal; MCP-1, keratinocyte-derived monocyte chemotactic protein-1; ESWT, extracorporeal shockwave therapy; KSiNPs, KGM-modified SiO<sub>2</sub> nanoparticles; miRNAs, microRNAs; MSC, mesenchymal stem cell; iPSCs, induced pluripotent stem cells; ASCs, adipose stem cells.

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## Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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