Chapter

Macrophages as a Target for Treating Diabetic Foot Ulcers

Lingyan Zhu, Yu Xiao, Yao Xiao, Yinan Jiang, Maha Adama and George K. Gittes

Abstract

In all stages of wound healing, macrophages play a pivotal role by coordinating the repair steps in a timely and accurate fashion. The successful completion of wound healing requires proper spatiotemporal presence and function of macrophages. Diabetes significantly alters the proliferation, polarization and functionality of macrophages, leading to a suboptimal but prolonged pro-inflammatory M1-like phenotype in wound macrophages and a failure of their late transition to a reparative M2-like phenotype. This defect in macrophage phenotype and the proper transition results in delayed or even failure of wound healing. Specifically in the diabetic foot ulcer (DFUs), this macrophage dysfunction results in chronic infection and potentially amputation. The abnormal macrophage phenotype in diabetes is not fully understood but is believed to mainly result from epigenetic changes in macrophages and altered interactions between macrophages and other cell types, such as fibroblasts, endothelial cells, neutrophils and T-cells. Recent research on DFUs has focused on developing strategies to improve diabetic wound repair through modulation of macrophage polarization. Treatment of DFUs will greatly benefit from a multi-modal therapy that includes controlling high blood glucose, topical support, prevention of secondary infection, resolution of sustained inflammation and application of cellular therapies targeting macrophages.

Keywords: diabetic foot ulcer, diabetic wound healing, macrophage polarization, epigenetics, inflammation

1. Introduction

Diabetes is a metabolic disease that affects over 300 million people worldwide [1]. Diabetes is characterized by high blood glucose levels due to inadequate amounts of insulin produced and secreted by pancreatic beta cells, plus the loss of sensitivity to insulin in peripheral tissues [2]. There are two major types of diabetes, type 1 diabetes (T1D) and type 2 diabetes (T2D) [3]. T1D constitutes about 5% of all diabetes and is known as a T-cell mediated autoimmune invasion and destruction of insulin-producing beta cells in the pancreas, characterized by a significantly reduced beta cell mass and a significantly reduced secretion of insulin [4]. T2D accounts for about 90% of diabetes, and results from the failure of beta cells to compensate for the insensitivity of cells in responsive to insulin, which is called insulin resistance [5].

Diabetic patients can develop severe complications due to impairment in cell proliferation, differentiation, migration, immune responses, angiogenesis, etc., under a sustained hyperglycemic status [6]. Non-healing wounds, or diabetic foot ulcers (DFUs), are one of the most severe diabetic complications, and represent the leading cause of amputations, with an associated greater than 50% 5-year mortality [7]. Correspondingly, the treatment of DFUs comprises the highest annual US medical expenditure for any diagnosis [8]. Therefore, great effort has been made to understand the pathological processes during diabetic wound healing and to create novel therapies.

During the study of the mechanisms underlying diabetes-related impaired wound healing, accumulating evidence suggests that macrophages play a pivotal role in orchestrating proper wound healing [9]. In the early stages of normal wound healing, macrophages polarize to an M1-like phenotype, whereby they remove pathogens, dead cells and debris, and promote inflammation through secreting pro-inflammatory cytokines [10]. Later in the repair process, macrophages transition to more of an M2-like phenotype to resolve the inflammation and secrete trophic factors that promote the proliferation, differentiation and migration of fibroblasts, keratinocytes, mesenchymal cells and vascular endothelial cells, leading to tissue regeneration, neovascularization and wound repair [10]. These wound macrophages originate from different sources and interact with several other cell types through which they develop diverse functions to properly and efficiently assist with the repair process [11]. It is noteworthy that in diabetes there are alterations in the baseline function of macrophages, as well as the corresponding phenotypic changes in macrophages during the wound healing process [10]. Early in wound healing macrophages are responsible for the initiation and progression of inflammation and removal of pathogens, dead cells and debris. However, at later stages, macrophages instead contribute to the resolution of inflammation, reorganization of extracellular matrices (ECM), re-epithelialization, angiogenesis, cell and tissue regeneration and tissue remodeling through secreting a number of factors at late stages [12]. In a normal healthy situation, macrophages initially polarize to a pro-inflammatory M1 subtype to assist in the early stages of wound healing but then re-polarize to an alternative anti-inflammatory M2 subtype to assist in the later stages of wound healing [13]. Interestingly, diabetes leads to impairment of the M1-to-M2 transition in the later stages of wound healing, resulting in sustained inflammation and compromised cell proliferation, differentiation and migration, abnormal immune responses and inadequate angiogenesis [14]. Therefore, different strategies have been generated to target macrophages in order to reverse this pathologic inflammation during diabetic wound healing through modulating macrophage polarization [15]. In this book chapter, we discuss the role of macrophages in normal wound healing and their impairments during diabetic wound healing. We also discuss the present approaches to enhancing the repair of DFUs through targeting macrophages.

2. Macrophages and their role in inflammation

Neutrophils, macrophages and other cells involved in the innate immune system constitute a first line of defense against microorganisms and are critical for the control and resolution of common infections [16]. However, not all infectious organisms can be recognized by macrophages, for which lymphocytes of the adaptive immune system are present to create a more versatile defense system [17]. The innate and adaptive immune systems cooperate through many interactions among different

cell types. For example, cells of the innate immune system such as macrophages, dendritic cells and natural killer (NK) cells orchestrate the initiation and the subsequent progression of lymphocyte-mediated adaptive immune responses, and then receive feedback signaling from lymphocytes to adapt their phenotypes and functions for the direct involvement in the removal of pathogens targeted by adaptive immune responses [18]. Moreover, the innate immune response by macrophages is the critical response to control infections before the adaptive immune response takes effect a few days after the infection [19]. Furthermore, macrophages also contribute to directing the adaptive immune response through antigen presentation and production and secretion of cytokines and chemokines [20].

Classical macrophages display a pro-inflammatory phenotype, which has been designated as "M1" macrophages, while another subtype of macrophages that are alternatively differentiated and exhibit anti-inflammatory properties or contribute to resolution of inflammation, tissue regeneration and remodeling, have been designated as "M2" macrophages [21]. The overall M1 or M2 characteristics in a given macrophage is called its "polarization" [22]. When a macrophage changes its expression pattern to fit a more M1 or M2 phenotype, it is called a polarized macrophage [22]. Macrophage polarization can either occur in undifferentiated macrophages (naïve macrophages) or occur in polarized macrophages, which is then called "re-polarization" [22]. It is now known that polarization of macrophages into the precise definition of "M1" or "M2" macrophages rarely occurs. Instead, macrophage typically polarize into a wide spectrum of phenotypes that exhibit distinct gene and protein expression patterns [23]. This broad range of differentiation pattern allows macrophages to perform diverse tasks throughout the body [23]. M1 macrophages are characterized by high levels of proinflammatory markers such as reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), nitric oxide (NO), CD11c, tumor necrosis factor (TNF)α, interleukin (IL)-6, IL-1β and major histocompatibility complex (MHC)-II [24]. In contrast, M2 macrophages express markers associated with healing and inhibition of inflammation such as high levels of arginase 1, CD163, CD206, CD301, IL-10, resistin-like molecule alpha1 (Fizz1) and chitinase-like protein 3 (Ym1) [25]. Microenvironmental autocrine and paracrine signals, together with epigenetic changes, seem to influence macrophage activation and polarization [26].

3. The role of macrophages in normal wound healing

Wound healing is a very complex process encompassing 4 specific phases: hemostasis, inflammation, proliferation and remodeling. A successful wound closure requires the process to be well orchestrated by multiple cell types including keratinocytes, fibroblasts, endothelial cells, mesenchymal cells and inflammatory cells (macrophages, lymphocytes, neutrophils, NK cells, etc.) in a dynamic interactive way [10]. These 4 phases occur in a coordinated, linear and partially overlapping manner, in which one earlier phase is required for the completion of later phases [11]. The hemostasis phase is initiated immediately after injury, which involves vasoconstriction, platelet aggregation and activation of the clotting cascade resulting in clot formation and degranulation of platelets to convert soluble fibrinogen to insoluble fibrin and to release factors like P-selectin to recruit neutrophils to initiate the inflammatory phase [27, 28]. Next, the recruited neutrophils (peaking at 2 days after injury) release ROS, antimicrobial peptides and neutrophil extracellular traps in addition to chemokines to attract monocytes/macrophages [27]. Meanwhile,

tissue-resident macrophages and other antigen-presenting cells like dendritic cells are activated and release factors [11]. The monocytes/macrophages from either the tissue or the circulation thus become the cell type dominating the inflammatory phase and are the central regulators of this inflammatory phase [29]. Here, the macrophages interact with many cell types, activate a number of signaling pathways, and release many soluble mediators, such as growth factors, chemokines, cytokines and metabolites that signal to other cell types. Thus, the macrophages orchestrate the complex tasks and biological activities to enhance wound healing [30]. At this phase of wound healing, which typically lasts 3 days, macrophages are mainly pro-inflammatory, or M1-like, and produce cytokines such as IL-1β, IL-6, IL-12 and TNFα [10]. However, as early as day 1 after initiation of wound healing, anti-inflammatory or M2-like macrophages start to appear and their numbers increase little by little until they become dominant by about 4 days after wounding when the proliferative phase starts [14]. During the proliferative phase, M2-like macrophages (peaking at 7 days after wound) produce and secrete factors to activate fibroblasts and keratinocytes to proliferate, differentiate and migrate to the wound and deposit collagen and other ECM proteins, though keratinocytes start to proliferate immediately after wound due to loss of contact inhibition [13]. Moreover, M2-like macrophages also promote angiogenesis through interactions with vascular endothelial cells [13]. A requirement for macrophages in the activation and functions of fibroblasts at this phase was proven by a study applying macrophage-depletion [31]. Interestingly, a very recent report showed a myeloid origin of about 10% of wound fibroblasts in a mouse skin wounding model, further supporting the important role and plasticity of macrophages during wound healing [32]. The final remodeling phase is the longest phase of wound healing, during which type I collagen gradually replaces type III collagen to increase tensile strength [33].

Macrophages are key regulators for the overall wound repair process [34]. The wound monocytes/macrophages have different origins. Before injury occurs, skin monocytes/macrophages consist of the tissue-resident macrophages as predominantly Langerhans cells in the epidermal layer, and also as dermal macrophages in the dermis [35]. Wounds without macrophages have less cell proliferation, inadequate differentiation, compromised migration, delayed re-epithelialization, impaired angiogenesis and reduced collagen deposition [27]. Moreover, reduced secretion of vascular endothelial growth factor (VEGF)-A and transforming growth factor (TGF)β1 renders the wounds less conducive to angiogenesis and cell proliferation, which are critical for a proper completion of wound repair [36]. The plasticity of macrophages allows them to be first polarized to a pro-inflammatory M1-like phenotype, and then transition or repolarize to an anti-inflammatory M2-like phenotype [29]. Indeed, M1 macrophages are primarily responsible for destruction of pathogens and production and release of inflammatory cytokines in the wound. Meanwhile, M2 macrophages are associated with the late repair and regeneration phases of wound healing, and they are critical for proper angiogenesis, regeneration and remodeling of ECM, cell growth and replacement, production of anti-inflammatory and trophic cytokines and resolution of the inflammation [37]. Actually, as stated above, macrophages are not all polarized uniformly throughout wound healing [23]. At any given time point they may be in a wide spectrum of phenotypes, and only the predominant phenotype presented as the overall macrophage phenotype at any given time in the repair process (**Figure 1**) [10, 23, 29, 38].

Dermal wound healing is a very complex process encompassing 4 specific phases: hemostasis, inflammation, proliferation and remodeling. These 4 phases occur in a coordinated, linear and partially overlapping manner, in which one earlier phase is

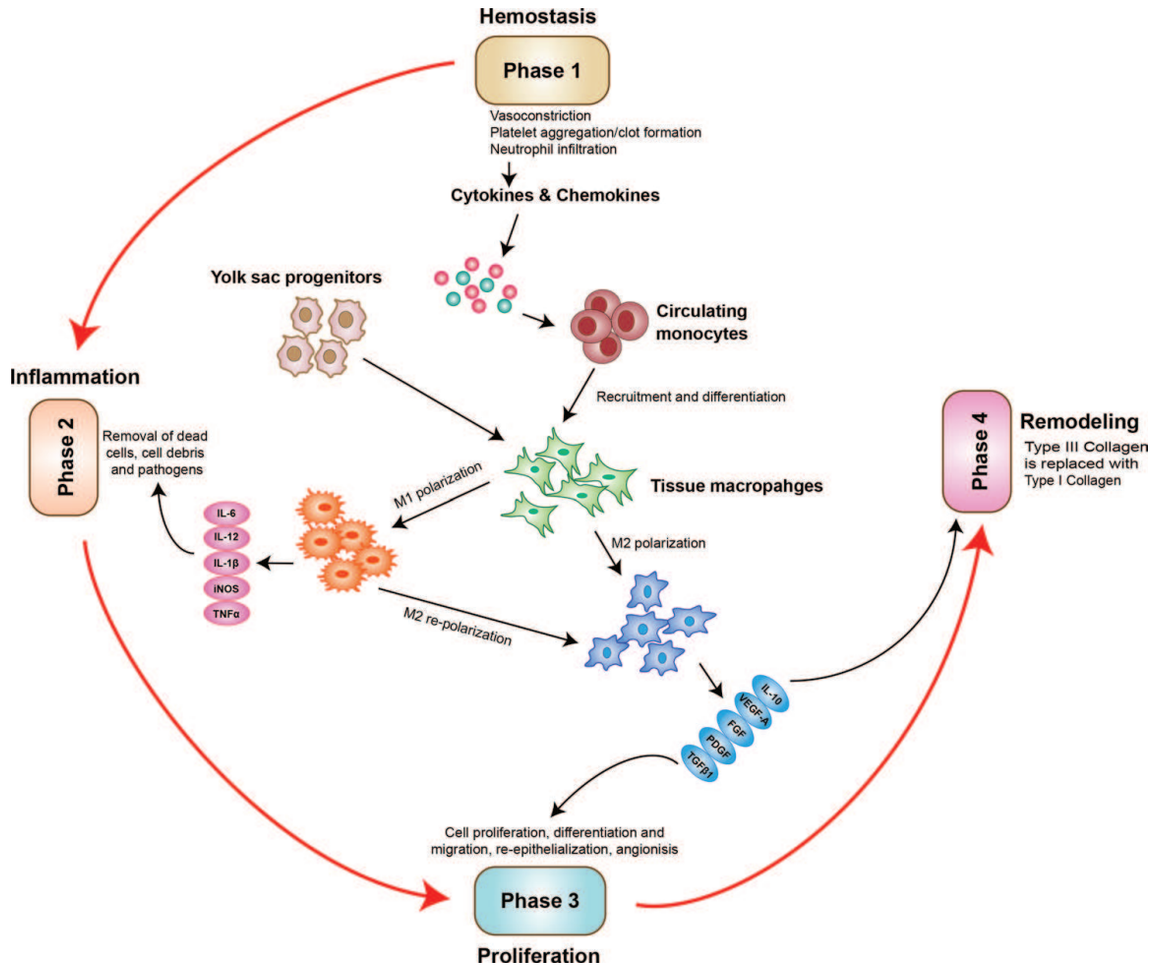

Figure 1.
Role of macrophages in wound healing.

required for the completion of later phases. The hemostasis phase is initiated immediately after injury, and involves vasoconstriction, platelet aggregation and activation of the clotting cascade, resulting in clot formation and degranulation of platelets to convert soluble fibrinogen into insoluble fibrin and to release factors like P-selectin to recruit neutrophils to initiate the inflammatory phase. Next, the recruited neutrophils (peaking at 2 days after injury) release chemokines to attract monocytes/macrophages. Meanwhile, tissue-resident macrophages and other antigen-presenting cells like dendritic cells are activated and release factors. The monocytes/macrophages from either the local tissue or the circulation thus dominate this phase of wound healing, which typically lasts 3 days. At this time, macrophages are mainly pro-inflammatory or M1-like, and produce cytokines such as IL-1β, IL-6, IL-12 and TNFα. However, as early as day 1 after the initiation of wound healing, anti-inflammatory or M2-like macrophages start to appear and their numbers increase little by little until they become dominant by about 4 days after wounding, the point when the proliferative phase starts. In the proliferative phase, M2-like macrophages (peaking at 7 days after wounding) produce and secrete factors to activate fibroblasts and keratinocytes to proliferate, differentiate and migrate to the wound and deposit of collagen and other ECM proteins. Moreover, M2-like macrophages also regulate angiogenesis through interactions with vascular endothelial cells. The final remodeling phase is the longest phase of wound healing, during which type III collagen is gradually replaced with type I collagen to increase tensile strength.

4. Alterations in macrophage polarization in diabetic wounds

Innate immune cells including macrophages have been shown to exhibit a pro-inflammatory phenotype with production and secretion of inflammatory cytokines, factors and chemokines. In diabetic wounds these processes are pathologically exaggerated as a possible contributor to the poorly healing DFU [39]. High susceptibility of diabetic patients to bacterial infections and impaired wound repair is well known [11]. The molecular mechanisms underlying this weakness are not fully understood but have been extensively studied in the past. Now it is believed that disorders in glucose metabolism and related alteration in metabolic pathways may be the reason for this susceptibility [40–42]. Interestingly, for unclear reasons the number of Langerhans and dermal macrophages significantly increases in uninjured diabetic skin [43]. Moreover, the effects of diabetes on macrophages and the related wound healing process are profound, and likely stem from changes in many aspects of the diabetic environment [13, 15, 44–48]. Macrophages that are generated in a high glucose culture system exhibit a reduction in their phagocytic potential and are less capable of clearing an infection [49, 50]. Furthermore, macrophages derived from diabetic mice and human patients appear to have increased responsiveness to inflammatory stimulants and secrete more proinflammatory cytokines than normal, which seems to prevent their later transition into the more reparative M2-like phenotype [51–55]. Indeed, at the initiation of wound healing, the phagocytotic capacity of diabetic M1 macrophages is reduced due to suboptimal differentiation, which happens before the impaired transition of M1 macrophages to M2-like macrophages later on [49, 50]. The failure of their transition or repolarization in the later stages of wound healing prevents regeneration and the repair process, resulting in a delay or even failure to heal [12].

The mechanisms underlying these alterations in the diabetic macrophage phenotype have been extensively studied. Hyperglycemia affects macrophage polarization in vitro and in vivo [46, 56–62]. For example, it has been shown that diabetic mice or human patients have an increased ratio of chemokine (C-C motif) receptor (CCR7) to CD48 3 days after wound formation [46]. CCR7 is an M1 macrophage marker whereas CD48 is an M2 macrophage marker [24]. Moreover, M1 macrophages in diabetes express less matrix metalloproteinases 1 (MMP1) and more pro-inflammatory cytokines like TNFα, resulting in an impairment in keratinocyte migration and subsequent delay of wound repair [46]. Furthermore, a hyperglycemic environment has been shown to lead to an increase in many pro-inflammatory cytokines, including TNFα, IL-1β, IL-12 and IL-6 [63], rendering these M1 macrophages more metabolically active and pro-inflammatory, but less phagocytic [63, 64]. This specific alteration in the phenotype of M1 macrophages under hyperglycemic conditions further increases the sensitivity of macrophages to cytokine stimulation and starts a vicious cycle that maintains M1 macrophage polarization and leads to a prolonged inflammation during the wound healing process [64, 65].

The role of interleukins in macrophage differentiation and polarization has been recently studied [66–73]. Some interleukins have been targeted in a therapeutic modality, exhibiting a significant impact on treatment outcomes. For example, depletion of a pro-inflammatory cytokine, IL-23, causes a significant increase in M2 macrophage polarization through loss of IL-17, which leads to improvements in diabetic wound healing [74]. Similar results have been obtained using IL-17-knockout mice or using antisera against IL-17 [74]. IL-1β is highly expressed in activated M1 macrophages in a hyperglycemic environment [63, 64]. Interestingly, experiments have shown that IL-1β expression is regulated by a protein complex called NOD-, LRR- and

pyrin domain-containing protein 3 (NLRP3), which is an inflammasome [75] that controls the dimerization and activation of caspase-1, leading to the subsequent transformation of the IL-1β precursor (pro-IL-1β) into its activated form IL-1β to be secreted [76]. Knockdown of NLRP3 with siRNA-mediated gene silencing reduced the production and secretion of IL-1β, which is beneficial to diabetic wound healing [77].

Recent research has also shed light on epigenetic alterations in macrophages in a hyperglycemic environment. These epigenetic changes can induce enhanced expression of proinflammatory cytokines to promote and sustain M1 macrophage polarization [78]. Now it is believed that epigenetic modifications are the main cause of the alterations in macrophage phenotype in diabetes [79]. The epigenetic modifications include histone modifications, DNA modifications and other post-transcriptional controls like microRNAs [10]. Moreover, regulation of macrophage polarization requires interactions with other cell types such as adipocytes, keratinocytes, fibroblasts and other immune cells (Neutrophils, T-cells, dendritic cells, etc.) that secrete factors to modulate macrophage polarization [14]. In the setting of diabetes, these cell-cell interactions are altered, leading to a suboptimal polarization of macrophages [46, 80–82].

A specific role for histone modification in the control of macrophage polarization has been recently highlighted. In eukaryotes, DNA and histones gather together to generate units called nucleosomes [83]. When histones are tightly wrapped with DNA, the access to transcriptional machinery is blocked to prevent transcription [84]. However, when DNA-histone machinery is disassembled, transcriptional binding is allowed. An N-terminal "tail" with lysine (K) residues on histones can be modified by some enzymes through catalyzing methylation and acetylation [84]. Histone methylation and demethylation are controlled by histone methyltransferases (HMTs) and histone demethylases (HDMs), respectively, which regulate macrophage differentiation and polarization [84] and are responsible for the M1 to M2 repolarization during wound healing [85]. For example, mixed-lineage leukemia 1 (MLL1) is a methyltransferase that catalyzes H3K4me3 deposition to affect macrophage polarization and the induction of expression of proinflammatory genes in macrophages [86]. Mechanistically, MLL1 is found to regulate changes in macrophages partially via Toll-like receptor 4 (TLR4) in both diabetic humans and diabetic mice [87, 88]. On the other hand, Suppressor of variegation, Enhancer of Zeste, Trithorax and myeloid-Nervy-DEAF-1 domain-containing protein 3 (SMYD3), which is another H3K4me3 methyltransferase, has been shown to regulate M2-like polarization of macrophages [89]. Besides HMTs, HDMs also play a critical role in macrophage polarization during wound healing. For example, Jumonji domain-containing protein 3 (JMJD3) is a H3K27 demethylase that regulates a context-dependent polarization of macrophages towards either a proinflammatory M1-like or an anti-inflammatory M2-like macrophage phenotype [90–95]. JMJD3-mediated release of H3K27me3 is compromised in diabetic wound macrophages, resulting in enhanced and sustained expression of genes associated with inflammation [96, 97]. However, lipopolysaccharides (LPS) and IL-4 have been shown to induce JMJD3 for directing M2-like macrophage polarization [96]. Together, a lot of data have demonstrated the importance of histone methylation and demethylation by HMTs and HDMs in controlling macrophage polarization during wound repair [98, 99]. Transcriptional repression is often regulated by DNA methylation, which is catalyzed by DNA methyltransferases (DNMTs) to transfer a methyl group to the cytosine ring of DNA at clusters of CpG islands [100]. The potential binding of transcription factors to a promoter region is significantly altered via methylation of CpG islands on the promoter [101]. For example, DNMT1 has been shown to induce M1-like polarization of macrophages [102]. Moreover, genetic

depletion of DNMT1 or chemical suppression of DNMT1 by 5-aza-2′-deoxycytidine promotes M2-like macrophage polarization [103] and improves wound healing in diabetic mice [104]. Macrophage polarization during wound healing has also been shown to be affected by histone acetylation and deacetylation. Transcriptional activation is enhanced by acetylation of the lysine residue on the histone tail, for which an acetyl group is transferred from acetyl CoA to the lysine residue catalyzed by histone acetyltransferases (HATs) [105]. In diabetes, it has been shown that histone deacetylase 6 (HDAC6) alters the phenotype of macrophages through IL-1β but not IL-10 [106].

Post-transcriptional control is also an important regulator of macrophage polarization in diabetes. For example, microRNAs have been shown to be important regulators of gene expression during macrophage polarization [9]. MicroRNAs (miRNAs) are non-coding small RNAs about 20 base pairs in length. miRNAs control protein levels of an expressed gene through Watson-Crick pairing to the 3′-untranslated region (3′-UTR) of the mRNA of a specific gene, resulting in altered protein translation [107]. It has been reported that M2 macrophages express high levels of miRNA-146, while M1 macrophages express low levels of miRNA-146 [108, 109], and the levels of miR-146 appear to alter macrophage polarization and their production and secretion of proinflammatory and anti-inflammatory cytokines [108, 109]. It has also been shown that miR-155 can induce an M1-like macrophage polarization through suppressing antagonists of proinflammatory cytokines [110]. Moreover, miR-33 was shown to favor M2 macrophage polarization through suppressing NLRP3 [111], a key inducer of IL-1β and inflammation [76, 77]. Similarly, long noncoding RNAs (lncRNAs) have an emerging role in regulating macrophage polarization [112–116]. Besides miRNAs, long non-coding RNAs also play essential roles in macrophage phenotypic determination and control of inflammation [117]. The role of lncRNA GASS in macrophage polarization and associated wound healing has been reported recently [113].

5. Strategies to improve diabetic wound healing through manipulating macrophages

DFUs will likely require multimodal therapies for optimal treatment. Novel therapeutics are being generated, including specific targeting of macrophages. Since the initial trigger of all the macrophage defects in diabetes appears to be sustained high blood glucose, correction of the primary problem, the hyperglycemia, would appear to be the first approach to treat problems related to diabetic wound healing [7]. However, only half of diabetics can reach the recommend standard hemoglobin A1c (HbA1c) level of <7.0% (issued by the American Diabetes Association (ADA)) [118]. Thus, it is important to search for novel therapies beyond control of blood glucose [7].

Insulin administration is a regular and effective therapy for those unresponsive to diet changes or non-insulin medications. Insulin has been found to reduce the number of M1 macrophages. Moreover, insulin has been shown to induce M1 to M2 macrophage polarization through peroxisome proliferator-activated receptor-gamma (PPAR-γ) and phosphatidylinositol-3 kinase (PI3K)/Protein kinase B (Akt)/Ras-related C3 botulinum toxin substrate 1 (Rac-1) signaling pathways [47]. Interestingly, the PPAR-γ pathway that reduces proinflammatory cytokine expression and enhances M2 macrophage polarization in normal wound healing is impaired in diabetes and could be partially recovered by insulin [119]. Metformin is a commonly used medication for diabetes. Metformin treatment has been shown to increase M2 macrophages and decrease M1 macrophages [120–125], likely through NLRP3 inflammasome

suppression, which was discussed above [123]. Melatonin is a medication not for diabetes. However, it was found to affect macrophage polarization in diabetic wound, likely through effects on insulin [47, 126].

Treatment of chronic wounds benefits largely from localized therapies, which have the advantage of avoiding systemic effects and allowing for local and direct treatment [127]. A hydrocolloid dressing to provide moisture to the wound has been shown to improve M1-to-M2 macrophage transition to favor wound healing, especially in diabetes [128]. In another study, use of a modified dressing in diabetic mice led to an earlier appearance of M2 macrophages at the wound [129]. Thus, certain dressings to induce an M2 macrophage polarization appear to be an attractive strategy for treating chronic diabetic wounds. These dressings help the wounds heal through moisture provision, prevention of infection, induction of anti-inflammatory effects and generation of trophic factors. Along with these dressing benefits, Collagenase Santyl Ointment (CSO), with an important component called Clostridial collagenase, has been shown to increase local expression of IL-10 and arginase 1 that are both critical for M2-like macrophage polarization and functionality [130]. Another modified collagen gel has also been shown to increase IL-10 expression and macrophage migration, resulting in a substantial increase in M2 macrophages in the wound at different time points [128]. In addition, increases in IL-10 expression have also been detected after use of docosahexaenoic acid to treat diabetic wounds, with the therapeutic outcome correlated to the degree of M2 macrophage polarization [45, 131, 132].

Multipotent stem cells (MSCs) have been used in the treatment of DFUs, taking advantage of the capacity of MSCs to differentiate into different cell types such as endothelial cells, fibroblasts and smooth muscle cells that are critical for wound healing. These newly formed cells can produce and secrete trophic factors like VEGF-A, fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) [133–135]. Recently, we have reported that human MSCs express high levels of miR-205-5p, which inhibits protein translation of VEGF-A through 3'-UTR interactions [136]. Expressing antisense of miR-205-5p (as-miR-205-5p) in human MSCs significantly improved the therapeutic effects of human MSCs on diabetic wound healing in rodent models [136]. Next, we reported that MALAT1 is a lncRNA acting as a competing endogenous RNA (ceRNA) for miR-205-5p and is absent in human MSCs [137]. Expression of MALAT1 significantly attenuated the high expression of miR-205-5p in human MSCs, resulting in upregulation of VEGF-A production and improved therapeutic effects of human MSCs on diabetic wound repair [137]. Of note, these improvement in treating diabetic wounds through increasing VEGF-A levels in human MSCs was shown to be associated with increased vascularization of the wounds [136, 137]. Macrophages express high levels of VEGF receptor 1 (VEGFR1), which is one of the major receptors on macrophages to respond to chemoattractants. Thus, it is possible that the therapeutic effects of increased VEGF-A levels in human MSCs may be due, at least partially, to alterations in macrophage proliferation, differentiation and polarization [21]. Further studies of the exact alterations in macrophages caused by VEGF-A are needed. We have recently shown that another VEGF family member, placental growth factor (PlGF), is capable of altering macrophage migratory capacity and polarization [138]. Moreover, we and others have recently shown that PlGF is decreased in DFUs [139, 140]. PlGF injection significantly improved angiogenesis and diabetic wound healing, with both positive effects being abolished by macrophage-specific depletion of VEGF1R to block the effects of PlGF on macrophages [139]. Together, these approaches showed promise as strategies for treating diabetic wound through targeting macrophages.

6. Conclusions

The treatment of diabetic wounds will benefit from a multi-modal approach including control of hyperglycemia, topical treatment, prevention of secondary infection and inflammation and cellular therapy targeting macrophages.

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Conflict of interest

The authors declare no conflict of interest.

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