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Macrophage-mediated inflammation in diabetic wound repair

Sonya J. Wolf[a,1], **William J. Melvin**[a,1], **Katherine Gallagher**[a,b,*]

[a]Section of Vascular Surgery, Department of Surgery, University of Michigan, Ann Arbor, MI, USA

[b]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI, USA

Abstract

Non-healing wounds in Type 2 Diabetes (T2D) patients represent the most common cause of amputation in the US, with an associated 5-year mortality of nearly 50%. Our lab has examined tissue from both T2D murine models and human wounds in order to explore mechanisms contributing to impaired wound healing. Current published data in the field point to macrophage function serving a pivotal role in orchestrating appropriate wound healing. Wound macrophages in mice and patients with T2D are characterized by a persistent inflammatory state; however, the mechanisms that control this persistent inflammatory state are unknown. Current literature demonstrates that gene regulation through histone modifications, DNA modifications, and microRNA can influence macrophage plasticity during wound healing. Further, accumulating studies reveal the importance of cells such as adipocytes, infiltrating immune cells (PMNs and T cells), and keratinocytes secrete factors that may help drive macrophage polarization. This review will examine the role of macrophages in the wound healing process, along with their function and interactions with other cells, and how it is perturbed in T2D. We also explore epigenetic factors that regulate macrophage polarization in wounds, while highlighting the emerging role of other cell types that may influence macrophage phenotype following tissue injury.

Keywords

Diabetes; Epigenetics; Macrophage; Wound; Inflammation; Phenotype

1. Introduction

Around 34.1 million adults in the US have diabetes, and the incidence has been increasing by 5% per year [1]. These patients develop several complications, with impaired lower extremity wound healing being one of the most common causes of hospitalization. Standard therapy for these wounds often leads to amputation, as over 70% of diabetic wounds fail to heal with the current standard of care [1]. The significant morbidity following amputation in these patients leads to an associated 50% mortality at five years [2]. Current literature supports a role for decreased microcirculation and peripheral neuropathy in

*Correspondence to: Department of Surgery, University of Michigan, 1500 East Medical Center Drive, SPC 5867, Ann Arbor, MI 48109, USA. kgallag@med.umich.edu (K. Gallagher).

[1]Equal contribution.

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impaired diabetic wound healing [3]. Additionally, chronic inflammation plays a vital role in this process, with macrophage functional impairment serving a crucial role in the hyper-inflammatory phenotype seen in non-healing diabetic wounds [4].

Wound healing is a complex process regulated by multiple cell types. Our lab and others have demonstrated that macrophages are critical for this healing process. Macrophages' plasticity allows them to be pro-inflammatory initially and then transition to an anti-inflammatory phenotype to perform functions vital for tissue repair [5,6]. Diabetic wounds exhibit a chronic inflammatory state where imbalances in macrophage phenotype prevent wound inflammation resolution [7]. Molecular mechanisms that control macrophage phenotype are not well understood. However, recent studies by our lab and others have suggested epigenetics plays a critical role in controlling macrophage plasticity following tissue injury [8]. Current literature demonstrates that gene regulation through histone modifications, DNA modifications, and microRNA can influence macrophage plasticity during wound healing [8,9]. Further, there is increasing evidence that cells such as adipocytes, infiltrating immune cells (PMNs and T cells), and keratinocytes secrete factors that may help drive macrophage polarization during wound healing [10–14].

This review will examine the role of macrophages in the wound healing process, their function and interactions with other cells, along with how these functions are disturbed in T2D. It will also explore epigenetic factors that regulate macrophage polarization in diabetic wounds and highlight emerging evidence on the role of other immune cells and structural cells that may influence macrophage phenotype following tissue injury.

2. Phases of wound healing

Dermal wound healing is an intricate process encompassing four classically described phases of wound healing: hemostasis, inflammation, proliferation, and remodeling [15]. Under normal conditions, these phases occur in an overlapping, linear fashion. The hemostasis phase is initiated following injury; this involves the aggregation of platelets and solubilized coagulant proteins in the blood to form a clot at sites of active bleeding. These platelets degranulate to release factors critical for the initial inflammatory phase, such as P-selectin, allowing for neutrophil adherence to the wound [16].

Early in the inflammatory phase, neutrophils are the primary cell type within the dermal wound. They release antimicrobial peptides (*e.g.*, LL37), reactive oxygen species (ROS), and neutrophil extracellular traps (NETs). Additionally, neutrophils secrete chemokines to attract monocytes/macrophages [17,18]. These monocytes/macrophages, in addition to tissue-resident macrophages, then become the dominant cell type in the inflammatory phase and are critical in regulating this inflammation [7,19–21]. Initially, macrophages are pro-inflammatory or M1-like, producing cytokines such as IL-12, IL-1β, IL-6, TNFα, and iNOS. During normal wound repair, anti-inflammatory or M2-like macrophage subtypes become dominant over time to trigger the proliferative phase.

During the proliferative phase, fibroblasts and keratinocytes are stimulated by growth factors secreted by macrophages and other cells, to re-epithelialize and form granulation

tissue within the wound; this is *via* the secretion of collagen and other extracellular matrices (ECM) proteins. Macrophages play a critical role stimulating these fibroblasts, this is demonstrated by the ablation of macrophages leading to a significant delay in the appearance of fibroblast during the proliferative phase of wound healing [22]. Interestingly, recent single-cell analysis has also revealed that in a murine skin wounding model, about 6–11% of fibroblasts are myeloid-derived, demonstrating the important role and plasticity of myeloid cells through the stages of wound healing [23].

Lastly, the remodeling phase is the longest phase of wound healing in which type III collagen is replaced with type I collagen for higher tensile strength. Depending on the depth, mechanism of injury, and species, dermal structures such as hair follicles and pre-injury tensile strength do not entirely recover [24].

3. Monocytes/macrophages during wound healing

3.1. Monocytes

There are two types of circulating monocytes, classical ($CD16^{-}/Ly6C^{hi}$) and nonclassical monocytes ($CD16^{+}/Ly6C^{Lo}$). In response to initial signals of Damage Associated Molecular Patterns (DAMPs) or Pathogen Associated Molecular Patterns (PAMPs), classical monocytes ($CD16^{-}/Ly6C^{Hi}$) extravasate at the site of injury and immediately release inflammatory cytokines and chemokines to recruit more myeloid cells [25]. These infiltrating myeloid cells perform phagocytosis, efferocytosis, and autophagy function to ‘clean’ the wound, priming it for the placement of granulation tissue and proliferation. They also massively proliferate within the wound and bone marrow, peaking in murine dermal skin wound models on day six following injury in a highly conserved fashion [26,27]. Following proliferation, these monocytes will differentiate into ‘M1’ inflammatory macrophages and undergo *in situ* differentiation to $Ly6C^{Lo}$ monocyte/M2-like ‘anti-inflammatory’ macrophages and are the primary source of cells within the wound [28].

In contrast to classical monocytes, nonclassical monocytes ($CD16^{+}/Ly6C^{Lo}$) are ‘anti-inflammatory’ and express VEGF, TGFβ, and IL-10. They are biased progenitors of ‘M2’ macrophages [29], which are key promoters of the proliferative and remodeling phases of wound healing. However, fewer of these monocytes are circulating; thus, they are not the major contributors to myeloid-function within the wound healing setting.

3.2. Macrophages phenotypes in wound healing

Macrophages play a critical role in normal wound healing. Indeed, macrophage-specific ablation resulted in delayed re-epithelialization and exhibited a reduction in the secretion of VEGF and TGF-β1, making wounds less conducive to angiogenesis and cell proliferation [30,31]. Macrophages are classically identified based on *in vitro* studies into subsets based on their markers, phenotype, and role. These *in vitro* phenotypes are the ‘M1’ or “classically-activated” macrophage and the ‘M2’ or “alternatively-activated” macrophage. ‘M1’ macrophages ($CD86^{+}$) are the primary players in pathogen destruction, secretion of inflammatory cytokines, and driving a Th1-type response in wound healing. Meanwhile, ‘M2’ macrophages ($CD206^{+}$) are associated with critical aspects of wound healing,

including angiogenesis, ECM remodeling, production of anti-inflammatory cytokines, and inflammation resolution [32]. However, more recent work has identified multiple unique subtypes that are host and context dependent – and demonstrate that a 'spectrum' of macrophage phenotypes exist, especially in the immunomodulatory phenotypes, rather than a dichotomous M1 and M2 polarization seen with acute *in vitro* stimulation [33]. Further, the *in vitro* 'M1' and 'M2' phenotype classification omits the role and context of the *in vivo* wound on influencing macrophage phenotypes. *In vivo*, heterogenous individual cells exhibit varying characteristics of traditional M1/M2 phenotypes on a spectrum [33,34]. These subtypes and others' specific roles continue to be discovered, especially in how they function in tissues *in vivo*. As a whole, in the physiologic dermal wound, *in vivo* macrophages dynamically change their phenotypes from pro-inflammatory to reparative over time, rather than a dichotomous switch between M1 and M2 [23,28]. One recent study shows that wound angiogenesis in murine and zebrafish models, is dependent on pro-inflammatory TNFα-secreting macrophages[35], highlighting the complexity of the wound microenvironment. Further, specific macrophage subtypes continue to be discovered. For example, one recent study established two novel *in vivo* macrophage subtypes ($CX3CR1^{Hi}$ *vs.* $CX3CR1^{Med/Lo}$) and showed how a loss of $CX3CR1^{Hi}$ macrophages in T2D contributes to poor wound healing and a pro-inflammatory state [36]. Kinetic regulation of these subtypes is critical for physiologic maturation of the dermal wound. Recent work examining *in vivo* macrophage transcriptional networks showed that the *Runx2* locus had strong control over the transition from pro-inflammatory *in vivo* macrophages to reparative phenotypes in a murine ear wound model [37]. Loss of function of *Runx2* led to persistence of pro-inflammatory phenotypes and delayed wound healing [37]. This highlights the importance of managing *in vivo* macrophage phenotypes in a kinetic fashion to progress the wound through the stages of wound healing, and how the artificial *in vitro* M1/M2 dichotomy, although easy to describe, does not apply perfectly to the wound.

Another active area of research focuses on differences between tissue-resident macrophages *vs.* monocyte-derived macrophages in the context of wound healing. Dermal macrophages are essential for promoting the initial inflammatory response to DAMPs/PAMPs in wounds [38,39]. They are quickly outnumbered by monocyte-derived macrophages during wound repair, but upon resolution, they will self-renew to return the environment to homeostasis.

3.3. Macrophage dysregulation in type II diabetic wounds

Wound macrophage dysregulation in T2D is characterized by the decreased host response to pathogens in the wound [40], sustained presence of inflammatory phenotypes [28], increased number of monocytes/macrophages at late time points in wounds [41], and a dampened initial inflammatory response [42].

Diabetic wounds are known to have enhanced susceptibility to bacterial infections [40], and this is in part due to a dysfunctional macrophage host response in diabetes [43]. Diabetic wounds are colonized in a polymicrobial fashion, and *Staphylococcus aureus* is typically present [44]. Peritoneal macrophages isolated from diabetic mice demonstrated impaired phagocytosis of fluorescently labeled *S. Aureus* compared to controls [43]. Furthermore, bone marrow-derived macrophages (BMDMs) cultured in high glucose media exhibited

decreased phagocytic ability and were less bactericidal than controls [43]. They also have a dampened inflammatory response to lipopolysaccharide (LPS), a component of the gram-negative bacteria cell wall, despite their increased basal inflammatory activity [45]. The interplay between the microbiota colonizing wounds and macrophages likely plays a role in altering their phenotype and subsequent wound repair ability [44]; thus, this deserves further study, especially on identifying the specific epigenetic changes on macrophages exposed to various microbiomes. Hyperglycemic conditions have been shown to impair macrophage efferocytosis. In particular, macrophage clearing of apoptotic neutrophils and necrotic cells in the tissue is dampened by local hyperglycemia in the form of advanced glycosylated end products *via* the RAGE receptor [46,47]. This dysregulated macrophage function contributes to the well-described pro-inflammatory diabetic wound milieu *via* failure to remove pathogens and inflammatory apoptotic neutrophils.

Increased persistence of M1-type ($Ly6C^{Hi}$) monocytes/macrophages in diabetic wounds following the monocytes initial recruitment phase is well established [28]. Several studies have demonstrated their role in contributing to delayed healing, showing that reversal of this persistent inflammatory phenotype improves wound healing. One study demonstrated that administration of anti-monocyte chemoattractant protein-1 (MCP-1) to wounds prevented this influx of late inflammatory monocyte/macrophages ($Ly6C^{Hi}$) and enhanced wound healing in diabetic mice [28]. Our group has recently illustrated this *via* single-cell RNA sequencing of human wound skin, demonstrating increased NFκB–mediated inflammation in diabetic wounds compared to control; this was in part due to increased cyclooxygenase 2/prostaglandin E_2 (COX-2/PGE_2). Subsequent inhibition of the COX-2/PGE_2 pathway genetically ($Cox2^{fl/fl}$ $Lyz2^{Cre+}$) in mice or with macrophage-specific nanotherapy targeting COX-2 in murine tissue macrophages reversed the inflammatory macrophage phenotype and improved wound repair [48]. Another study reversed epigenetic modifications priming NFkB-mediated inflammation in diabetic macrophages and subsequently improved wound healing [11,49]. The wealth of translational studies showing improved diabetic healing with reversal of prolonged macrophage-mediated inflammation underscores their important role during *in vivo* wound repair.

There is also mounting evidence involving regulation in the timing of macrophage presence in diabetic wounds; however, whether M1 macrophages are increased or decreased initially following an injury is unclear. Several studies using an unsplinted wound model demonstrate an increased presence of inflammatory or ‘M1’ macrophages at early time points in diabetic wounds [27,50]. In contrast, using a splinted wound model in $Lepr^{db/db}$ mice, *Yan* et al. showed a decreased presence of both ‘M1’ and ‘M2’ macrophages at early time points when the wounds were less than 20% healed by size [51]. This difference in the presence of ‘M1’ macrophages early in the diabetic wound may be due to the ‘chronic’ splinted wound model used by *Yan* et al., compared to an acute unsplinted model used by the first groups. Unsplinted wounded models can lead to healing *via* contracture instead of secondary intention and granulation, seen in the splinted model. One study highlights the importance of the initial tissue macrophage response early after injury. Increasing ‘M2’ macrophage phenotypes in unsplinted diabetic wounds for the first three days leads to increased keratinocyte migration, increased granulation tissue formation, increased angiogenesis, and faster-wound healing [52]. In fact, our group has also found delayed

epigenetic regulation of inflammation by Mixed lineage leukemia 1 (MLL1) in T2D leads to a decrease in inflammatory macrophages early following injury and impaired diabetic wound healing [42]. Thus, the effects here lend credence to the significance of a dampened initial macrophage response to tissue injury in diabetes contributing to poor wound healing. However, the exact nature of macrophage dysregulation and timing in early wound healing merits further investigation, and is likely model dependent. This could be achieved by directly comparing *in vivo* macrophage phenotypes between various sized murine skin wounds and comparing splinted *vs.* unsplinted wounds at multiple time points. Further, linking murine findings with human data from diabetic wounds is critical to establish clinical relevance of differing models.

The changes contributing in part to macrophage dysregulation in the wound stem from alterations in hematopoietic stem cells in the bone marrow. One study showed that, following adoptive transfer, there was dysregulation of both ‘M1’ and ‘M2’ macrophages early on in the wound course and a subsequent preference to ‘M1’ macrophages later [51]. Epigenetic changes to hematopoietic stem cells are conserved, contributing to M1 and M2 macrophage dysregulation and impaired wound healing [27,50].

As single-cell RNA sequencing becomes more accessible and the technical challenges performing this in human diabetic wounds is overcome, more targets affecting macrophage plasticity and phenotype in diabetes will be discovered [53]. Furthermore, the regulators of macrophage phenotypes – transcriptional networks, epigenetic modifications, and other wound cells in the wound microenvironment will need to be analyzed and offer exciting opportunities for potential therapeutic targets for management of diabetic wounds.

4. Epigenetic modifications of macrophages in type 2 diabetic wounds

Epigenetic modifications regulate monocyte/macrophage phenotype during wound healing by altering the chromatin to activate or silence a gene without changing the genetic code [8,54–56]. Studies demonstrating the importance of epigenetic regulation in macrophage polarization are summarized in Table 1. There are three main types of epigenetic modifications: histone modification, DNA modification, and ATP-dependent remodeling [57], with the former two being more studied in wound healing. Additionally, miRNA can change the macrophage inflammatory profile by regulating gene expression (Fig. 1).

4.1. Histone modifications

Current literature demonstrates a role for histone modification in regulating macrophage polarization. In eukaryotes, DNA and histones form units called nucleosomes [58]. When DNA is tightly wrapped around histones (heterochromatin), transcription is inhibited by blocking transcriptional machinery access. However, when DNA-histone interactions are relaxed (euchromatin), transcriptional proteins can bind to allow transcription to occur [58–60]. Histones have an N-terminal “tail” with lysine (K) residues that histone-modifying enzymes can methylate and acetylate to direct formation of heterochromatin and euchromatin [59].

Histone methylation and demethylation are the most highly studied histone modifications and play a role in macrophage polarization. Methylation of a histone activates or represses transcription depending on the number of methyl groups added and their location on the histone tail. For example, the tri-methylation of lysine 4 on histone 3 (H3K4me3) causes the chromatin to open, promoting transcription. On the other hand, the tri-methylation of lysine 27 (H3K27me3) or lysine 9 (H3K9me3) is associated with promoting heterochromatin formation [14,56,60,61]. These histone methylation marks play a part in regulating the macrophage switch during wound healing [14,56].

Histone methyltransferases (HMTs) and Histone demethylases (HDMs) respectively achieve methylation and demethylation of a histone. Several HMTs have been ascribed with roles in macrophage polarization during wound healing. One methyltransferase necessary for catalyzing H3K4me3 deposition driving macrophage polarization is MLL1. Our lab has shown that MLL1 is associated with promoting pro-inflammatory gene expression in macrophages during the inflammatory phase of wound healing. Use of an MLL1 inhibitor or myeloid specific deletion of MLL1 delayed wound healing and decreased pro-inflammatory cytokine production [42]. We further demonstrate that monocytes isolated from T2D mice exhibited impaired MLL1 expression early during wound healing, followed by overexpression in the latter phase [42]. Additionally, we show in T2D patients and mice that MLL1 regulates macrophage phenotype in part through TLR4 receptor signaling, as myeloid-specific knockout of MLL reduced TLR4 expression and improved wound healing [62,63]. Conversely, SMYD3, another H3K4me3 methyltransferase, seems to regulate M2-like polarization [64]. We have recently shown that SETDB2, a H3K9me3 methyltransferase, negatively regulates pro-inflammatory genes, inducing a tissue repair macrophage phenotype. Further, we demonstrate that SETDB2 expression is regulated by IFN-β [14]. Not only do HMTs play a role in macrophage polarization in wound repair, but HDMs also control this process. The H3K27 demethylase Jumonji domain-containing protein 3 (JMJD3) has roles in activating both pro-and anti-inflammatory macrophage phenotypes depending on the environment/tissue [56,65,66]. Our lab and others have shown that in T2D murine models, wound macrophages exhibit decreased H3K27me3 expression mediated by JMJD3 release of H3K27me3 to promote inflammatory gene expression [49,67]. JMJD3 expression has also been shown to increase in response to inflammatory stimuli such as LPS and IL-4 [67]. These data indicate that histone methylation and demethylation regulated by HMTs and HDMs influence macrophage polarization following injury.

Histone acetylation and deacetylation are other mechanisms important in macrophage polarization during wound healing. Acetylation of the lysine residue on the histone tail interferes with the interaction between the DNA and histone, promoting transcriptional activation. Histone acetyltransferases (HATs) carry out the acetyl group’s transfer from acetyl CoA to the lysine residue [68]. Our lab has shown that Males-absent on the first (MOF), a histone acetyltransferase, is elevated in T2D mice and is regulated by TNF-alpha stimulation [69]. The primary substrate for MOF activity, H4K16, has increased deposition associated with promoters of inflammatory genes of diabetic macrophages [69]. Others have demonstrated that histone deacetylase 6 (HDAC6) influences macrophage production of IL-1β, while inhibiting IL-10 production, under hyperglycemic conditions [70]. Though

histone acetylation can induce macrophage polarization, further studies into upstream regulation are needed to elucidate this mechanism fully. Overall, since histone modifications demonstrate an important role in the macrophage “switch” required for wound healing, targeting these modifications directly or indirectly may prove useful in improving wound healing.

4.2. DNA methylation

DNA methylation is predominantly associated with transcriptional repression. It is characterized by the transfer of a methyl group to the cytosine ring of DNA by DNA methyltransferases (DNMTs), which occurs at clusters of CpG islands. Methylation of CpG islands within the promoter can directly silence transcription by impeding transcription factor binding [71]. DNMT1 can regulate macrophage and bone marrow progenitor cells’ inflammatory profile towards an ‘M1’ phenotype [51, 72]. DNMT1 inhibition by 5-aza-2’-deoxycytidine promoted ‘M2’ macrophage formation and suppressed inflammation in bone marrow-derived macrophages (BMDMs) [72]. This inhibition of DNMT1 also resulted in protection against obesity-induced inflammation and insulin resistance [72]. Further, DNMT1 is elevated in BMDMs from type 2 diabetic murine models and promotes a pro-inflammatory macrophage phenotype. Indeed, the knockout of DNMT1 improved wound healing in these mice [51]. DNMT3b has also been shown to regulate macrophage polarization towards a ‘M1’ phenotype [73]. The expression of DNMT3b is elevated in macrophages isolated from diet-induced obese mice compared to control [73]. Additionally, knockout of DNMT3b in macrophages *in vitro*, induced polarization towards a ‘M2’ phenotype [73]. While DNMT3b may regulate macrophage polarization, how this contributes to wound repair is unclear.

4.3. miRNA regulation

Research recently has shown microRNA as an additional regulator in gene expression during macrophage polarization [9]. One group demonstrated that microRNA-146a (MiR-146a) expression is elevated in ‘M2’ macrophages but attenuated in ‘M1’ macrophages [74]. MiR-146a exhibits protective effects on macrophages by inhibiting the activation and secretion of pro-inflammatory cytokines [74,75]. Meanwhile, microRNA- 155 (miR-155) expression promotes an ‘M1’ macrophage phenotype by repressing negative regulators of inflammatory cytokine expression [76]. Additionally, overexpression of microRNA-21 (miR-21) in diabetic wound macrophages was associated with the upregulation of pro-inflammatory genes such as IL-1β and TNFα [77]. Similarly, long noncoding RNA may also demonstrate a role in regulating macrophage polarization [78], though further studies are needed to elucidate the mechanism fully.

5. Cellular regulation of macrophage phenotype in wounds

Current research has demonstrated that the pro-inflammatory microenvironment in diabetic wounds contributes to the persistent ‘M1’ macrophage phenotype exhibited following injury. In particular, our lab and others have shown that blocking or inhibiting cytokines (*e.g.*, IL-1β) and chemokines (MCP-1) elevated in the wound microenvironment can reduce the overabundance of inflammatory macrophages in diabetic wounds [4,28]. Additionally,

keratinocytes, adipocytes, T cells, and neutrophils exhibit altered phenotypes in diabetic wounds [79–83]. The factors secreted by these cells have been suggested to be involved in macrophage polarization (Fig. 2).

5.1. Adipocytes

Adipocytes in obese patients and mice release MCP-1, TNF, and free fatty acids [10]. Specifically, dermal adipocytes can release palmitic acid and oleic acid, and fatty acid levels are elevated in T2D patients [84–87]. Our lab and others have shown that fatty acids can manipulate the macrophage inflammatory profile [10,11]. In particular, we demonstrate that palmitate can increase JMJD3 expression in macrophages in a TLR4/MyD88-dependent manner, releasing H3K27me3 deposition on the promoters of inflammatory genes [11]. Fatty acids can also increase the expression of SIRT3, a primary mitochondrial deacetylase in macrophages [88]. We found that SIRT3 negatively regulates inflammatory cytokine production in macrophages and is important for normal wound healing. In diabetic wounds, macrophages exhibit attenuated SIRT3 expression in a fatty acid-binding protein (FABP4)--dependent manner [89]. However, the mechanism by which FABP4 is regulated in diabetic wounds is unclear. Together these studies suggest following injury, adipocyte secretion of fatty acids may manipulate macrophage phenotype through inducing epigenetic modification.

5.2. Infiltrating immune cells

Infiltrating immune cells into the skin is also associated with manipulating macrophage phenotype during wound healing. Neutrophils are among the first cells entering the wound, secreting neutrophil extracellular traps (NETs). When healthy and diabetic skin was compared, diabetic patients exhibited increased NETosis. NETosis is associated with activation of NLRP3 inflammasome and IL-1β production by macrophages [12]. Within the wound microenvironment, research suggests lymphocytes also contribute to macrophage polarization [90]. Increased T cells are present in diabetic wounds, particularly gamma delta and Th17 cells [13]. In diabetic wounds, IL-17, a cytokine produced mainly by Th17 cells, demonstrates a role in macrophage polarization as knockout of IL-17 improved wound healing in a diabetic mouse (db/db) model by reducing ‘M1’ macrophages and maintaining ‘M2’ macrophages [91]. Further studies are warranted to identify the specific signaling pathways or epigenetic enzymes that are driving Th17 polarization, and whether there are viable therapeutic targets to improve wound healing by preventing excessive Th17 polarization in wound T cells.

5.3. Keratinocytes

Keratinocytes not only function as a barrier in the skin, but they are also primed to respond to environmental stimuli and serve as the first link in cutaneous immunity through the release of cytokines/chemokines. During chronic skin inflammation, keratinocytes secrete NFκB regulated cytokines such as IL-18 and IL-6, in addition to type I IFNs. Although other chronic inflammation skin models demonstrate keratinocyte production of type I IFNs influences immune cell inflammatory profiles [92], less is known about their role in tissue repair. Diabetic studies to date have focused on IFN-β, demonstrating that administration of IFN-β to high-fat-diet (HFD) mice has been shown to attenuate inflammation in adipose

tissue, reduce weight gain, and improve glucose tolerance [93]. We found that knockout of the type I IFN receptor impairs wound healing; this impaired wound healing is associated with an increased inflammatory macrophage phenotype [14]. Additionally, we demonstrate that IFN-β induces Setdb2 expression in macrophages resulting in the turning off of inflammatory genes; however, in diabetic wounds, this IFN-β/Setdb2 axis is impaired [14]. These data suggest a role for IFN-β in regulating macrophage phenotype during wound healing. As keratinocytes are known for type I IFN production, further investigation is needed to understand how keratinocytes may contribute to macrophage polarization to lead to novel topical therapies.

6. Conclusion

Dysregulation of diabetic macrophages leads to heightened inflammation and poor wound healing in diabetic wounds. Here, we have described the epigenetic changes and regulations by other cell types in the wound microenvironment, leading to these pathologic changes. However, there remains significant questions about the interactions between macrophages and their environment, especially with other immune cells and structural cells. In particular, very little is known about the interplay between keratinocyte cytokine production on macrophage polarization during wound healing, and filling in this gap in knowledge may lead to the development of potential topical therapies. Further studies investigating the therapeutic potential of targeting epigenetic enzymes such as MLL1 or MOF in a local, cell-specific manner are also warranted. Given that the current standard of care leaves 70% of diabetic wounds unhealed [94], it is critical to investigate the therapeutic potential of these recently discovered pathways triggering pathologic inflammation.

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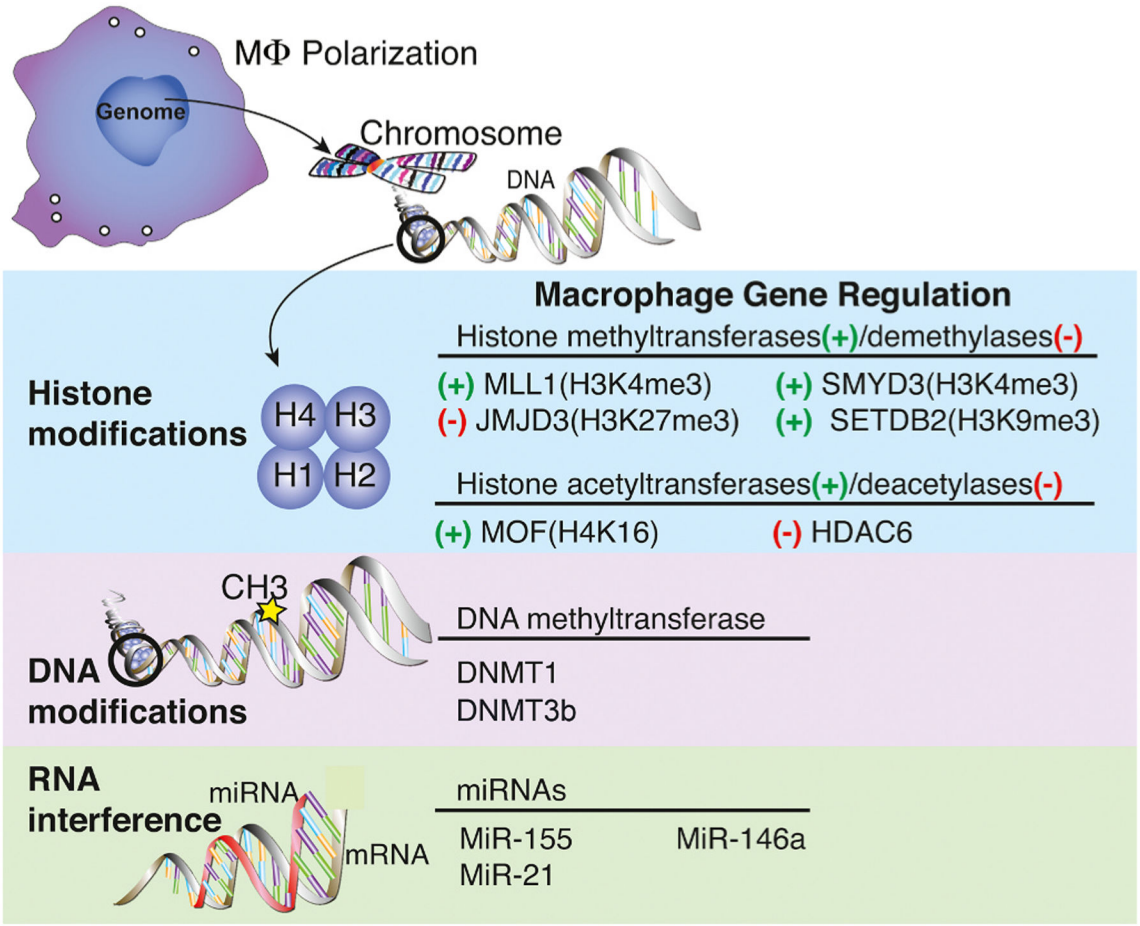

Fig. 1.

Gene regulation enzymes associated with inducing macrophage polarization. Histone modifications, DNA modifications, and microRNAs associated with influencing macrophage phenotype. Histone deacetylase (HDAC); Jumonji domain-containing protein (JMJD); Mixed-lineage leukemia (MLL); SET domain bifurcated (SETDB); SET and MYN domain (SMYD); Males-absent on the first (MOF); DNA methyltransferases (DNMT).

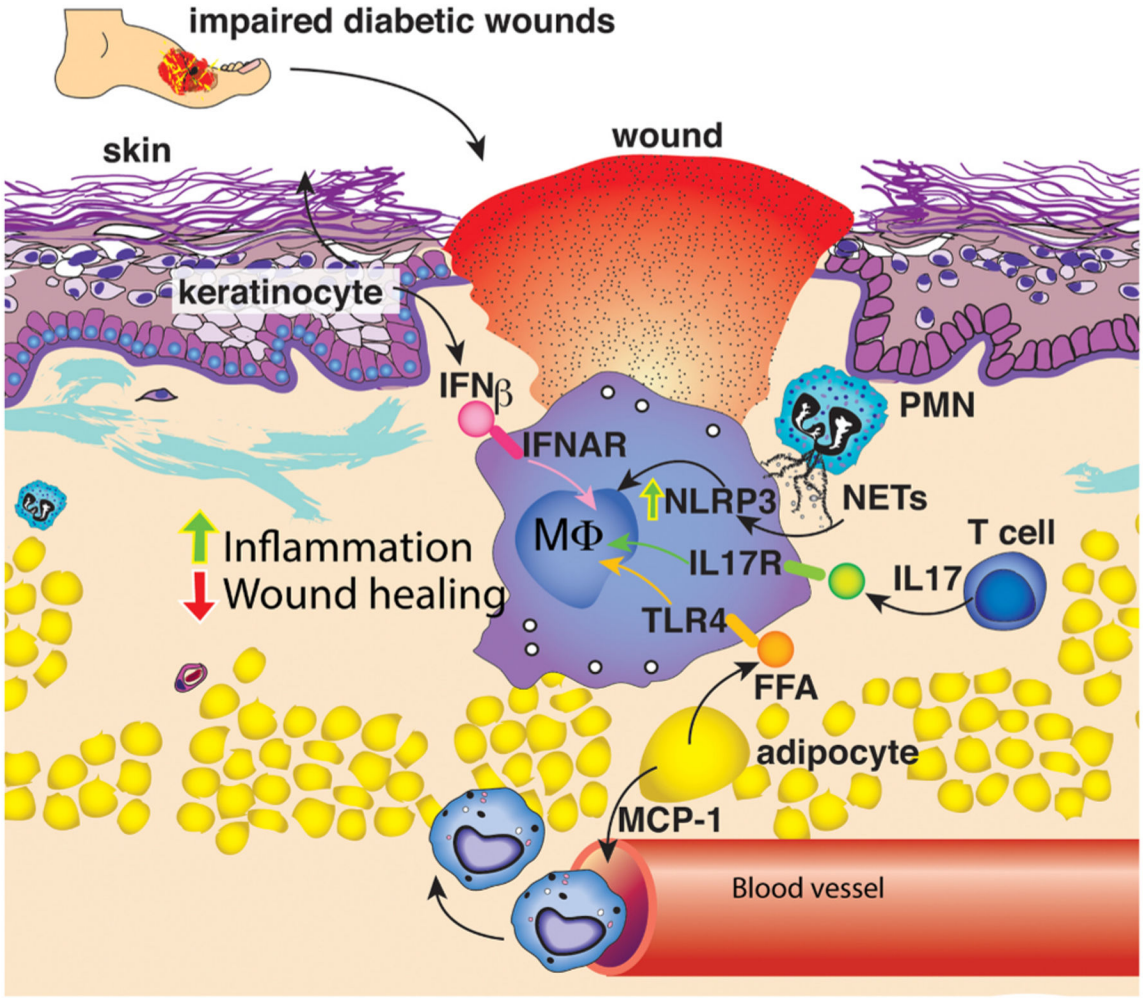

Fig. 2.

Cellular regulation of macrophage phenotype in the wound microenvironment. Following injury infiltrating immune cells and structural cells within the wound microenvironment secrete factors that influence macrophage polarization.

Table 1

Epigenetic enzymes that regulate macrophage polarization

Name	Function	Upstream regulators
Histone methyltransferases		
MLL1	• InducesTLR4 signaling and proinflammatory cytokine expression [42,62,63] • Regulates the COX-2/PGE2 pathway [48]	
SMYD3	• Positively regulates M2 polarization [64] • Induces Alox15 expression [64]	• Upregulated with exposure to M-CSR, IL4 and IL13 [64]
SMYD2	• Suppresses *Tnf, Il6*, MHC-II and CD40/80 expression [95]	• Downregulated with LPS stimulation [95]
SMYD5	• Represses transcription of *Il1α, Il1β*, *Ccl4, Tnf*, and *Cxcl10* [96]	
SETDB2	• Suppresses transcription of inflammatory cytokines *(Il1β, Il6, Il12, Tnfα)* and regulates the expression of xanthine oxidase [14]	• Upregulated with exposure to IFNβ [14]
SETDB1	• Suppresses TLR4 mediated *Il6* expression [97]	
SET7	• Regulates TNFα-induced inflammatory genes [98]	• Upregulated with TNFα stimulation [98]
PRMT1	• Promotes expression of NFκB regulated genes [99]	
Histone demethylases		
JMJD3	• Activates both pro and anti-inflammatory macrophage phenotypes [49,56,65–67]	• Upregulated with exposure to IL4 or LPS [67]
UTX	• Regulates LPS-induced pro-inflammatory cytokine production [66]	
JMJD1A	• Represses Ccl2, *Ccr1* and *Ccr5* expression [100]	• Upregulated with exposure to hypoxic enviroment [100]
Histone acetyltransferases		
MOF	• Increases *Il1β, Il12, Tnfα* expression [69]	• Upregulated following stimulation with TNFα [69]
SIRT1	• Inhibits inflammatory pathways [101]	
SIRT6	• Activates anti-inflammatory macrophage phenotype [102]	• Downregulated with exposure to increased levels of glucose [102]
Histone deacteylases		
HDAC6	• Induces *Il1β* expression and inhibits *Il10* expression [70]	• Upregulated with exposure to increased levels of glucose [70]
HDAC2	• Promotes LPS-mediated expression of *Il12, Tnfα* and *iNOS* [103]	• Upregulated following stimulation with LPS [103]

HDAC3	• Promotes pro-inflammatory macrophage phenotype [104]		
DNA methyltransferases			
DNMT1	• Promotes pro-inflammatory macrophage phenotype [51,72]		
DNMT3t	• Represses PPARγ and promotes pro-inflammatory macrophage phenotype [73]	• Upregulated following exposure to increased levels of saturated fatty acids [73]	