

Research Progress on the Mechanisms of Neutrophil Extracellular Traps in Diabetic Wound Healing

Lin Wu, Zhiyong Yu, Ketao Xiao, Lijin Zhao *

Department of General Surgery, Digestive Disease Hospital, Affiliated Hospital of Zunyi Medical University, Zunyi Guizhou, 563000, China

* Corresponding author: Lijin Zhao

Abstract: Diabetes is a prevalent metabolic disease that poses a significant challenge to public health due to the development of difficult-to-heal skin ulcers in approximately one-quarter of patients. Neutrophil extracellular traps (NETs) are mesh-like structures released by neutrophils, initially believed to play a crucial role in combating microbial infections. However, an increasing body of research suggests that NETs also contribute to the pathogenesis of various diseases, including diabetic wounds. Studies have demonstrated that NETs can trigger excessive inflammatory responses, impede re-epithelialization and angiogenesis, and disrupt normal wound healing processes in individuals with diabetes. This article provides an overview of NETs, their mechanisms of action, and explores potential treatment strategies for diabetic wounds, aiming to offer novel insights for improving diabetic wound management.

Keywords: Diabetic Wounds; Neutrophils Extracellular Traps; Wound Healing.

1. Introduction

Diabetes is a major metabolic disease that affects human health worldwide. It is estimated that by 2045, the number of people with diabetes will exceed 750 million [1]. As diabetes progresses, patients may develop various complications. Diabetic wounds are among the most common complications, with the majority occurring in the feet. In severe cases, they can lead to amputation or even death². Due to prolonged hyperglycemic conditions, the skin of diabetic patients is more susceptible to damage and infection, resulting in chronic inflammation, excessive oxidative stress, and impaired angiogenesis at the wound site. These factors contribute to delayed wound healing and recurrent ulceration, posing significant challenges to treatment [2,3]. Frequent hospitalizations and multiple surgeries severely impact the quality of life of patients and impose a significant economic burden on society.

Diabetic patients face significant health challenges due to the difficulty in wound healing, a problem that has become a global public health concern. Recent studies have identified a critical role for Neutrophil Extracellular Traps (NETs) in the pathogenesis of diabetic wounds. NETs represent a distinct form of cell death, in which DNA webs and proteins are released, contributing to the regulation of inflammation and immune responses [4]. However, during the process of diabetic wound healing, NETs may become excessively activated, accumulate abnormally, or exhibit an imbalance, resulting in persistent inflammation and hindering the healing process². Therefore, understanding the precise role of NETs in diabetic wound healing and exploring related therapeutic strategies is of great significance. This review aims to discuss the current research progress on the mechanisms underlying the role of NETs in diabetic wounds, with the goal of providing new insights and directions for addressing the challenges in diabetic wound treatment.

2. Overview of NETs

Neutrophil Extracellular Traps (NETs) are a unique form of

cell death, first discovered by Brinkmann et al. in 2004. Unlike traditional forms of cell death, such as apoptosis and necrosis, NETs represent a distinct form of cell death. They are formed by neutrophils in response to infection or stimulation, resulting in the release of a fibrous DNA network structure that carries various proteins, including myeloperoxidase, citrullinated histone H3, neutrophil elastase, and free double-stranded DNA [4]. The primary function of NETs is to participate in immune defense by capturing and killing pathogens⁴. However, overactivation or dysfunction of NETs has been observed in certain disease states, where they may contribute to tissue damage and inflammatory responses [5].

The formation of NETs involves four steps: activation, adhesion, membrane rupture, and release [6]. First, neutrophils are activated, typically by stimuli such as bacteria, viruses, or inflammatory mediators. Next, activated neutrophils adhere to endothelial cells and release cytokines and histamine proteins, which enhance NET formation by increasing vascular permeability and activating platelets. During the adhesion process, neutrophils undergo apoptosis and release nuclear chromatin. Finally, the cell membrane ruptures, releasing DNA and proteins to form a net-like structure.

There are two pathways for NETs formation [4-7]: (1) NADPH oxidase-dependent pathway: The intracellular NADPH oxidase is the key component in this pathway. Activated neutrophils produce large amounts of reactive oxygen species (ROS), such as superoxide anions, via NADPH oxidase, which induces nuclear chromatin formation. This process leads to the rupture of the nuclear membrane and the release of chromatin, which then interacts with cytoplasmic proteins to form NETs. (2) NADPH oxidase-independent pathway: This pathway is independent of NADPH oxidase activation and occurs when the neutrophil membrane remains intact. In this case, chromatin is expelled from the cell through vesicular budding, resulting in an enucleated neutrophil with functions such as chemotaxis and phagocytosis.

3. The Mechanisms of NETs in Diabetic Wound Healing

NETs release is strictly regulated by glucose levels and is, to some extent, dependent on glycolysis [8]. Diabetic patients, who are chronically in a hyperglycemic state, exhibit an enhanced response of neutrophils to stimuli, which subsequently triggers the abnormal release of NETs. NETs, to some extent, possess antimicrobial properties, as they can capture and kill pathogens, thereby controlling infections in diabetic wounds [9]. However, the DNA and proteins released from NETs can exacerbate the inflammatory response in diabetic wounds, hindering normal wound repair and healing. Furthermore, excessive NET release may sustain inflammation, triggering a vicious inflammatory cycle and leading to chronic wound formation, which impedes reepithelialization and angiogenesis [10, 11]. Studies have reported elevated levels of NET markers, such as CitH3, PAD4, and the neutrophil marker MPO, in the wound sites of diabetic patients, with significant increases compared to non-diabetic skin [12,13]. Additionally, higher levels of serum double-stranded DNA and increased neutrophil activity have been observed in the blood of diabetic patients compared to non-diabetic individuals, suggesting that neutrophils in diabetic patients are more likely to undergo NETosis, thereby generating more NETs [13].

3.1. Excessive Inflammatory Response

In normal wound healing, the inflammatory response plays a crucial role in protecting and promoting tissue repair [14]. However, excessive or prolonged inflammation can lead to tissue damage and chronic inflammation [15]. Evidence suggests that the activation of the NOD-like receptor protein 3 (NLRP3) inflammasome in macrophages contributes to sustained inflammation, which is associated with impaired wound healing in diabetes [16]. Liu et al. [17] reported that excessive NETs in diabetic wounds activate the NLRP3 inflammasome and trigger IL-1 β release in macrophages through the TLR-4/TLR-9/NF- κ B signaling pathway. The use of deoxyribonuclease I (DNase I) to clear NETs resulted in reduced NLRP3 inflammasome levels, decreased macrophage infiltration, and accelerated wound healing in a diabetic rat model.

MFG-E8 (milk fat globule epidermal growth factor VIII) plays a crucial role in the resolution of inflammation, angiogenesis, and wound healing. Studies have shown that MFG-E8 attenuates NET formation and NET-induced NLRP3 activation, while also reducing the release of IL-8 and IL-18, thereby modulating the activity of the NLRP3 inflammasome-NET axis [17]. Furthermore, research has revealed that diabetic mice lacking MFG-E8 exhibit increased neutrophil infiltration, higher NET abundance, impaired angiogenesis, and delayed wound closure, suggesting that MFG-E8 plays a regulatory role in the increased formation of NETs in diabetic wounds [17]. Additionally, Yang et al. [18] demonstrated through *in vitro* experiments and a diabetic wound healing mouse model that the activation of the NLRP3/caspase-1/GSDMD pathway promotes the generation of more NETs by neutrophils in diabetic wounds.

Leucine-rich α -2-glycoprotein 1 (LRG1) is a leucine-enriched blood protein that plays a significant role in various physiological and pathological processes. LRG1 influences biological processes such as cell proliferation, migration, and differentiation by modulating the inflammatory response and

immune cell functions [19]. According to Liu et al. [19], LRG1 significantly increases during the inflammatory phase of wound healing in non-diabetic mice, and its deficiency leads to immune cell infiltration and exacerbated inflammation. However, further research has revealed that LRG1 is highly induced in both diabetic mice and diabetic patients. When LRG1 is absent, diabetic wound healing is enhanced, possibly because LRG1 mediates NETosis through the TGF β type I receptor kinase ALK5 in an Akt-dependent manner, thereby promoting NET formation. This finding suggests that LRG1 may play a unique regulatory role in diabetic wound healing, providing valuable insights into the regulatory mechanisms of diabetic wound healing.

3.2. Angiogenesis and Vascular Dysfunction

During wound healing, the formation of new blood vessels provides oxygen and nutrients to the cells, promoting tissue repair [20]. However, in diabetic wound tissue, the degree of neutrophil infiltration is negatively correlated with the expression levels of CD31, VEGF, and VEGF receptor 1, suggesting that neutrophil infiltration may inhibit angiogenesis and impair the wound healing process [12]. Under diabetic conditions, the overactivation of protein kinase C (PKC) β II is associated with angiogenesis and NET formation by neutrophils. Studies have shown that in diabetic mice, treatment with a specific PKC β inhibitor reduces NET formation in the wound, increases capillary density and endothelial progenitor cell (EPC) numbers, and accelerates wound healing [21]. Additionally, during diabetic wound healing, the gonadotropin-releasing hormone (GnRH) receptor expressed on neutrophils plays a role, and the enhancement of GnRH leads to neutrophils undergoing phorbol myristate acetate-induced NET formation, thereby impairing wound healing [22].

Endothelial cells regulate the tension of vascular smooth muscle by producing nitric oxide, and a reduction in nitric oxide levels is a hallmark of preclinical vascular dysfunction, which may manifest before clinically significant vascular diseases [20]. Liu et al. [23] reported that compared to normal Akita mice, neutrophil elastase and PAD4 knockout mice exhibited reduced circulating NETs and improved vascular function. Neutrophil elastase inhibitors have the potential to reverse pre-existing diabetic vascular dysfunction, and cell transfer experiments suggest that neutrophils are at least one of the driving factors of vascular dysfunction. NETs have been shown to play a significant role in the vascular dysfunction characteristics of several diseases. For example, NETs promote the development of hypertension by inducing vascular smooth muscle cell phenotype changes [24] and impair normal vascular remodeling during stroke recovery [25].

3.3. Inhibition of Reepithelialization

During skin wound healing, fibroblasts produce a temporary extracellular matrix (ECM) to promote granulation tissue formation, while keratinocytes are responsible for migration, proliferation, and differentiation to remodel functional epidermis, thus driving the closure and recovery of the vascular network, protecting tissue from further damage [26].

NETs have a complex impact on cell migration and proliferation. NETs contain several chemotactic cytokines, such as interleukin-8 (IL-8), which can attract other immune cells to specific areas. This is important for immune defense

and inflammation, as it facilitates the recruitment of immune cells to infection or injury sites for repair and defense [2]. However, excessive NET release can lead to excessive cell infiltration and inflammation, thereby hindering normal cell migration and wound repair. Wong et al. [11] found that PAD4 expression was significantly increased in neutrophils of diabetic patients, with an associated increase in NET release. Compared to wild-type mice, PAD4 knockout mice showed accelerated wound healing, unaffected by hyperglycemia. Additionally, there were no significant differences in the Ki67 cell proliferation index and TUNEL apoptosis index in diabetic mouse wounds, suggesting that the mechanism by which NETs affect wound healing might be due to the inhibition of keratinocyte migration, thus impairing reepithelialization [11].

NETs release may also influence cell proliferation. The DNA and certain protein molecules in NETs can interact with signaling pathways related to cell growth and proliferation, directly or indirectly affecting cellular proliferative capacity. One study showed that low concentrations of NETs induce human keratinocyte proliferation through NF- κ B activation [27], while diabetic wounds often exhibit higher concentrations of NETs, which may impair cellular proliferation. Under normal glucose conditions, LRG1 promotes keratinocyte proliferation and migration [19]. Interestingly, Liu et al. [19] found that LRG1 is highly expressed in diabetic wounds and promotes NET release through the TGF β /ALK5/Akt-dependent pathway, thereby impairing wound healing. Therefore, targeting LRG1 in diabetic wounds could suppress excessive NET formation and promote keratinocyte proliferation and migration, accelerating wound healing.

However, in contrast to the aforementioned view, Yang et al. [28] found that, compared to normal rats, the peak expression of LRG1 around diabetic rat wounds was delayed. Exogenous administration of LRG1 significantly accelerated wound closure and promoted angiogenesis in diabetic rats. Mechanistically, LRG1 primarily promotes angiogenesis and diabetic wound healing by activating the Wnt/ β -catenin pathway, which is suppressed in diabetic wounds. Thus, the mechanisms of LRG1 in diabetic wounds may require further investigation to clarify these differing viewpoints.

4. Strategies and Prospects of NETs in Diabetic Wound Healing

Diabetic patients often face difficulties with chronic wound healing, and the role of NETs in diabetic wound treatment has attracted significant attention. Currently, strategies to promote diabetic wound healing involve inhibiting NET formation and release, clearing existing NETs, and regulating the inflammatory response. The most common pharmacological approach targeting NETs is the use of DNase I, which can dismantle the structural framework of NETs. Recombinant human DNase I is cost-effective and has no known side effects [29]. Studies have shown that systemic administration of DNase I enhances reepithelialization and accelerates wound healing in diabetic mice. However, DNase I has little effect on histones, elastase, or other components associated with NETs, which are released into the bloodstream and may lead to proteolysis, resulting in tissue damage [30]. Furthermore, Perrault et al. [31] created two 6-mm full-thickness wounds on the backs of diabetic mice and treated them with subcutaneous injections of interleukin-1 receptor

antagonists. This treatment downregulated MPO protein expression in neutrophils, reduced neutrophil infiltration, and accelerated wound healing. Additionally, Yang et al. [32] found that local insulin application normalized the function of impaired neutrophils in diabetic wounds, inducing macrophages to shift their polarization from the M1 to M2 phenotype, thereby promoting wound healing.

Meanwhile, research in materials science has also driven the advancement of diabetic wound treatment. Local hydrogel dressings can increase the number of neutrophils in diabetic wounds while reducing abnormal neutrophil infiltration, thereby mitigating the inflammatory response [33]. For example, Tu et al. [33] designed a multifunctional hydrogel for use in a diabetic rat wound model, which significantly reduced ROS levels and alleviated inflammation, accompanied by decreased neutrophil infiltration and enhanced M2 macrophage polarization *in vivo*. In terms of cell therapy, Zhang Chunlin et al. [34] found that human amniotic epithelial cells may promote diabetic wound healing by inhibiting NET formation. Recently, acellular therapies, such as extracellular vesicles, have gained attention in wound repair. Chu et al. [35] reported that excessive NETs are present in diabetic wounds, and high concentrations of NETs impair fibroblast function by activating endoplasmic reticulum stress. However, extracellular vesicles derived from mesenchymal stem cells preconditioned in hypoxia contain miR-17-5p, which targets the TLR4/ROS/MAPK pathway to block NET formation, thus accelerating wound healing in diabetic mice.

Currently, strategies targeting NETs in diabetic wound treatment are still in the research and exploration stages. With a deeper understanding of the mechanisms underlying NETs and continuous optimization of therapeutic approaches, it is expected that more innovative treatments will emerge in the future, along with related clinical studies, providing more effective solutions for wound healing issues in diabetic patients.

5. Conclusion

Diabetic wounds are one of the common complications of diabetes, and their pathogenesis is complex. Studies have found that NETs contribute to excessive inflammation, inhibit reepithelialization, impair angiogenesis, and cause functional abnormalities, thereby delaying wound healing. Current research on NETs in treatment primarily focuses on inhibiting NET formation and release, clearing existing NETs, and modulating the inflammatory response. Although this area is still in the research and exploration phase, a deeper understanding of the mechanisms underlying NETs, along with continuous optimization of therapeutic approaches, is expected to provide better treatment options and improve the quality of life for diabetic patients.

Conflict of Interest

The authors declare no conflict of interest.

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