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Molecular immunological mechanisms of impaired wound healing in diabetic foot ulcers (DFU), current therapeutic strategies and future directions

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ABSTRACT

Diabetic foot ulcer (DFU) is a foremost cause of amputation in diabetic patients. Consequences of DFU include infections, decline in limb function, hospitalization, amputation, and in severe cases, death. Immune cells including macrophages, regulatory T cells, fibroblasts and other damage repair cells work in sync for effective healing and in establishment of a healthy skin barrier post-injury. Immune dysregulation during the healing of wounds can result in wound chronicity. Hyperglycemic conditions in diabetic patients influence the pathophysiology of wounds by disrupting the immune system as well as promoting neuropathy and ischemic conditions, making them difficult to heal. Chronic wound microenvironment is characterized by increased expression of matrix metalloproteinases, reactive oxygen species as well as pro-inflammatory cytokines, resulting in persistent inflammation and delayed healing. Novel treatment modalities including growth factor therapies, nano formulations, microRNA based treatments and skin grafting approaches have significantly augmented treatment efficiency, demonstrating creditable efficacy in clinical practices. Advancements in local treatments as well as invasive methodologies, for instance formulated wound dressings, stem cell applications and immunomodulatory therapies have been successful in targeting the complex pathophysiology of chronic wounds. This review focuses on elucidating the intricacies of emerging physical and non-physical therapeutic interventions, delving into the realm of advanced wound care and comprehensively summarizing efficacy of evidence-based therapies for DFU currently available.

1. Introduction

Skin wounds can be classified into acute and chronic, depending upon their pathogenesis and healing duration. Chronic wounds (CWs) are generally referred to as the ones that do not follow a sequential order of wound healing phases and usually take more than three months to heal and produce functional and anatomic integrity [1]. Chronic wound management still appears to be a significant issue and continues to be a clinical, economic, and social challenge. CWs affect almost 2.21 per 1,000 individuals and have deeper impacts on their quality of life along with increased chances of infection [2]. Chronic wounds in individuals having diabetes or vascular diseases can cause prolonged pressure, arterial diseases, severe venous insufficiency, and neuropathy followed by amputation [3]. Diabetic foot ulcer commonly referred to as DFU located on the lower extremity of the foot occurs as an open wound experienced by approximately 15 to 25 percent of people suffering from diabetes. DFU has a global incidence rate of 9.1 to 26.1 million per annum [4]. As the rate of diabetic diagnosis increases in the coming years, DFU incidence is also expected to rise accordingly. Diabetic foot ulcers can have multifactorial causes. The most common etiologies of DFU include improper foot care, poor circulation, foot deformities, dry skin, poor glycemic control, underlying neuropathies, and calluses.

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Diabetic foot ulcers usually develop in three stages. The first stage is callus development. Neuropathy resulting in prolonged pressures and compromised vascular efficacy is generally considered an underlying cause of callus. As it progresses, patients may develop other risk factors for ulceration as well, including reduced angiogenesis as well as impaired healing and inflammation. Lastly, frequent callus trauma causes subcutaneous hemorrhage leading to ulcer formation [5]. Microbial population invades the site of the ulcer, making their control strenuous, ultimately leading to severe infections [6]. Wagner classification system has divided DFUs into five different grades, depending upon condition severity. Grade 0 involves no open lesions. Grade 1 involves superficial skin or subcutaneous tissue ulcer with partial or full thickness. Grade 2 includes ulcer extending deep involving fat, tendons and ligament with no bone involvement or abscess, Grade 3 ulcers have deep abscess with significant bone involvement with or without joint sepsis. Grade 4 diabetic ulcers involve partial localized gangrenes and Grade 5 ulcers are determined by extensive whole foot gangrenes [7]. Antibiotic misuse during false diagnosis of ulcer infections lead to widely spread antimicrobial resistance, causing dramatically increased pathogen infiltration at wound site, influencing its healing. Poor blood circulation, causing inefficient blood transport to the wound site often adds to delayed healing, necrosis, and gangrene, followed by amputations. Almost 3 percent of the total healthcare budget is spent on chronic wound management in developed countries which causes a huge financial burden [8]. Various treatment strategies including combination therapies have been designed to target mechanisms of inefficient healing in diabetic wounds including direct immune cell applications, platelet therapy, stem cell approaches, nanotherapeutic applications as well as physical methodologies aiding the process of healing.

2. Molecular immunology of wound healing

Body's immune system is actively involved in response to an injury. Some of the major immune cells involved in the wound healing process are platelets, macrophages, neutrophils, and lymphocytes (natural killer cells, B and T cells,). Wound healing takes place in four distinct phases; hemostasis, pro-inflammatory phase, anti-inflammatory also known as proliferative phase, followed by remodeling phase [9,10].

2.1. Phases of wound healing

2.1.1. Hemostasis phase

Vasoconstriction, platelet aggregation, and formation of thrombus are the main events of initial hemostasis phase. Following injury, a cascade of events occurs to prevent blood loss which involves the activation of precursor enzymes called zymogens into completely functional proteases leading to activation of platelets and fibrin clot formation [11]. Activated platelets are accumulated on subendothelial collagen, releasing various growth factors and cytokines causing hemostatic plug formation. This serves as a matrix for the migration of thrombospondins, vitronectin, and fibronectin as well as provides signaling for locating fibroblasts, immune cells, and keratinocytes [12]. Activation of platelets is followed by the release of platelet-derived growth factor (PDGF) as well as immune regulators, instigating the immune system. Degranulation of platelets also results in the release of inflammatory cytokines including tumor necrosis factor (TNF-α), CXCL8, IL-6, IL-8, and IL-1, causing complement system's activation. Complement cascade releases histamine, which dilates capillaries allowing inflammatory cells to migrate to the wound, shifting healing to the inflammatory phase [13,14]. Fig. 1 illustrates the initial response mechanisms activated during hemostasis phase after an exposure to injury.

2.1.2. Inflammatory phase

After hemostasis comes the inflammatory phase. In this particular phase, phagocytosis, neutrophil and lymphocyte infiltration and macrophage activation takes place. It involves the release of molecular signals causing monocyte and neutrophil infiltration. M1 (pro-inflammatory macrophages) are the most prominent cells during this phase. Cells undergoing necrosis release damage associated molecular patterns (DAMPs), recruiting neutrophils to wound sites by allowing tissue-resident cells to release neutrophil chemoattractants. These signals also activate neutrophils by binding to their surface receptors [15,16]. Keratinocytes present at the site of wound release certain factors such as CXCR, causing neutrophil arrival at the wound site. Neutrophils play a major role in preventing infection by removing debris [17]. Pathogen associated molecular patterns (PAMPs) help deal with pathogens at wound sites [18]. Several cell surface receptors are present on neutrophils that mitigate their activation. The neutrophil requirement is important in chronic wounds where there are increased chances of infection [19,20]. CXCL8 is the most common neutrophil attractant

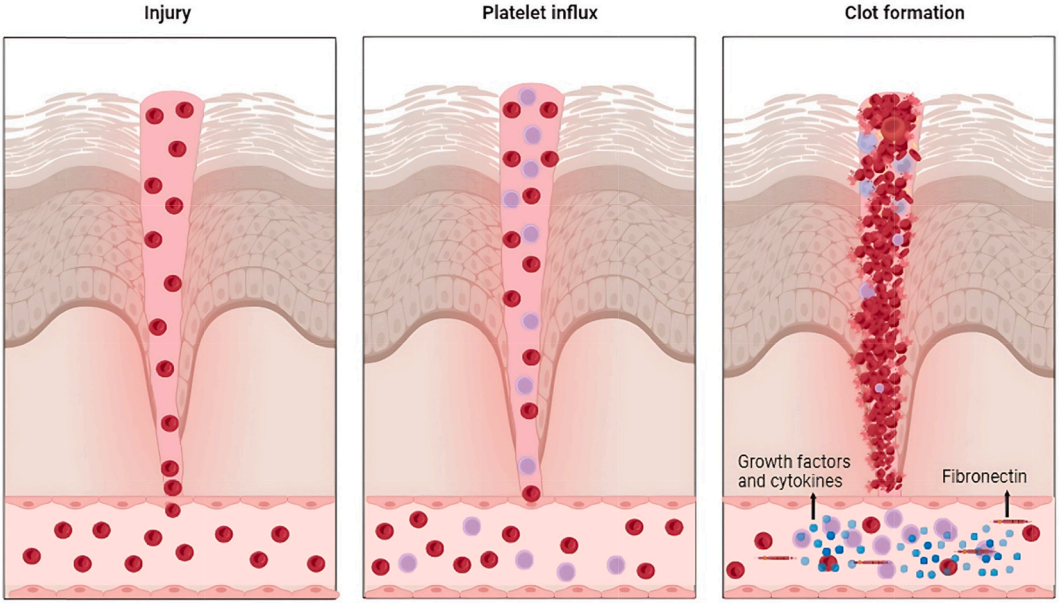

Fig. 1. Hemostatic phase of wound healing involves the activation of platelets causing clot formation to hinder blood loss post injury.

released by fibroblasts and tissue-resident cells, recruiting neutrophils to the tissue injury site [21]. Neutrophils also start secreting CXCL8 after their arrival at the injury site to create a pro-inflammatory environment by increasing endothelial permeability to facilitate the influx of inflammatory cells [22]. Although neutrophils are not the most significant cells of wound healing, they do perform certain functions to aid the healing process. For example releasing specific proteases, reactive oxygen species (ROS), and certain antimicrobial peptides for killing microbes invading the injury site. Neutrophils are also responsible for the creation of neutrophil extracellular traps (NETs) involved in controlling the population of pathogens either by immobilizing them for phagocytosis or by killing them directly [23,24].

Monocytes arrive at the wound site after the release of extracellular matrix (ECM) fragmenting enzymes by creating a path for monocyte migration. Monocyte differentiation results in the formation of macrophages in response to the wound microenvironment. They increase during the inflammatory and tissue granulation phase and decline during the remodeling phase [25]. Anaphylatoxins including C3a and C3b released by the complement system cause further infiltration of neutrophils, mast cells, and macrophages [26]. Two types of monocytes participate in the healing process, tissue resident monocytes and the ones recruited from blood. Blood monocytes are further divided into two classes i.e. pro-inflammatory and anti-inflammatory monocytes. $CD14^{+}$, and $CD16^{-}$ are referred to as pro-inflammatory and give rise to M1 macrophages thus enhancing inflammatory responses. On the other hand, $CD16^{+}$, and $CD14^{-}$ differentiate into anti-inflammatory M2 macrophages involved in the proliferative healing phase [27]. M1 phenotype releases many pro-inflammatory cytokines including TNF-α, IL-1, IL6, IL12, and IL-23 causing enhanced response of natural killer (NK) cells and T cells. Moreover, M1 macrophage signaling causes the recruitment of lymphocytes to the damaged site as well as plays a role in clearing cellular debris [25,28,29]. Fig. 2 summarizes the molecular events occuring in a specific order during an inflammatory healing phase.

2.1.3. Anti-inflammatory or proliferative phase

The proliferative phase starts once the inflammation has been resolved. One of the significant events of proliferative phases is the M1 to M2 phenotype shift. Inflammatory regulators that cause this phenotype transition include prostaglandins, glucocorticoids, specific TLRs (toll-like receptors), IL4, IL-10 and IL-13 pathways [30]. Fibroplasia, angiogenesis, matrix deposition, and re-epithelialization occur in this stage of wound healing. Adenosine signaling, regulator T cells, and noncoding microRNAs (miRNAs) also play a critical role in the M1-M2 phenotype shift [31,32]. M2 macrophages initiate the tissue repair process by reducing inflammation through the generation of anti-inflammatory signals such as IL-10 and receptor antagonist of IL-1 as well as by inducing fibroblast proliferation, ECM synthesis, angiogenesis promoting growth factors including transforming growth factor (TGF) and vascular endothelial growth factor (VEGF) [33]. Resolving inflammation through a pro-inflammatory to anti-inflammatory environment shift is necessary for an effective wound repairing process [34]. Keratinocytes present at the wound boundary start accumulating in the center a few hours post-injury. After 2 to 3 days of injury, proliferation of epithelial stem cells from the roots of hair follicles and epidermis' basal layer takes place [35,36]. Re-epithelialization and angiogenesis occurs after the release of chemical signals by immune cells including pro-repair, anti-inflammatory macrophages. Blood vessel formation plays a huge part in the proliferative phase of wound repair as it allows the transport of chemical signals as well as nutrients and oxygen through the blood, required at the healing site. Angiogenesis occurs in two steps; vessel sprouting and then vessel network formation in which they join together also known as anastomosis [37]. Proliferative macrophages promote angiogenesis by releasing growth factors and also by expressing cell membrane proteins involved in the initiation of vessel sprouting and anastomosis. Experimental models deficient in macrophages exhibit impaired or inefficient angiogenesis which dictates their importance in the wound repair process [37,38]. Angiogenesis is coupled with granulation tissue formation, comprising fibroblasts and collagen. Fibroblasts are the key cells in the re-epithelialization and tissue granulation

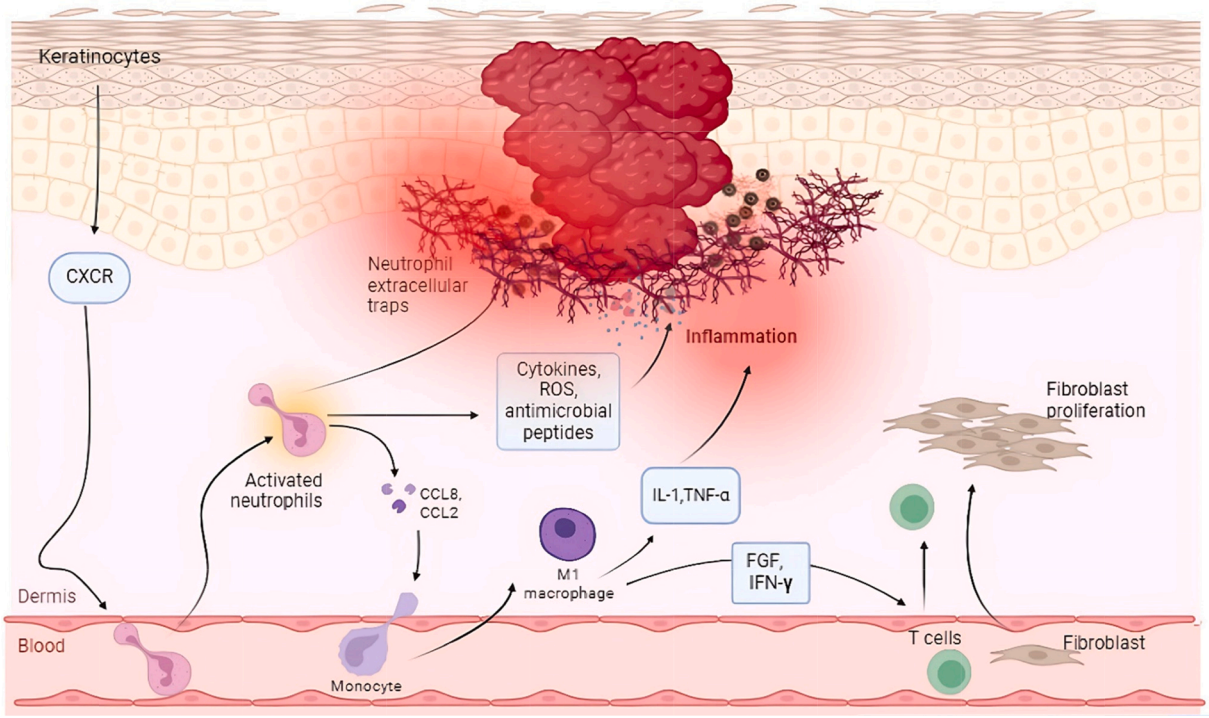

Fig. 2. Keratinocytes at wound site release neutrophil attracting factors, inhabiting neutrophils which release NETs and other antimicrobial peptides as well as cytokines. It also causes the arrival of monocytes which differentiate into M1 macrophages; releasing pro-inflammatory factors causing influx of fibroblasts, T cells and keratinocytes.

process. Pro-repair macrophages release certain factors that influence the activity of fibroblasts for example TNF-α, IL-1, IL-6, PDGF-β, and TGF-β [39,40]. M2 macrophages, keratinocytes, and platelets also produce keratinocyte growth factor (KGF), epidermal growth factor (EGF), and transforming growth factor (TGF), required for re-epithelialization. Keratinocytes also activate fibroblasts by releasing tenascin C and fibronectin [41,42]. Fig. 3 comprehends the immune cell signaling associated with attenuated inflammatory response and induced proliferation in wound microenvironment.

2.1.4. Remodeling phase

Wound remodeling starts a few weeks post-injury and can continue up to several months depending upon the severity of damage. It involves ECM remodeling, increased tensile strength, epithelialization, and granulation tissue is replaced with scar formation. Collagen type III is also substituted by stouter and tougher collagen type I [43]. Fibroblasts transition into myofibroblasts and are accountable for the physical strength and shrinkage of wounds when exposed to TGF-β [44]. Smooth muscle actin (SMA) expressed by myofibroblasts generates this mechanical tensile strength for wound closure [45]. In addition, myofibroblasts release matrix metalloproteinases (MMPs) that degrade collagen during the formation of granulation tissue, thus aiding in remodelling [46–48]. After completion of wound healing, myofibroblasts undergo apoptosis, and scar formation is observed. Collagen, ECM, and other proteins become nicely organized, and a balance between degradation and synthesis is maintained in the remodeling phase. Vascularity level decreases and scar color changes as time passes [49,50]. The same level of tissue strength is never obtained after healing as it was before injury. Fig. 4 visually represents the molecular events occuring during the remodeling phase of wound healing.

3. Impaired healing in chronic wounds

When wounds fail to experience sequential order of healing phases, get stuck in a single one and are not progressed to the next phase, they become difficult to resolve. Impaired wound healing can be because of many factors including repeated infection, reduced re-epithelialization, defective angiogenesis, tissue necrosis, ROS overproduction, exudation, and pro-inflammatory environment. Generally, three different categories of chronic wounds have been observed which include vascular ulcers, diabetic foot ulcers, and pressure ulcers. Elderly people facing health conditions like diabetes, obesity, and vascular diseases are more prone to suffering from these ulcers [51,52].

DFU being the most severe complication of diabetes occurs as a deformity in lower extremities of diabetic patients. Various pathological alterations associated with diabetes contribute to worse healing outcomes. Chronic hyperglycemic conditions severely affect vasculature, thus inhibiting systemic blood perfusions. Hyperglycemia causes accumulation of glycation end products that compromise the functioning of fibroblasts and keratinocytes [53]. Moreover, high blood sugar levels induce oxidative stress. Increased ROS can disrupt blood circulation as well as structure and function of peripheral nerves, causing neuropathy [54]. The existence of diabetic neuropathy and peripheral vascular disease also reduces the likelihood of early detection of wounds in diabetic patients. Diabetic patients are more prone to infections, which obstructs smooth wound healing and recovery [55]. Poor immune response, hindered angiogenesis, increased inflammation and altered migration as well as proliferation of cells involved in wound repair and remodeling; all act as contributing factors to delayed healing in DFUs. However, immune cell dysfunctioning plays a major role in DFU pathogenesis [56].

3.1. Immunological factors associated with diabetic foot ulcers

The complete pathophysiology of chronic wounds still remains undiscovered but generally impaired healing in DFUs is characterized by complications of the immune system, ischemia, infections, underlying neuropathies and peripheral vascular disorders. Abnormal production of growth factors, collagen accumulation, increased foot pressure, reduced angiogenesis and prolonged inflammatory responses collectively add to

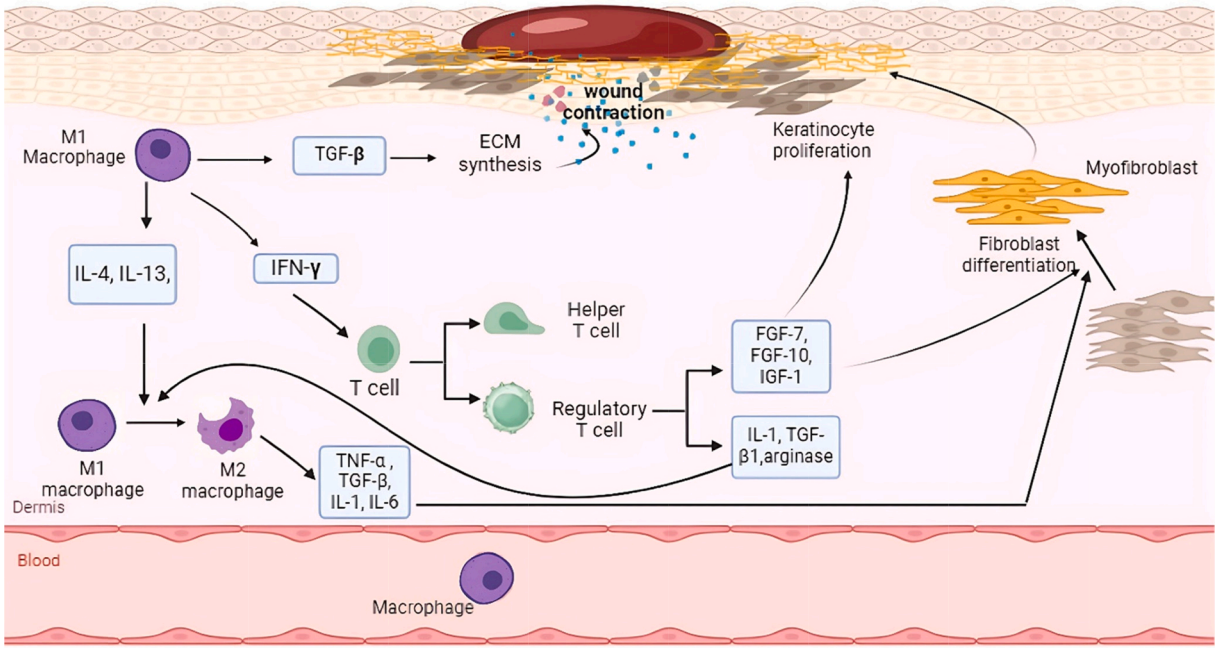

Fig. 3. M1 macrophages release certain growth factors and cytokines responsible for generating a number of mechanisms involved in proliferation and differentiation of cells. Release of IFN-γ results in T cell differentiation into helper T cells and regulatory T cells. Later is involved in releasing anti-inflammatory interleukins and growth factors for fibroblast differentiation and keratinocyte proliferation. M1 also releases interleukins to generate a macrophage phenotype shift from M1 (pro-inflammatory macrophages) to M2 (anti-inflammatory macrophages), thus resolving inflammation through myofibroblast proliferation, resulting in ECM synthesis and wound size reduction.

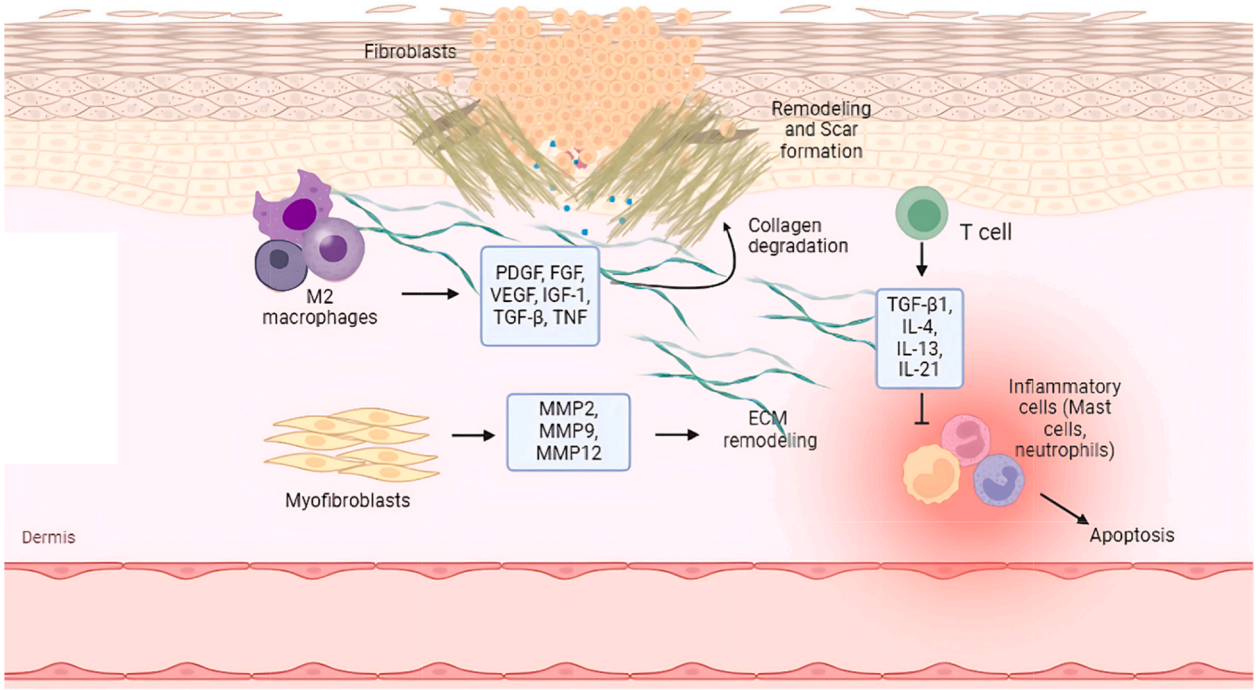

Fig. 4. Matrix metalloproteinases released by myofibroblasts result in balanced synthesis and degradation of collagen at wound site. M2 also releases certain chemokines and growth factors including VEGF, TNF-α, PDGF, FGF and IGF-1, influencing mesenchymal myofibroblasts to undergo ECM remodeling. T cells release TGF-β1 and various interleukins i.e. IL-4, IL-13, 1L-5 and IL-21 for fibrogenesis and inhibition of inflammatory cells, which either leave the wound site or undergo apoptosis.

disease severity.

3.1.1. Role of macrophages in development of DFU chronicity

The main factor for non-resolving chronic wounds in diabetic patients is the persistent and increased M1 macrophage phenotype polarization [57]. M1 to M2 phenotype shift is generally believed to determine the healing fate of diabetic wounds. However, on the contrary, recent evidence suggests no significant association between number of M2 macrophages and healing strength of wound in diabetic rat models [58]. Similarly, a recent single cell profiling study has shown that there is an increased polarization of M1 macrophages in healing ulcers and an abundance of M2 macrophages in non-healing ones. Transcriptomic based study revealed a number of genes differentially expressed in M1 macrophages of healing group [59]. Mast cells also play a part in reduced wound healing by maintaining a pro-inflammatory environment and are also involved in decreased contraction of wounds. They are generally present during the early stages of injury but their persistence for longer periods is a factor in diabetic ulcers [60]. Experimental models have shown that there is a decrease in IL-36 receptor antagonists because of overproduction of CXCL1, TGF-β, and IL-36γ leading to excessive infiltration of macrophages as well as neutrophils in non-healing chronic wounds [61]. Depletion of another chemokine receptor CCR4 involved in the release of pro-inflammatory cytokines i.e. TNF-α, IL-1, IL-6, and IL-12 in diabetic mice negatively affects wound healing [62]. Matrix metalloproteinases also add to the pathogenesis of chronic wounds. MMPs are released by immune cells as well as keratinocytes and fibroblasts in small quantities in response to growth mediators and cytokines including interferons, interleukins, EGF, and VEGF [63]. They are responsible for cellular proliferation and epithelialization. Their increased expression can result in delayed healing because of inefficient epithelialization at the injury site. In diabetic patients, increased glucose levels activate the ERK/AP1 pathway, resulting in increased MMP expression [64–66].

3.1.2. T cell involvement in pathogenesis of chronic wounds

T cells are also involved in maintaining the proinflammatory status of impaired healing wounds. CXCR3 receptor's ligand is overexpressed in helper T cells in chronic wounds. Moreover, diabetic patients have higher levels of circulating proinflammatory T cells, thus contributing to retarded healing in DFUs [67]. Effector T cells are accumulated and naïve T cells are significantly reduced in diabetic foot ulcers, which also add to the severity of non-healing chronic wounds [68]. CCR4 is a chemokine receptor involved in the migration of Treg cells. CCR4 is overexpressed in diabetic foot ulcers in mouse models leading to the increased population of regulatory T cells, thus causing delayed healing of wounds [62]. In general, T cells are involved in accelerating pro-inflammatory responses to attenuate repair in chronic diabetic wounds.

3.1.3. Neutrophils' role in determining wound chronicity

Neutrophils are required during the initial phase of wound healing and after fulfilling their desired function, they systematically undergo apoptosis and make way for other injury-responsive cells for example macrophages. If this ordered manner becomes faulty at any point, it can lead to neutrophil infiltration for longer and disrupt the healing process. Prolonged neutrophil presence can cause the release of ECM-degrading proteases in diabetic foot ulcers. Induced depletion of neutrophils decreases the levels of pro-inflammatory cytokines and curtailed infiltration of $CD4^+$ T cells and M1 macrophages was observed, leading to reduced inflammation [69]. Neutrophils also release NET traps to kill or immobilize pathogens at the wound site to control infection. NET-specific biomarkers are present in large amounts in non-healing diabetic ulcers as compared to acute wounds. NETs that fail to resolve over time are proved to be contributing to extended healing in chronic wounds [70]. Blocking of an enzyme PAD4 (peptidyl arginine deaminase) in diabetic mouse models resulted in an improved rate of healing and wound closure by blocking the process of NETosis [71]. Moreover, excessive release of MMP-9 by neutrophils in diabetic ulcers has been reported [72]. Another growth factor Mfge8 enhances the rate of healing in cutaneous wounds in diabetic patients. Its decreased levels are

associated with non-healing DFUs [73,74].

3.1.4. Role of NK cells in chronic wound pathogenesis

Natural killer cells also have their role in chronic wound inflammation. They promote a pro-inflammatory environment by releasing interferon-γ, perforins, and granzymes, thus posing a threat to effective wound healing. This specific IFN released by NK cells also facilitates M1 macrophage polarization and influences them to release pro-inflammatory cytokines including IL-12, IL-6, IL-1β, TNF-α and IL-23. IFN released by NK cells also causes increased neutrophil infiltration during the proliferative phase, thus enhancing fibrosis [75,76]. Innate lymphoid cell type 3 attracts macrophages at the injury site to initiate epidermal proliferation [77]. Deficiency of ILC3 has been reported in diabetic mouse models resulting in loss of epidermal proliferation and macrophage polarization contributing to delayed healing [78]. ILC type 2 however has a protective role in the process of wound healing as they improve anti-inflammatory responses through M2 macrophage polarization [79].

3.1.5. Role of langerhan cells in pathogenesis of chronic wounds

Another study identified the role of $CD207^{+}$ Langerhans cells (LCs) in the healing of chronic wounds. They are a part of the immune barrier of the epidermal layer of skin. They proved that there is a decreased amount of $CD207^{+}$ LCs in non-healing diabetic wounds and thus the population of these cells at the wound site can determine the healing outcomes [80].

3.2. Diabetic conditions influencing immune efficacy in DFU

Poor glycemic control is yet another factor contributing to the reduced healing of wounds in diabetic patients [81]. It causes decreased macrophage functions by reducing the expression of interleukin-2, leading to an increased infection rate [82]. A glycoprotein called GM-CSF (Granulocyte-Macrophage colony-stimulating factor) known for its role in immune modulation is not completely activated in DFUs. It results in an impaired response of the immune system in patients, suffering from diabetes, adding to the severity of ulcers [83]. Another study proposed that decreased amounts of IL-37 aid in inefficient healing in diabetic ulcers and non-healing chronic wounds as they are important mediators for resolving inflammation [84]. A recent transcriptomic study has revealed that transcription factors STAT3 and FOXM1, involved in immune cell activation are inhibited in diabetic conditions, causing reduced recruitment of macrophages and neutrophils at wound site aiding chronicity [85,86]. Persistent population of myeloid cells including monocytes, macrophages, neutrophils as well as mast cells during the late inflammatory phase of wound repair and reduced amounts of eosinophils, dendritic cells (DCs), and LCs result in chronic wounds.

3.3. miRNAs influencing healing outcomes in DFUs

miRNAs are also involved in regulating immune responses, thus playing a role in chronic wound pathogenesis. Many studies have reconnoitered the significant role of miRNAs in influencing the immunological responses in wound healing. They are involved in the regulation of the immune system by monitoring the response of macrophages, B as well as T cells, neutrophils, lymphocytes, and fibroblasts by governing gene expression. This implicates the potential role of non-coding microRNAs in altered immune mechanisms of impaired healing in chronic wounds including DFUs.

In diabetic mouse models, the reduced levels of miR-146a resulted in increased infiltration of neutrophils at the wound site, causing an interruption in the wound healing [87]. Downregulated miR-129 causes delayed healing of diabetic chronic wounds. Upregulation of miR-129-2-3p was connected with increased expression of CCR2 and Casp6 that initiates apoptosis and proliferative responses in diabetic patients causing improved healing of chronic wounds in diabetic mice [88]. miR-21 overexpression in M1 macrophages leads to its enhanced polarization and release of increased inflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-12, attenuating the healing of wounds [89]. It was also responsible for the decreased amounts of fibroblasts at the site of injury, causing wound healing impairment [90]. miR-132 is known to be extremely up-regulated in the inflammatory wound healing phase. It is associated with the suppression of inflammatory cytokines production by keratinocytes. It also promotes the re-epithelialization and keratinocyte growth and is significant for the phase shift from pro-inflammatory to proliferative one by inhibition and enhancement of NF-κB and ERK pathways respectively. Its altered expression causes delayed healing of wounds [91,92]. Another microRNA, miR-203 was found to be significantly upregulated in diabetic foot ulcer samples and its levels had a positive correlation with DFU severity [93]. It controls B cell apoptosis [94] and inhibits keratinocyte proliferation and migration at wound site [95]. miR-126 is associated with the proliferation as well as migration of leukocytes at chronic wound site as well as angiogenesis and endothelial cell growth. Reduced levels of this non-coding RNA in diabetic patients result in adverse healing outcomes [96,97]. miR-223 is involved in the employment of neutrophils at the site of injury by actively targeting CCL3 and CXCL2 chemokine ligands [98]. Moreover, the downregulation of miR-93 and upregulation of miR-155 in DFUs was associated with the reduced expression of CXCR1 ligand IL-8. IL-8 has a positive effect on keratinocytes migration at site of injury, having a prognostic value in wound healing process [99,100]. Expression of another microRNA miR-210 is increased in diabetic wounds because of hyperglycemic conditions and hypoxia. This miRNA is linked to the keratinocytes' differentiation during the process of wound healing [101,102]. Enhanced expression of miR-155 has been stated in non-healing wounds of diabetic mice. Inhibition of this miRNA led to decreased inflammatory cell infiltration and an increased population of proliferative macrophages, thus accelerating the healing process [103]. Increased miR-483 and miR-198 expression also hinders the infiltration and proliferation of keratinocytes during wound healing in diabetic patients [104]. All this experimental evidence dictates how microRNAs can influence the potential activity of cells involved in the wound healing and how alterations in their expression levels can directly determine the healing outcomes in chronic wounds, specifically DFUs.

4. Therapeutic strategies for DFUs

Chronic wounds are characterized by damage in the structural integrity of skin that fails to heal within a certain time frame with the help of standard wound care and generalized skin treatments. The most common chronic wound is diabetic foot ulcer. DFU is a chronic impediment of diabetes mellitus. Approximately 40 % to 60 % of diabetic patients experiencing chronic wounds have to undergo amputations because of the damage severity [105]. DFU therapeutic strategies should address the multidisciplinary pathogenesis of DFU. It requires identifying and assessing the etiologies and co-morbidities to facilitate the patients with the targeted therapeutic approach so that the amputation risk of lower extremities can be reduced. Currently, different therapeutic approaches are under trial for effective chronic wound healing which include stem cell therapies, tissue regeneration through transplantation, skin grafting, immune-based therapies as well as specific hydrogels and wound dressings but achievement of effective healing of wound ulcers remains a challenge. The use of biomaterials for medication delivery, instrument-based technologies, therapies targeting inflammation to aggravate the healing process, use of growth factors in wound dressing, and antibacterial ointments to control infection at the injury site; all these different approaches are being studied for effective clinical outcome in treating non-healing wounds. None of these therapeutic strategies have been able to provide 100% efficient results which mimic the need for the availability of personalized treatments depending upon the pathophysiological conditions of the wound that can vary from one

patient to another. This review focuses on the analysis of the current advancements and the clinical approaches to tissue regeneration and wound healing practices.

4.1. Hydrogels

Nowadays, various natural and synthetic biomaterials are being used for the development of hydrogels including alginate, collagen, hyaluronic acid, and chitosan providing efficient cell affinity and biocompatibility. Synthetic biomaterials having high homogeneity such as polyurethane (PU), poly(vinyl alcohol) (PVA), polyacrylic acid (PAA), poly(vinyl pyrrolidone)(PVP), poly(hydroxyethyl methacrylate) (PHEMA) and poly(ethylene glycol) are also being used for making hydrogels [106]. Studies have explored that hydrogels consisting of a decellularized extracellular matrix (ECM) have the ability to influence the migration and differentiation of immune cells at wound sites [107]. Hyaluronic acid (HA) hydrogels have also been applied for DFU treatment. Low molecular weight HA gels induced M1 (Pro-inflammatory macrophage) polarization, which was detected by increased secretion of inflammatory cytokines (TNF-α and nitric oxide) and enhanced expression of pro-inflammatory factors including TNF, CD80, and NOS2. On the contrary, high molecular weight HA hydrogels promoted M2 (proliferative macrophage) polarization, confirmed by overexpression of anti-inflammatory cytokines as well as genes such as MRC1 and ARG1 [108]. This explains how altered hydrogel densities can influence the polarization of different macrophages controlling immune responses and thus can facilitate targeted therapies. This was confirmed by another study which explained that HA/PVA-based high molecular weight hydrogels caused M1 to M2 phenotypic shift and resolved the inflammatory microenvironment of the wound to accelerate healing, which can serve as an effective therapy for treating DFUs [109]. Gelatin-hyaluronic acid-based hydrogels accelerated healing in chronic ulcers by reducing inflammatory immune cell responses and improving cell adhesion and survival at wound site in diabetic mice [110]. Silk fibrin (SF) is a natural polymer that has immunomodulatory properties by influencing NF-κB pathway [111,112]. Chouhan et al. (2018), formulated an SF hydrogel and did a comparative analysis of wound healing characteristics between SF and collagen hydrogels. Results demonstrated that SF hydrogels were a better and more cost-effective way for the treatment of chronic wounds as they facilitated the migration of keratinocytes and the proliferation of primary human fibroblasts. SF hydrogel application also resulted in enhanced expression of CD163 and TNF-α which means the successful progression of wound from inflammatory to proliferative healing stage [113]. Hyaluronic acid methacrylate modified with phenyl boronic acid was fabricated to form HMPC hydrogels which exhibited 3D network structure when applied to chronic diabetic wounds and showed a visible reduction in inflammation (reduced IL-6 and enhanced IL-10 levels) and also enhanced the expression of angiogenesis promoting genes including CD31 and VEGF [114]. Synthetic Mxene nanoparticles have been of great interest because of their immune-modulating abilities. Titanium Carbide Mxene quantum dots have shown positive results in healing wounds by promoting the proliferation of immunoproliferative regulatory T cells and inhibiting T cell lymphocyte activation [115]. Elastin-based and chitosan/dextran hydrogels promote healing by facilitating the migration of neutrophils and macrophages and also by invading microbes to help control the spreading of infection [116]. Sodium alginate and dendritic hydrogels facilitate the survival of fibroblasts, keratinocytes, and endothelial cells by attenuating local inflammatory responses through M2 macrophage polarization at the wound site [117].

This is how naturally derived as well as synthetic hydrogels have been providing promising results in the treatment of DFUs. The application of polymer hydrogels holds a significant interest in modulating immune responses and is a cost-effective way to lessen the severity of wound chronicity and lead them toward a progressive healing path. Physical properties of hydrogels like pore size, stiffness, etc can also influence immune cell infiltration, their migration as well as polarization at the site of injury. Therefore, caution needs to be taken in determining such factors [118,119].

4.2. Immune cells applications

Immune cells play a significant part in the pathogenesis of chronic wounds. Immune cells are majorly involved in transitioning of inflammatory to the proliferative phase, therefore they are responsible for attenuating the onset of the healing phase in wounds having chronic outcomes. So, therapeutics based on the immune cells' regulation can bring favorable results in the effective treatment of diabetic foot ulcers. Various types of immunocytes including antigen-loaded DCs, cytotoxic as well as regulatory T cells, Natural Killer (NK cells), and chimeric (CAR T cells) have been formulated in hydrogels and have been applied for the treatment of chronic diabetic wounds. They have been proven effective in generating cell-specific responses and designing immune suppressive strategies to control the spreading of wounds and increase healing through cellular mechanisms and signaling pathways. M2-seeded hyaluronic acid hydrogels have been shown to increase wound healing of diabetic ulcers by enhancing the secretion of anti-inflammatory cytokines (VEGF, IL-4) and reducing levels of inflammatory cytokines for example TNF-α, IL-1, and IL-6, ultimately resulting in improved angiogenesis and accelerated tissue regeneration [109,120]. However, on the contrary, some other experimental research has found a non-significant associationbetween M2 macrophages and healing of diabetic ulcers [121,122]. Table 1 enlists the immunomodulatory therapies designed for healing of diabetic ulcers, along with their associated response and treatment outcomes.

4.3. Stem cell-based therapies for DFU treatment

Because of their diverse biological functions, hemostasis maintenance, and self-renewal capabilities, stem cells have been used for tissue regeneration and are of keen interest to researchers in designing advanced therapeutic approaches for chronic wounds. Adult stem cells, embryonic stem cells, and induced pluripotent stem cells have applications in the treatment of wounds. Stem cells can be extracted from bone marrow, umbilical cord blood and tissue, and adipose tissues and exhibit immune modulation properties by controlling the growth, differentiation, and proliferation of immune cells. Research has proved the constructive role of stem cells in regenerating tissues after injury exposure when targeted to a specific area with the help of a carrier medium. Hydrogels are considered ideal as a carrier medium for the efficient delivery of different kinds of stem cells.

Kim et al. (2007), obtained adipose-derived stem cells (ADSCs) and used them to assess their potential role in treating chronic ulcers in vivo and in vitro models. ADSCs improved healing outcomes by releasing cytokines and growth factors for fibroblast proliferation, promoted angiogenesis as well as regulated the levels of ECM proteins, and enhanced the secretion of collagen type I from human dermal fibroblasts, thus allowing re-epithelialization and wound closure [129]. Zheng et al. (2017), stated that amniotic stem cells when localized to the targeted wound area in diabetic mice, accelerated healing by secreting chemotaxis and growth-related factors that induced neovascularization, and regulated macrophage polarization by recruiting $CD34^{+}$ progenitor cells. This study showed that cryopreserved amniotic cells can be used as dermal substitutes to treat chronic ulcers in diabetic patients [130]. Lv et al. (2017), did a comparative analysis of the potential roles of two different stem cell types; mesenchymal stem cells (MSCs) and stem cells derived from human-exfoliated deciduous teeth. The results showed significant downregulation of IL-10 and increased expressions of MMP2, MMP9 VEGF, and NOS2. They proved that both types were able to promote healing by suppression of inflammatory immune responses as well as accelerating the rate of angiogenesis in diabetic mouse models with chronic ulcers [131]. Application of fetal aorta-derived $CD133^{+}$

Table 1
Immune cell/Macrophage-modulation approaches for DFU treatment.

Immune cell type/ Immunomodulatory therapy	Diabetic Model	Hydrogel Characteristics	Administration Route	Molecular Changes	Outcomes	Healing Impact	References
Macrophages	STZ-induced DM C57BL/ 6J mice	HA@MnO_2/FGF-2/ Exos hydrogel	Local injection	↑ M2-derived Exosomes (M2 Exos) ↑ FGF-2 growth factor	Angiogenesis Epithelialization	Accelerated wound healing	[123]
Macrophages	Diabetic mice	HTHE-M@D hydrogel	Injection along with mild heat stimulation	↑ M1 to M2 phenotype transformation	Angiogenesis Antibacterial, Antioxidant, Anti-inflammatory Hemostatic properties	Reduced healing time	[124]
Macrophages	C57BL/6 mice	HA-JK1 hybrid hydrogel	Topical application	↑ M2 polarization	Re-epithelialization Collagen deposition Angiogenesis Cell proliferation	Accelerated wound regeneration	[125]
Macrophages	STZ induced T1DM C57BL/6 mice	Cu-HHA/PVA@MΦ2 hydrogels	Topical application	↑ M1 to M2 phenotype transformation ↑ M2 enrichment	Angiogenesis Improvement of immunocompromise	Reduced healing time	[109]
Macrophages	STZ induced DM rats	CS-AT-Exo hydrogel	Injection	↑ M2 polarization	Re-epithelialization Angiogenesis Collagen deposition	Promoted wound thickness and healing efficiency	[126]
Macrophages	C57BL/KsJ db/db mice	HA-MA-NB (HNM) hydrogel encapsulating AM-CM	Topical application	↑ M2 polarization ↑ VEGF ↑ TGF-β1 ↓ IL-1β ↓ TNF-α	Angiogenesis Re-epithelialization	Enhanced skin regeneration	[127]
Macrophages	STZ induced DM Sprague-Dawley rats	GelMA-SF hydrogel	Topical application	↑ M2 polarization	Angiogenesis Re-epithelialization	Accelerated wound healing	[128]

Hyaluronic-acid-based hydrogel (HA hydrogel), M2 macrophage-polarized anti-inflammatory hydrogel (HTHE-M@D), JK1 encapsulating hyaluronic acid hydrogel (HA-JK1 hybrid hydrogel), High molecular weight Hyaluronic acid/Poly vinyl alcohol hydrogel treated with Cu and Macrophage (HHA/PVA@MΦ2 hydrogels), Chitosan-graft-aniline tetramer hydrogel loaded with exosomes (CS-AT-Exo hydrogel), Methacrylic anhydride (MA) and N-(2-aminoethyl)-4-[4-(hydroxymethyl)-2-methoxy-5-nitrophenoxy]-butanamide (NB) hydrogel encapsulating amnion-derived conditioned medium (AM-CM, HA-MA-NB (HNM) hydrogel encapsulating AM-CM, Microbial lipopeptide-surfactin (SF) reinforced gelatin methacrylate (GelMA) hydrogel (GelMA-SF hydrogel).

progenitor cells proved efficient in promoting wound healing in diabetic ulcers by secreting elevated levels of interleukins and growth factors associated with the angiogenesis process and increased wound closure rates by activation of the Wnt signaling pathway [132]. Placenta-derived MSCs when targeted to chronic wounds in diabetic mouse models through intradermal injection released proangiogenic molecules including TGF, FGF, IGF-1, as well as VEGF, causing reduced wound severity to noticeable levels [133]. Embryonic stem cells and induced pluripotent stem cells have shown promising results in the treatment of diabetic ulcers by resolving drastic immune responses and elevating the proliferation and regenerational wound healing phases [134,135]. Genetically modified mesenchymal stem cells formed after transfection with vector loading IL-4 gene applied through microribbon hydrogels showed enhanced IL-4 expression and M2 macrophage phenotype shift, thereby strengthening immunosuppressive properties [136].

4.4. Platelet therapy for DFUs

Blood-derived platelets play a significant role in hemostasis, thrombosis, inflammatory, and remodeling phases of wound healing. They secrete granules that release growth factors, cytokines, and extracellular matrix modulators which cause differentiation and proliferation of MSCs into desired cell types and also revascularize injured tissue sites. They are also involved in playing a defensive role against invading pathogens through the secretion of antimicrobial peptides such as kinocidins as well as cytokines and chemokines. Moreover, they accelerate wound healing by stimulating the proliferation and migration of human fibroblasts [137–139]. Platelets contain an increased number of growth and blood coagulation factors regulating clot formation, hemostasis, and tissue repair [140]. Platelet concentrations prepared in vitro through blood centrifugation can accelerate the wound regeneration process by releasing molecules and growth factors at the wound site [141]. Platelet-rich plasma (PRP) contains increased amounts of platelets and growth factors than normal blood plasma and is associated with reduced risks because it is obtained from the patient's blood. PRP is being used clinically to treat chronic wounds [142,143]. Secretory substances present in PRP such as growth factors including VEGF, CTGF, EGF, TGF-β, IGF, and PDGF as well as cytokines can accelerate wound healing in skin lesions including diabetic foot ulcers by stimulating collagen synthesis, and angiogenesis as well as epithelial, endothelial and epidermal tissue regeneration and repair [144,145]. Certain anti-inflammatory and proliferative cytokines are also released from platelet-rich concentrates for activation of wound repair. PRP contains abundant amounts of VEGF and is involved in stimulating angiogenesis and the production of matrix metalloproteinases for the healing of chronic ulcers [146].

Ahmed et al. (2017), treated DFUs with plasma-treated gels and proved that this therapy was more effective in accelerating wound closure rates and reducing the chances of infection at clean wound sites as compared to antiseptic wound dressings [147]. PRP when coupled with stem cell therapy for ulcer treatments showed promising results in terms of increasing wound closure rates, thus accelerating healing [145]. PRP gel treatments also have the efficacy to regulate autophagy and reduce inflammation at the wound site, therefore can be effective in accelerating wound healing in DFUs [148]. Huang et al. (2023), used a compatible way of PRP delivery to the wound site. They prepared

bioactive hydrogels by coupling the Ca+ crosslinking method with 3D bio-printing technology and checked for their effects in healing diabetic foot ulcers. These fibrous bioactive hydrogels promoted granulation tissue growth, generating an ordered collagen network and enhanced vascularization at the wound site, supporting healing [149]. An experimental study compared the effects of injectable PRP with conventional wound dressing methods to treat chronic ulcers in diabetic patients. Targeted PRP therapy showed better results with higher frequency of wound size reduction [150]. Table 2 enlists the stem cell-based treatment implications in different diabetic models, addressing their molecular outcomes affecting diabetic ulcers' healing.

4.5. Nano-medicinal approaches for improved healing in DFUs

Chronic wound management is far more complex and challenging because of its multifaceted pathophysiological complications as well as microbial infections as compared to acute wounds. DFU treatment strategies utilizing hydrogels, immune modifications and application of stem cells have been associated with potential healing properties but these methods have certain inadequacies for example local application and drug penetration into deeper tissues, resulting in deficient healing conditions. Advanced nano-therapeutic models comprising nanomaterials have been designed to minimize such shortcomings and have come up with promising results in pathing ways for improved drug penetration into target tissues eradicating wound chronicity, essential for fast healing. A nano model comprising of poly (lactic-co-glycolic acid) acting as a carrier of human EGF resulted in improved proliferation of fibroblasts and rapid healing in mouse models compared to the control group [168]. A recent research elucidated the efficacy of gelatin nanofiber composed of CaCO3/SiO3 and quercetin against DFUs in cell lines. MTT assay revealed that the use of this nanotechnological approach was coupled with increased cellular proliferation and was effective against major bacterial strains causing infection at wound site [169]. Another study designed a nano-strategy comprising ceria nanoparticles loaded with clindamycin drug for effective treatment of DFU by targeting ROS. The drug release in vitro targeted sites was assessed and the study concluded that these nanoparticles were associated with lowering serum glucose levels as well as attenuated release of inflammatory cytokines in diabetic rat models. It also lowered the levels of oxidative stress markers, accelerating healing of chronic wounds [170]. Moreover, oxygen loaded nanoparticles when delivered through radial electric shockwave technology (rESW) showed pronounced biocompatibility in vivo and vitro models, expediting healing in DFUs through improved supply of oxygen at wound site [171]. Nanotechnology based wound healing strategies provide significant benefits including enhanced shelf life of active drug compounds, better penetration and bioavailability across skin and tissue barriers as well as increased susceptibility of microbes to drugs over traditional approaches. Table 3 enlists various nanotherapeutic approaches including nanogels, polymeric micelles, carbon nanotubes, nanofibres, nanosheets, liposomes as well as solid and liquid phase nanoparticles that have influenced the efficacy for chronic wound management and healing by helping achieve promising results in terms of drug delivery and skin regeneration.

4.6. Growth factor-based strategies to target DFUs

The significance of growth factors including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) in maintaining an optimum healing environment after injury cannot be denied. They are actively involved in processes such as cell proliferation and migration, collagen synthesis, stimulating angiogenesis, inflammatory response modulation, epithelialization as well as wound contraction during healing. Their optimum amounts are critical for transitioning of sequential healing phases, therefore their application for treatment of non-healing chronic wounds have provided promising results.

4.6.1. Human epidermal growth factor EGF

Several studies have shown the importance of EGF growth factor for the process of wound healing and alterations in its expression can promote impaired healing and progression of wound chronicity [194]. Glycolic acid microspheres loaded with EGF were tested in diabetic foot ulcers in mouse models. The study revealed that application of EGF microspheres led to earlier inflammatory phase resolution, re-epithelialization and complete wound closure, leading to enhanced DFU healing [195]. Mari et al. (2022), demonstrated the role of EGF on fibroblast cells isolated from diabetic ulcers. They experimentally proved that EGF caused fibroblast proliferation, transcriptomic analysis revealed downregulation of pro-inflammatory cytokines (IL-6 and TNF), thus resolving inflammation even in hyperglycemic conditions [196]. Phase III clinical trial reported that the DFU group treated with recombinant EGF showed increased epithelialization and enhanced wound closure rates compared to the control placebo group [197]. Certain other studies for example [198 199] have also favored the constructive role of EGF in improved treatment of DFUs leading to reduced risk of skin graft applications and amputations, making it a potential therapeutic candidate to be considered during the formulation of effective treatment strategies against DFU.

4.6.2. Fibroblast growth factor FGF

FGF is a cell signaling protein, of which 23 subtypes have been identified till date. FGF expression has been keenly linked with progressive healing of wounds. Therefore, various therapeutic strategies are implying the use of FGF in treating chronic diabetic ulcers. FGF-1 was administered in obesity induced diabetic models NONcNZO10Ltj to evaluate its effects on the healing properties of wounds. Research showed that FGF-1, with or without heparin formulation was associated with reepithelialization and accelerated wound closure rates, suggesting its role as a potential drug against DFUs [200]. An experimental study showed that reduced expression of FGF-2 in diabetic wounds resulted in attenuated angiogenesis and decreased collagen formation, contributing to wound chronicity [201]. A recent study showed that FGF-2 retained hydrogels when applied directly to wound surface area showed improved epithelial and collagen formation, resulting in optimized healing in mice models [202]. Similarly, FGF-21 has been linked with apoptotic inhibition supporting reepithelialization, granulation tissue formation, suppression of inflammation through NLRP3 inhibition and regulation of various cytokines (IL-6, IL-10, TNF), thus progressing the wound into proliferative stages [203,204].

4.6.3. Platelet-derived growth factor (PDGF)

PDGF subfamily consists of PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, PDGF-DD homo and heterodimers. It is actively involved in the wound healing process by stimulating the proliferation and chemotaxis of various cells including fibroblasts, monocytes as well as neutrophils at wound site [205]. Becaplermin gel; the only FDA approved drug for healing of chronic wounds consisting of recombinant PDGF-BB variants [206]. A recent study explored the role of PDGF-BB regulator gene TUG1 in endothelial progenitor cells (EPCs) present in hyperglycemic conditions. The study experimentally proved that induced overexpression of TUG1 stimulated the increased production of PDGF-BB via regulation of miR-29c-3p, resulting in improved migration and proliferation of EPCs, angiogenesis and fast healing in ischemic mouse models [207]. More recently, Deptula et al. (2020), demonstrated a positive correlation between the application of PDGF2 (a PDGF-BB derived peptide) and healing efficiency of wounds in in-vitro models. PDGF peptide was associated with reduced cytotoxicity and increased proliferation of cells with more immune tolerance at the wound site [208]. Another experimental study has proved that miR-145-5p regulated over-expression of PDGFD accelerated the healing of diabetic ulcers; the reverse attenuated the healing process in mouse models by influencing migration, cell viability and proliferation at wound site [209]. Various clinical trials utilizing PDGF growth factor to treat DFUs are being actively conducted

Table 2
Stem cell-based therapeutic approaches for DFU treatment.

Stem cell Type	Stem cell Subtype	Diabetic Model	Cell Concentration/ Dosage	Administration Route	Molecular Outcomes	Histological Parameters	References
Adult stem cell	Adipose-derived stem cells (ADSC)	STZ induced DM Sprague Dawley rats	1×10^6 cells/1mL DMEM	ADSC-ADM graft	↑ VEGF ↑ HGF ↑ TGFb ↑ bFGF	Tissue regeneration Re-epithelialization Neovascularization	[151]
		STZ induced DM Sprague Dawley rats	5×10^6 ZsGreen-hASDCs	Intravenous injection	↑ VEGF ↑ bFGF ↑ Ki-67 ↑ IL-1β ↑ IL-6 ↑ IL-13 ↑ CCL3	Angiogenesis Re-epithelialization Collagen deposition Granulation tissue formation Cellular proliferation	[152]
		Zucker diabetic fatty (ZDF) rats	$\sim 9 \times 10^5$ cells /sheet	ADSC sheet with artificial skin transplantation	↑ VEGF ↑ HGF ↑ TGF-β1 ↑ IGF-I ↑ EGF ↑ KGF	Angiogenesis Re-epithelialization	[153]
		STZ induced DM rats	1×10^6 cells/100ul Pluronic F127 gel solution	Topical injection	↑ CD31 ↑ Ki67 ↑ TGF-β1	Angiogenesis Cell proliferation Granulation tissue regeneration	[154]
	Adipose tissue-derived mesenchymal stem cells (AD-MSCs)	Diabetic BKS *db/db* mice	1×10^6 DFX-preconditioned cells/50 μL CM	Intravenous	↑ Type 1 collagen ↑ IGF-1 ↑ Angiopotetin-1 ↑ PDGF, ↑ VEGF-α	Angiogenesis Collagen deposition Re-epithelialization	[155]
		STZ induced DM Wistar rats	0.05 mL cell suspension	Intramuscular and subcutaneous injection	↑ CD31 ↑ Ki-67 ↑ VEGF	Angiogenesis Cell proliferation Granulation tissue formation Re-epithelialization	[156]
	Human umbilical cord-derived mesenchymal stem cells (hUC-MSC)	STZ induced T1DM Sprague Dawley rats & T2DM Goto-Kazizaki rats	1×10^6 cells/1mL HA gel	Topical gel application, hUC-MSC-CM	↑ SOD1 ↑ COL3A1 ↑ COL4A1 ↓ TNF-α ↓ IL1-β ↓ IL-6 ↓ p-16 ↑↓ PCNA	Re-epithelialization Collagen deposition Angiogenesis. MAPK & AKT pathway Reduced oxidative stress	[157]
		Diabetic *db/db* mice	5×10^6 cells/100 μL in hydrogel	Dermal transplantation	↑ CD31 ↑ α-SMA ↑ VEGF ↓ TNF-α ↓ IL1-β	Collagen deposition Angiogenesis Re-epithelialization	[158]
		Diabetic *db/db* mice	1–4 $\times 10^6$ cells/cm^2 on PLGA scaffold	Dermal tissue sheet application	↑ Collagen III: Collagen I ↑ CD31	Granulation tissue formation Re-epithelialization collagen synthesis Angiogenesis Reduced inflammation	[159]
	Placenta-derived mesenchymal stem cells (PMSC)	Diabetic Goto Kazizaki rats	1×10^6 cells/200 μL PBS	Intradermal injection	↑ VEGF ↑ HGF, ↑ bFGF ↑ TGF-β ↑ IGF-1	Vascular regeneration Angiogenesis	[133]
		T2MD female patient	1×10^6/cells/cm^2 on hydrogel	Topical application	–	Granulation tissue formation Neovaascularization	[160]
	Human amniotic fluid derived mesenchymal stem cells (AF-MSC)	STZ induced DM NOD/SCID mice	1×10^6 cells/100 μL PBS	Intradermal injection	↑ IGF-1 ↑ EGF ↑ IL-8	Angiogenesis Re-epithelialization Increased engraftment	[161]

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Table 2 (*continued*)

Stem cell Type	Stem cell Subtype	Diabetic Model	Cell Concentration/ Dosage	Administration Route	Molecular Outcomes	Histological Parameters	References
		IRC mice	–	Subcutaneous injection & topical application	↑ TGF-β ↑ IL-8 ↑ IL-6 ↑ TNFRI ↑ VEGF ↑ EGF ↑ MMP3	Fibroblast migration Angiogenesis Re-epithelialization	[162]
		STZ induced DM mice	Cultured AF-MSCs on the hydrogel	Topical application	↓ IL1-β ↓ IL-6 ↓ CXCL-1 ↓ CXCL-2	Re-epithelialization Reduced macrophage & neutrophil infiltration Enhanced regulatory T cells infiltration	[163]
	Epidermal stem cells (EPCs)	STZ induced DM mice	1.0×10^6 cells/100 μL PBS	Subcutaneous injection	↑ Jag1 ↑ Notch1 ↑ Hes-1	ESC migration Increased vessel density	[164]
	Hair follicle stem cells (HFSC)	Wistar rats	1×10^6 cells/rats	Intradermal	↑ CD31	Granulation tissue Re-epithelialization Collagen fibers Neovascularization	[165]
	Human fetal aorta-derived $CD133^+$ progenitor cells	STZ induced DM CD1 mice	2×10^4 $CD133^+$ cells on collagen or hydrogel	Topical application	↑ VEGF-A ↑ IL-8 ↑ Wnt genes	Angiogenesis Endothelial cell migration	[132]
	Umbilical cord blood-derived CD34 + stem cells	STZ induced DM albino rats	0.5×10^6 cells/rat	Local injection	–	Revascularization Epidermal thickness Collagen deposition Keratinocytes production	[166]
Embryonic stem cell (ESCs)	–	STZ induced DM Sprague Dawley rats	5×10^6 cells/mL saline	Topical injection	↑ VEGF ↑ EGF ↑ Fibronectin	Epidermal regeneration Angiogenesis Granulation tissue thickness	[134]
Induced pluripotent stem (iPS) cells	iPS cell-derived exosomes (iPS-Exos)	C57BLKS/J-Leprdb (db/db) mice	4 μg iPS-Exos /20 μl PBS	Injection	↑ α-SMA ↑ CD31	Fibroblast migration Angiogenesis	[167]

Diabetes Mellitus (DM), Streptozotocin (STZ), Acellular dermal matrix (ADM), Hyaluronic acid (HA), Poly acetic co-glycolic acid (PLGA), NOD/Severe Combined Immuno-Deficiency (SCID) mice, ICR mice, Induced pluripotent stem (iPS) cell-derived exosomes (iPS-Exos), ↑-increased expression, ↓- decreased expression, ↑↓-abnormal expression

and have shown promising results [210,211]. These evidence suggests potential candidacy of PDGF as a novel agent for designing efficient DFU treatment strategies.

4.6.4. *Vascular endothelial growth factor VEGF*

VEGF is essential for the growth and proliferation of vascular endothelial cells, hence considered a potent factor for angiogenesis. VEGF expression levels are directly linked to the healing outcomes in DFUs. VEGF regulated angiogenesis is involved in formation of granulation tissue as well as the supply of oxygen and other nutrients at wound site, promoting tissue regeneration [212,213]. Peng et al. (2021), in their recent experimental study revealed that long non-coding GAS5 RNA mediated activation of VEGF through its interaction with upstream gene TAF15 accelerated healing in DFU mouse models by enhancing cellular proliferation and tubule formation, facilitating the formation of blood vessels [214]. More recently, a research focused on finding the association between hyperglycemic (HG) conditions and angiogenesis stimulation found out that HG induced attenuated wound healing occurs as a result of significant downregulation of STING protein. Sting is responsible for the regulation of VEGF expression levels, migration of epithelial cells and inhibition of apoptosis at wound site. Overall, the study stressed on the fact that abnormal VEGF levels can result in inhibition of the healing process, especially in hyperglycemic conditions, signifying its importance as a target in designing DFU treatments [215]. Clinical trials utilizing VEGF for diabetic limb ischemia and chronic DFUs resulted in reduced pain and improved healing respectively, suggesting constructive potential of VEGF in treating DFUs through modulation of biological healing activities [216–218].

4.7. *Skin substitutes for DFU healing*

Skin substitutes containing a combination of biomaterials, growth factors, and other components have been widely used to treat chronic non-healing wounds. Their efficacy in wound healing depends upon the type of bioactive materials used and their anatomical shape. Their use has been vastly accepted as an advanced treatment method. They act as a physical barrier by providing protection against pathogens, thus limiting wound site infections as well as creating optimal moisture conditions required for wound healing. An ideal skin substitute should possess certain qualities including cost-effectiveness, resistance to infection, durability, availability, and lack of antigenicity. Tissue engineering processes have been used to make a variety of skin substitutes. Apligraf is an example of an artificially prepared skin substitute known for wound healing through the production of various cytokines and growth factors [219]. Alloderm, another engineered substitute enhancing vessel formation in wound environment and accelerating the formation of new skin cells, consists of a dermal matrix of collagen [220].

Skin substitutes are biomaterials having heterogeneous compositions

Table 3
Nano-particles/material-based therapeutic approaches for DFU treatment.

Nanoparticle	Therapeutic Agent	Incorporated Agent	Diabetic Model	Route of Administration	Molecular Response	Histological Outcomes	References
NLC-rhEGF and SLN-rhEGF nanoparticles	Growth Factors	rhEGF	Diabetic db/db mice	Topical administration	–	Re-epithelialization Restoration of inflammatory process	[172]
NaCMCh-rhEGF nanoparticles		rhEGF	STZ induced DM in Sprague-Dawley rats	Hydrogel dressing	–	Re-epithelialization Higher cell viability	[173]
PLGA nanospheres/ plasmid complexes		PDGF VEGF	STZ induced DM in Sprague-Dawley rats	Subcutaneous injection	↑ CD31 ↑ VEGF-A ↑ PDGF-B	Angiogenesis Re-epithelization Granulation tissue formation	[174]
SDF1α-ELP nanoparticles		SDF1	BKS.Cg-Dock7^{m} +/+ Lepr^{db}/J mice	Topical administration	–	Increased epidermal layer thickness	[175]
Col–HA w/4GF nanofibers		VEGF PDGF bFGF EGF	STZ induced DM in Sprague-Dawley rats	Surgical sutures	↑Type I collagen ↓Type III collagen	Neovascularization Tissue regeneration Enhanced release of angiogenic factors Re-epithelialization	[176]
PCL/F-127 nanofiber 3D scaffold	Stem cells	BM-MSC	TALLYHO TSDM mice	Dermal implantation	↓ M1-type macrophages ↑ M2-type macrophages ↓ IL-6 ↓ TNF-α ↑ IL-4 ↑ IL-10	Granulation tissue formation Re-epithelialization Collagen deposition	[177]
FEP@Exo		ADSCs-derived exosomes	STZ induced DM in ICR mice	Topical administration	↑ Ki67 ↑ α-SMA ↑ Collagen I ↑ Collagen III	Angiogenesis Cell proliferation Granulation tissue formation Collagen deposition Re-epithelization	[178]
DsiRNA-AuNPs-CLRE and −HLRE	Nucleic acids	DsiRNA	STZ induced DM in Wistar rats	Topical administration	↑ PGE2 ↑ VEGF-A	Neovascularization Decreased inflammatory cells	[179]
CLN Lipoproteoplex nanoparticle		Keap1 siRNA	Diabetic mice	Topical administration	–	Nrf2 antioxidant function Accelerated tissue regeneration Augmented reduction–oxidation homeostasis	[180]
Peptide hydrogel doped with siRNA-loaded NPs		MMP-9 siRNA	Diabetic mice	Hydrogel dressing	–	MMP-9 silencing in keratinocytes Collagen deposition	[181]
Insulin-PLGA nanoparticles in PVA-borate hydrogel	Hormone	Insulin	STZ induced T1DM in Sprague-Dawley rats	Topical delivery	–	Angiogenesis Granulation tissue formation Re-epithelization Collagen deposition	[182]
MEL-NP		Melatonin	STZ induced DM in Wistar rats	Topical application	–	Angiogenesis Collagen deposition	[183]
FA-PLGA nanoparticles	Antioxidants	Ferulic acid (FA)	STZ induced DM Wistar rats	Oral and topical administration	↑ Hydroxyproline	Re-epithelialization Granulation tissue formation Collagen deposition	[184]
AuNP		EGCG	STZ induced DM BALB/c mice	Gas-injection	↑ VEGF ↑ EGFR ↑ Collagen I ↑ Collagen III ↑ Hyaluronic acid ↑ Cu/Zn superoxide dismutase	Fibroblast migration Collagen formation Angiogenesis Granulation tissue formation Re-epithelization	[185]
Cur-NP/HG	Polyphenolic Compound	Curcumin	STZ induced T1DM in albino rats	Topical delivery	↑ VEGF ↑ AQP3	Re-epithelization Collagen deposition Granulation tissue formation	[186]
CNPs@GMs/hydrogel		Curcumin	STZ induced T1DM in BALB/c mice	Topical application	↑ GPx ↑ α-SMA ↑ CD31 ↑ Ki67	Neovascularization Granulation tissue formation Collagen formation	[187]

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Table 3 (*continued*)

Nanoparticle	Therapeutic Agent	Incorporated Agent	Diabetic Model	Route of Administration	Molecular Response	Histological Outcomes	References
SLN-ATRA chitosan films	Vitamin	Retinoic acid	STZ induced DM C57BL/6J mice	Film insertion into wound	↓ MPO ↓ NAG	Collagen deposition Reduced leukocyte infiltration	[188]
AGN	Carbohydrate antigen	α-gal	STZ induced T1DM mice	Topical administration	–	Macrophage activation Keratinocyte migration Granulation tissue deposition Increased endothelial cell density	[189]
PLGA-VEGF NP	Growth factor & Carbohydrate	VEGF Lactate	Leptin receptor deficient db/db mice	Intradermal injection	↑ VEGFR2	Neovascularization Granulation tissue formation Keratinocyte migration Collagen Deposition Re-epithelialization	[190]
Se NPs-PRP	Plasma	PRP	Alloxan induced T2DM BALB/c mice	–	↑ Hydroxyproline ↑ GSH ↓ MDA	Re-epithelialization Collagen formation Fibroblast proliferation Angiogenesis	[191]
DS-PL electrospun membrane	Pro-angiogenic drug	DMOG	STZ induced DM C57BL/6J mice	Topical membrane application	↑ CD31 ↓ IL-1β ↓ IL-6 ↓ NF-κB	Neovascularization Collagen Deposition Granulation tissue formation	[192]
SM-PLGA nanosuspension	Phenolic compound	Sesamol	STZ induced T2DM in Wistar rats	Oral administration	↑ HSP-27 ↑ ERK ↑ PDGF-B ↑ VEGF ↑ CD-31 ↓ TNF-α	Re-epithelization Fibroblast migration Collagen deposition Reduced inflammatory cell infiltration	[193]

Selenium nanoparticles (SeNP), Platelet-rich plasma (PRP), Malondialdehyde (MA), Glutathione (GSH), Recombinant human epidermal growth factor (rhEGF), Nanostructure lipid carriers (NLC), Solid lipid nanoparticles (SLN), Sodium carboxymethyl chitosan-recombinant human epidermal growth factor conjugate (NaCMCh-rhEGF), Collagen-hyaluronic acid membrane with 4 growth factors (Col–HA w/4GF), Bone marrow mesenchymal stem cell (BM-MSC), Cold water extraction of *Lignosus rhinocerotis*-CLRE, Hot water extraction of *Lignosus rhinocerotis* (HLRE), Gold nanoparticles (AuNPs), Dicer substrate small interfering RNA (DsiRNA), Cationic lipid nanoparticle (CLN), Ferulic acid-poly(lactic-co-glycolic acid) (FA-PLGA), α-gal nanoparticles (AGN), Porous poly (L-lactic acid) (PLLA) electrospun fibrous membranes containing dimethyloxalylglycine (DMOG)-loaded mesoporous silica nanoparticles (DS), Epigallocatechin gallate (EGCG), VEGF encapsulated in Poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles (PLGA-VEGF NP), Stromal cell-derived growth factor-1 (SDF1), elastin-like peptide (ELP), Insulin-PLGA nanoparticles embedded in PVA-borate hydrogel, Adipose mesenchymal stem cells (ADSCs)-derived exosomes loaded on FEP hydrogel (FEP@Exo), Polylactic-co-glycolic acid (PLGA) nanospheres, Curcumin nanoparticles-hydrogel (Cur-NP/HG), Gelatin microspheres (GM), Sesamol-PLGA nanosuspension (SM-PLGA), Melatonin loaded lecithin-chitosan nanoparticles (MEL-NP), All trans retinoic acid (ATRA), Solid lipid nanoparticles (SLN).

designed specifically to accelerate healing in chronic wounds. Human-origin derived skin substitutes are called allografts and those originating from animal sources are called xenografts. Autographs are skin layers taken directly from the patient and applied to the wound area. Used excessively for wound healing, these temporary skin grafts do hold certain disadvantages i.e rejection risk by immune system as well as inefficient healing mechanisms because of different compositions and varying pathophysiological conditions of chronic wounds that differ from time to time and vary from person to person. Till date, numerous skin grafts have been prepared and are commercially available for the treatment of non-healing chronic ulcers, prepared by processing of either human derived placental and umbilical tissues, animal products as well as in vitro created synthetic grafts or a combination of both natural and synthetic components. Table 4 includes the list of commercially available skin grafts along with their respective compositions specifically formulated to improve healing of chronic ulcers.

4.8. Physical therapies for the treatment of DFUs

Modifications of the wound microenvironment are important in improving the healing mechanisms of chronic wounds. Physical factors can be altered in order to achieve the healing goal of DFUs. Certain physical therapies including oxygen therapy, use of offloading devices, nutritional modifications, physical exercise, pressure therapies, and debridement to clean affected wound sites have been applied in treating diabetic foot ulcers and have shown promising results.

4.8.1. Oxygen therapy

Oxygen is an important factor for wound healing mechanisms. In diabetic chronic ulcers, there is a depletion of angiogenesis and thus reduced blood vessels can lead to decreased or almost no oxygen supply to wound tissue, causing impaired immune functions and self-repairing mechanisms. This limits the energy required for the healing of the ulcer, encouraging the growth of bacteria and pathogens, and increasing the risk of infection [254]. Therefore, oxygen therapies are specifically designed to improve the hypoxic wound microenvironment [255]. Two kinds of oxygen therapies are in use nowadays for the therapeutic purposes for DFUs, which include topical oxygen therapies (TOTs) and hyperbaric oxygen therapies (HBOTs). In TOT, concentrated oxygen (approximately 93 %) is directly targeted to the affected wound site. Different primary factors determine the amount of O_2 delivered to chronic wound site including therapy time, pressure applied, and concentration [254]. On the contrary, In HBOT, patients are required to breathe in concentrated oxygen having a higher pressure than normal ATA (one atmosphere absolute), enhancing the distribution and supply of oxygen to the damaged wound site. It regulates immunity, microvessel and formation of granulation tissue as well as the production of collagen, playing a substantial role in reducing the risk of amputation by healing chronic ulcers in diabetic patients [256,257]. Compared to HBOTs, topical oxygen therapies are still believed to be a better strategy to treat chronicity of wounds especially in DFUs with better cost-effectiveness and small equipment requirement [258].

Table 4
Commercially available skin substitutes for healing of chronic wounds.

Name	Type	Composition	Manufacturer	References
OrCel	Epidermal and dermal	Type 1 Collagen sponge containing human dermal fibroblasts and epidermal keratinocytes	Forificell bioscience, NY, USA	[221]
StrataGraft	Epidermal and dermal	Allogenic epidermal skin substitute consisting of neonatal keratinocytes	Stratatech, a Mallinckrodt Company	[222]
Apligraft	Epidermal and dermal	Allograft-containing collagen gel composed of neonatal keratinocytes and fibroblasts	Organogenesis, Inc, Canton, Massachusetts	[223,224]
Hyalograft 3D	Dermal	Dermal autologous substitute enclosed with silicon membrane containing autologous fibroblasts	Fidia Advanced Biopolymers, Abano Terme, Italy	[225]
Hyalomatrix	Dermal	Non-woven hyaluronic acid benzyl ester pads embedded in silicon membrane	Fidia Advanced Biopolymers, Padua, Italy	[226,227]
Dermagraft	Dermal	Skin substitute using neonatal foreskins to derive keratinocytes and fibroblasts	Adnavced BioHealing Inc, La Jolla, California	[228,229]
TransCyte	Dermal	Thin biobrane silicon mesh and nylon layer featuring fibroblasts	Advanced Tissue Sciences, La Jolla, California	[230]
dCELL Human Dermis	Dermal	Split skin substitutes prepared by decellularizing human cadavers	NHS Blood and Transplant	[231]
Nevelia	Dermal	A porous 3D matrix containing stabilized collagen type 1 enclosed by semi-permeable membranes of silicon	SEMATESE Biomateriaux, ZI Les Troques, Chaponost, France	[232]
Integra Dermal Regeneration Template	Dermal	Bilayer membranes containing bovine collagen type 1, silicon pseudoepidermis, and chondroitine-6-sulphate	Integra LifeSciences, Plainsboro, NJ, USA	[232,233]
OASIS Wound Matrix	Dermal	Single and tri-layered substitute consisting of extracellular matrix obtained from mucosa of porcine jejunum	Smith & Nephew, Inc, Hull, UK	[234,235]
Biobrane	Dermal	Type 1 collagen and silicon thin layers enclosed in bilaminar mesh of nylon	UDL Laboratories, Inc, Rockford, Illinois	[236–238]
Matriderm	Dermal	1 and 2 mm thick substitutes containing type 1 collagen layers coated with elastin hydrolysate (3 %)	Skin and Health Care, AG, Billerbeck, Germany	[239]
Epicel	Epidermal	Cultured autograft; a fibrin mesh composed of human keratinocytes	Genzyme Tissue Repair Corp, Cambridge, Massachusetts	[240]
Epidex	Epidermal	Cultured epidermal autograft discs having a diameter of 1 cm containing keratinocytes extracted from hair follicles	EuroDerm Biotech and Aesthetics	[241,242]
Coll-e-derm	Dermal	Allograft containing angiogenin and type 4 collagen, initiating revascularization, assisting wound healing	Parametrics Medical, Leander, TX, USA	[243]
DermaPure	Dermal	DNA free human allograft encompassing native growth cytokines accelerating vascularization at damaged site	Tissue Regenix Group, San Antonio, TX, USA	[244]
GraftJacket	Dermal	Soft tissue skin graft predominantly comprising of collagen type 1 and 3 enhancing revascularization and cellular repopulation at the wound site	Wright Medical Group N.V., Memphis, TN, USA	[245]
AmnioBand	Dermal	Placental allograft layer comprising of chorion and amnion, retaining structural properties of ECM, providing cover to wound surface area	MTF Biologics, Edison, NJ, USA	[246]
AmnioExcel	Dermal	A tri-layered placental graft having a chorion layer enclosed in amnion layers, with extracellular matrix, growth factors and cytokines, enhancing wound closure	Integr LifeSciences Corp, Plainsboro, NJ, USA	[247]
Epicord	Dermal	Umbilical cord allograft containing hyaluronic acid and collagen ECM matrix for healing of chronic wound bed	MiMedx	[248]
NEOX	Dermal	A matrix containing amniotic and placental membranes providing wound covering for dermal ulcers	Amniox Medical., Inc, Miami, FL, USA	[249]
Endoform	Dermal	Xenograft containing ovine collagen ECM, particularly reducing MMP secretion at the wound site	Hollister Wound Care, Libertyville, IL, USA	[250]
Excellagen	Dermal	A gel composed of 2.6 % bovine fibrillar collagen, is applied directly to wound site	Taxus Cardium Pharmaceuticals Group, San Diego, CA, USA	[251]
Helicoll	Dermal	Acellular matrix free of contaminants, composed of bovine collagen	EnColl Corp, Fremont, CA, USA	[252]
Kerecis	Dermal	Sterile meshed sheet matrix containing fish collagen, applied for healing of chronic diabetic ulcers	Keresis, Arlington, VA, USA	[253]

4.8.2. *Offloading devices*

Peripheral neuropathy decreases the ability of diabetic patients to perceive skin pain. This can lead to unconscious repeated and prolonged pressure on the lower extremities of DM patients, resulting in blockage of blood flow, thus worsening the ulcer conditions. Reducing that pressure through offloading devices can help reduce the severity and enhance the healing of chronic wounds. Some of the most common offloading ways include removable cast walkers (RCW), crutch-assisted gait, wheelchair, bed rest and total-contact casts (TCC) [259]. TCC is considered an ideal method for reducing plantar pressure and treating DFUs. TCC is a casting designed specifically to reduce the weight load off the feet of diabetic patients suffering from DFU [260,261]. But because of its certain limitations including restricting patient's movement, it cannot be used frequently. Also, it is comparatively expensive and needs a professional medical staff having training in installing TCC [261]. RCW having knee-high lengths, is another offloading method that decreases peak plantar pressure. But still, high-quality clinical trials are needed to assess its working efficacy [262]. Offloading shoes is another alternative designed to help diabetic patients with chronic ulcers. Modifications can be done to the normal shoes for DFU patients but these alterations can lead to loss of dynamic balance and hence risk of falling appears. The use of properly modified and altered offloading shoes can be beneficial in reducing pressure and attaining ideal therapeutic effects [259,263,264].

4.8.3. *Compression therapies*

Compression therapy is yet another significant way of lessening the severity of DFUs. The presence of edema can worsen the chronicity of foot ulcers in diabetic patients. Therefore, compression bandaging can help manage and reduce edema in the lower limb characterized by DFU's presence [265,266]. This treatment therapy helps to enhance the flow of blood to the lower extremities by preventing the accumulation of fluid in that region. It involves the use of wraps, tight stockings, elastic bandages, hosiery as well as multifaceted compression systems. Literature studies have reported the positive influence of compression therapy in treating DFU and is considered comparatively safe in people having

no peripheral arterial defects or abnormalities [267]. Several experimental observations have shown that the use of offloading strategies in addition to compression therapy can add to the benefits and increased healing efficacy of chronic ulcers [268].

4.8.4. Negative pressure wound therapy

Negative pressure wound therapy (NPWT); a vacuum-sealed drainage and vacuum-assisted closure procedure was established in Germany for the first time in 1990. It is being used to treat chronic diabetic wounds and has been applied for the proliferation of granulation tissue and to ease edema by enhancing blood flow to the site of injury. It has also been proven beneficial in decreasing pro-inflammatory factors and removing tissue exudates from the wound microenvironment [269,270]. NPWT is an efficient cost-effective method with improved efficacy in treating DFUs with reduced amputation risk compared to conventional wound treatment options [271]. NPWT works by forcing sub-atomic pressure, thus helping to decrease inflammatory conditions to heighten wound healing physiology. A modified NPWT system has been introduced to eradicate the limitation of the use of negative pressure therapy to control infections at the wound site. The combination of NPWT with liquid infusions called NPWTi system; which contains an adaptable infusion solution applied to wound bed for washing and infiltration, has improved efficacy compared to traditional NPWT [272]. NPWTi gives us the liberty of adjusting and altering the residence time as well as the volume and type of liquid solution used, improving the healing mechanisms of the wound. The composition of liquid can also be modified for example addition of antibiotics to limit infection rate and to achieve wound cleaning [273]. In addition to that, negative pressure therapies have been recorded to enhance nerve growth factor expression by altering micro-stimulating forces at the surface of the wound [274]. NPWT also enhances granulation and vascular tissue formation by promoting the motility of endothelial progenitor cells [275].

4.8.5. Photobiomodulation therapy

Photobiomodulation therapy (PBMT) is a non-traditional energy-based physical method to treat DFU. It uses non-ionizing radiation i.e. near-infrared rays or red visible light to change the cell's mitochondrial redox potential to achieve effects like controlling inflammation, relieving pain, immune system modulation, and regeneration of skin cells [276]. PBMT uses LED lights and low-intensity lasers as non-thermal light sources. The efficacy of PBMT depends on certain controllable factors including energy and power density, wavelength, and time duration of irradiation exposure [277]. Numerous studies have reported a positive association of PBMT with enhancing metabolic activity by promoting metalloproteinase and cytochrome *C* oxidase expression as well as increasing migration and proliferation of cells involved in tissue repair [72,278]. PBMT also shows reduced inflammatory and amplified antioxidant capability of skin cells. Increased production of collagen, improved blood circulation through angiogenesis, pain reduction, wound remodeling, and microvessel formation at the wound site are all significant therapeutic effects of PBMT [279,280]. PMBT has also shown immunomodulatory effects for better healing of chronic wounds. A study found that PBMT can enhance the conversion of fibroblasts into myofibroblasts and can induce the synthesis of muscle glycogen in diabetic mouse models [281,282]. Recently, PBMT therapy has been used coupled with other wound-healing strategies. PBMT coupled with mesenchymal stem cell therapy has gained interest and has provided significant results with improved healing in chronic wounds and diabetic ulcers [283,284].

4.8.6. Electric stimulation therapy

Skin being the largest organ, possesses a skin battery in the form of cations and anions exchanged between the dermal and epidermal skin layers. This intact skin performs the role of a barrier and has *trans*-epithelial potential. When there is damage in the skin barrier, it results in partial loss of skin battery, causing a current of injury (COI) [285]. This COI results in the migration of cells to the damaged site for the healing of wounds. Hyperglycemic conditions in diabetic patients cause this COI's inhibition and disrupt the effective healing process. Electric stimulation (ES) therapy manages to re-establish the COI and enhance cellular proliferation to activate healing mechanisms [286]. ES therapy is a relatively cost-effective, non-invasive method to enhance healing in chronic wounds, especially diabetic ulcers. ES improves the blood circulation at the wound site, and has an antibacterial effect without causing resistance, thereby hurtling the progression of wound healing [287,288]. ES therapy is also involved in altering the wound microenvironment including nitric oxide, HIF-1α, and growth factors like VEGF to achieve improved healing in chronic ulcers [289]. ES is also associated with reduced pain perception of chronic ulcers in diabetic rats [290]. Factors including time duration and temperature can affect the working efficacy of ES therapy. Increased topical temperature up to 37 °C enhances blood flow to the wound site, speeding up the healing process [291].

5. Management approaches for DFU treatment

5.1. Hyperglycemic control

Poor hyperglycemic control is among many other contributing factors that lead to poor healing outcomes in diabetic foot ulcers [292,293]. Recently, different experimental as well as observational studies have been conducted showing a positive association between optimum blood glucose levels and improved healing in DFUs. Banerjee et al. (2023), in a recent study concluded that higher HbA1c levels were directly associated with reduced viability of macrophages as well as decreased IL-2 concentrations, contributing to persistent infections and wound chronicity in diabetic patients [82]. More recently, a follow-up study stated that DFU patients who had $>$ 8.15 % HbA1c levels, 4 weeks after the study initiated showed retarded healing at the 12th week. They summarized that hyperglycemic management during the initial stages of DFU affects its healing outcomes at later stages of follow-up, independent of the wound surface area [294]. Similarly, Lane et al. (2020), in their study focused on finding the association between glycemic control and wound healing outcomes and stated that patients whose HbA1c and fasting blood glucose concentrations were $\geq$ 8 % and $\geq$ 126 mg/dl respectively had increased chances of undergoing amputations as a result of poor healing in lower extremity wounds [295]. Based on literature evidence, the significance of glucose concentration management in designing proper treatment strategies for diabetic patients suffering from chronic wounds cannot be denied.

5.2. Ischemia treatment for DFU

Ischemia resulting from either reduced blood flow in macro and microvasculature or due to angiogenesis results in a reduced supply of oxygen and nutrients at the wound site, causing poor healing. Ischemia can be treated through revascularization of at least one of the major arteries supplying blood to the lower extremities to restore the flow of blood at the site of the ulcer, reducing amputation risk and enhanced healing [296]. Revascularization can be performed through either endovascular methods, anticoagulant therapies, or open bypass techniques [297]. Literature evidence suggests that bypass has been comparatively an effective method for the cause, ensuring prolonged patency in the femoral artery's bifurcation and obstruction, however, an endovascular approach might be more feasible for patients having continued angioplasty experience [298]. Atherectomy and angioplasty are common peripheral intervention techniques of revascularization for ischemic management in DFUs. Atherectomy is done by using a catheter having a rotational or directional blade, laser, or a sharp object to excise atheroma, whereas angioplasty is done by passing a small balloon from the narrow circumferenced artery and is inflated to open up the vessel,

enhancing the flow of blood [299]. However, no significant difference has been observed in clinical outcomes of open bypass and endovascular techniques, supporting the superiority of neither of the two [300]. Use of stents, beta-blockers, plaquetary inhibitors, hyperbaric oxygen therapies, and spinal cord stimulation strategies are used alone and as adjuvants after revascularization for improved response and to avoid recurrence and have been proven effective for treatment of ischemia and accelerated healing [301–303].

5.3. Diabetic peripheral neuropathy treatment

Diabetic peripheral neuropathy (DPN) causes protective sensational loss in the lower extremities, exposing diabetic patients to prolonged foot pressure, resulting in foot deformities and callus causing ulcers. Efficient glycemic control is a major factor contributing to neuropathy management [304]. Literature evidence has demonstrated pancreas transplant as an effective strategy for the restoration of normal glycemic levels as well as improved neuropathy in diabetic patients but because of response disparity and increased financial burden, the option is not opted by many [305]. Adipose tissue-derived mesenchymal stem cells have applications in DPN treatment as their use is associated with the release of neuroprotective, anti-inflammatory as well as pro-angiogenic factors having the potential to be effectively used for DPN management [306]. Biological therapies administering low-dose IL-6 have been associated with the regeneration of peripheral nerves, improved blood flow as well as reduced inflammation promoting healing, and might be an effective strategy for DPN treatment [307]. Certain analgesics have been used for the management of neuropathy-associated pain including oxycodone, acetaminophen, and tramadol but their use is coupled with adverse side effects and, therefore must be taken with care [308]. Alpha-lipoic acid (ALA) has also shown promising potential in treating DPN [309,310]. A recent study has demonstrated that ALA significantly downregulates TRPV1 receptors by regulating the NF-κB pathway, inhibiting neuropathic pain in diabetic mice models [311], showcasing their potential role in DPN management.

5.4. Infection-control strategies

5.4.1. Antibiotics

Uncontrolled infections at the wound site resulting in severe chronicity majorly lead to amputations. Therefore, managing bacterial load and controlling infections is the first and foremost step when initiating DFU treatments to maintain a healing environment. The use of antibiotics has been a traditional practice to achieve the cause. Oral, intravenous, and topical applications are the most common ways of antibiotic administration in the case of strategic infection control in DFUs. The recommended antibiotic and its dosage is dependent on additional factors including the host's immune state, hygiene conditions, the severity of the wound, previously administered antibiotics as well as type of infection to avoid resistance [312]. Antibiotic resistance has appeared to be a great threat in case of infected ulcers, especially carbapenems and β-lactamase producing gram-negative microbes developing resistance against vancomycin and methycycline [313]. To overcome this global threat, antimicrobial peptides (AMRs) are introduced and are potent against fungi, viruses, and a wide range of bacteria that have developed resistance [314]. Various AMRs have been tested for their effectiveness in controlling infections in DFUs including β-defensin-2 (hBD2), ubiquicidin (UB 29–41), nisin, pexiganan (MSI-78) as well as α-helical decapeptide (KSL-W) [315–317]. Gramicidin, nisin, and pexiganan have been under clinical trials to judge their effectiveness against infectious ulcers [318,319], however, none has been approved by the FDA to be used in humans.

5.4.2. Debridement

Wound debridement is a common practice for the control of infection at the wound site, accelerating the process of healing and preventing DFUs from progressing towards chronicity. Debridement is considered essential for the removal of necrotic, dead, and infectious tissue and to expose healthy underlying tissue to optimize healing at the affected site. Various clinical studies have signified the importance of debridement for granulation tissue formation [320], reduced bacterial loads and increased wound closure rates [321,322], enhanced cellular proliferation, and accelerated healing [323]. However, no positive correlation between the debridement approach and improved wound healing in diabetic ulcers was observed [322]. Moreover, the appropriate methods for the removal of narcotic tissue is still an ongoing debate [324]. Debridement along with other combinational therapies can be proven effective in providing the best possible treatments to accelerate healing in DFU. More experimental studies and clinical evidence are required to confirm the association.

5.4.3. Wound dressings

Wound dressings have been considered the foremost defense strategy against infections at the site of injury. Wound dressings are important to maintain a humid and healing microenvironment and their use is recommended by Clinical Practice guidelines to prevent and control infections, for exudate absorption, and to maximize healing [105]. Specific non-adherent bandages are advised for DFUs, however, recent advancements have led to the development of dressings such as hydrogels having increased potential for fighting infections at wound site [325]. Literature has shown that antimicrobial dressings with active biomaterials have been associated with better infection control and improved healing in DFUs [326–328], however, some studies have found no positive association between the use of engineered colloid or gel dressings and infectious control potential as compared to normal dressings, questioning their efficacy [329]. Therefore, more experimental studies are required in the context of confirming the applicability of advanced engineered dressings for improved healing and infection control at the wound site, especially in the case of DFUs.

6. Conclusion and future directions

Despite therapeutic advancements focused on improving complex healing mechanisms and long-term treatment necessities, the effective clinical management and assessment of chronic wounds still remains a challenge to healthcare system. Various therapies including immunotherapies, stem cell therapies and growth factor therapies, traditional ointments as well as skin substitutes have been designed for chronic wound healing, however they do not satisfy the healing requirements of distinctive chronic wounds. There is still a pertinent need for personalized and competitive treatments to address the complex nature of chronic ulcers which should be adaptable depending on the pathophysiological conditions of the wound.

DFU management requires multidisciplinary approaches, mainly through cooperation of physicians, vascular surgeons, orthopedics, shoe specialists as well as endocrinologists. Appropriate evaluation of DFUs through collaborative efforts of these field specialists is important to protect the patient's quality of life. Hyperglycemic management is considered the most important step in terms of avoiding diabetes-related complications. While choosing hypoglycemic drugs, their potential of healing ulcers as well as associated CVD risk factors should be considered. Debridement has been proven effective in increasing healing efficacy of DFU, however excessive debriding might not help. The combined use of wound dressings, antibiotics and physical therapies can accelerate healing in DFUs. Healing potentcy of some physical therapies including oxygen therapy must be tested in large sample studies to reveal its true efficiency. Exosomes, growth factors, biological scaffolds, stem cell therapies and nanotherapeutic approaches hold promising future, but further research should be conducted to understand their specific mechanisms of action. Identification of cell surface receptors and other wound microenvironmental factors influencing cellular polarization, differentiation, migration, and activation of wound healing cells in

diabetic patients needs to be done. Assessing the effectiveness of different combinational therapies can be considered and tested through clinical trials and their clinical potential can be explored to achieve maximum healing output in DFUs. The development of effective therapeutic strategies is crucial for providing improved healing outcomes, and reduced amputation risk contributing to overall physical health betterment in diabetic patients. Future research focused on advanced modalities promising effective DFU management can help reduce the healthcare burden associated with it.

CRediT authorship contribution statement

Fatima Mohsin: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Sheza Javaid:** Writing – review & editing, Data curation. **Mishal Tariq:** Writing – review & editing. **Muhammad Mustafa:** Writing – review & editing, Visualization, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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