Chapter 8

Biological Activity and Implications of the Metalloproteinases in Diabetic Foot Ulcers

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Abstract

Inadequate metabolic control predisposes diabetic patient to a series of complications on account of diabetes mellitus (DM). Among the most common complications of DM is neuropathy, which causes microvascular damage by hyperglycemia in the lower extremities which arrives characterized by a delayed closing. The global prevalence of diabetic neuropathy (DN) was 66% of people with diabetes in 2015, representing the principal cause of total or partial lower extremities amputation, with 22.6% of the patients with DN. Matrix metalloproteinases (MMPs) are involved in healing. The function that these mainly play is the degradation during inflammation that has as consequence the elimination of the extracellular matrix (ECM), the disintegration of the capillary membrane to give way to angiogenesis and cellular migration for the remodeling of damaged tissue. The imbalance in MMPs may increase the chronicity of a wound, what leads to chronic foot ulcers and amputation. This chapter focuses on the role of MMPs in diabetic wound healing.

Keywords: MMPs, wounds, diabetic foot

1. Introduction

Diabetes mellitus (DM) is a set of metabolic disorders, characterized by the presence of persistently elevated blood glucose levels caused by a deficiency of insulin production or insulin

resistance [1]. Chronic hyperglycemia is related to the appearance of microvascular complications, knowing as diabetic neuropathy (DN) that compromises the metabolism, inducing the formation of end products of advanced glycosylation and reactive oxygen species and reduction of the elimination of free radicals and endothelial dysfunction with neuronal damage. DN is a set of alterations that affect both the sensory and motor fibers as the autonomous system. Hyperglycemia is invariably associated with alterations in nerve conduction and the feet are highly susceptible to initiate phases of hypoesthesia. Vasomotor control is lost and blood flow to the extremity (vasodilation of the veins of the dorsum of the foot) is increased, but this flow is channeled into the skin and arteriovenous fistulas in the bone, which can cause hypoperfusion in other tissues. When normal capillary reflexes are lost, capillary hypertension of dependence and a decreased vasodilatory response to heat occur. Increased blood flow causes demineralization and bone osteopenia [2]. The diabetic foot ulcer (DFU) is the most common complication of lower limb DM. It is also the most disabling late complication of the disease. The World Health Organization (WHO) defines it as "a syndrome in which complications of a diverse etiology: neuropathic, vascular and infectious stemming from DM and predisposing to the suffering and development of ulcers."

There are more than 347 million people with DM in the world, of which 66% had DN in 2015, representing one of the principal causes of 22.6% total or partial lower extremities amputation [3]. DFU is considered as the major epidemic disease in the last decade; its etiological factors are DN and arterial disease. Neuropathy alone in 46%, ischemia in 12% being the most frequent neuroischemia (60%) and no risk factor identified in 12%. About 15% of diabetic patients will have ulcers in the lower extremities, half of these patients who have a single ulcer will subsequently develop another ulcer, and one third of these ulcers will cause limb amputation [4]. The worldwide DFU prevalence ranges from 1.3% to 4.8%. The current medical treatments for these chronic wounds continue to be somewhat opposed according to the country and the international guides that govern [5]. Part of the affections that these ulcers generate is in the extracellular matrix (MEC), where the matrix metalloproteases (MMP) are able to degrade all the components of MEC. The MMPs are indispensable for the healing process; among its functions is to eliminate the provisional extracellular matrix and facilitate migration to the wound center; they also participate in the remodeling of granulation tissue in the control of angiogenesis and the release of some growth factors [6, 7]. Different types of MMP expression patterns are present in a wound; it has been shown that immunity processes, such as cell migration, participate in the process of re-epithelialization and in the formation of scars [8]. Accordingly, the correct expression and regulation of MMPs are related with the healing process and therefore with a successful process of cicatrization.

2. Contents

2.1. Matrix metalloproteinases (MMPs)

The MMPs belong to a family of zinc-containing endopeptidases are calcium dependent, capable of degrading and remodeling the proteins that form the ECM and carry

out different biological and physiological functions; they are regulated by hormones, growth factors and cytokines [9]. Based on their specificity for the components of the MEC, MMPs are divided into collagenases, gelatinases, stromelysins and matrilysins. A numeric system has been adapted for the MMPs grouping them according to their structure and give place to eight different structural classes of MMPs. This system groups in five different groups those MMPs that are secreted, and in three groups to those MMPs according to their type of membrane, acquiring as MTP-MMP identification [10]. The first group of the minimal domain MMPs contains an amino-terminal signal sequence (Pre) that directs them to the endoplasmic reticulum, a propeptide (Pro) with a thiol group (SH) that interacts with the zinc and maintains them as inactive zymogens and a catalyst with a zinc binding site (Zn). The second group in addition to a minimal domain also contains a hemopexin-like domain that is connected to the catalytic domain by a hinge (H), which mediates interactions with the tissue inhibitors of the MMPs. The first and last of the four replicates in the hemopexin-like domain are linked by a disulfide bond (S-S). The third group of gelatinase-binding MMPs contains inserts resembling fibronectin (Fi) type II collagen-binding repeats. The fourth group of MMPs is furin (Fu) secreted and contains a recognition motif for serine and Fu type intracellular proteinases between their polypeptide and catalytic domains that allow intracellular activation by these proteinases. Within the fifth group is the vitronectin-like insert (Vn). The number group is included in membrane MMP (MT-MMP); these are conformed by a carboxy-terminal single chain (TM) transmembrane domain, a very short cytoplasmic domain (Cy). The seventh group has MMPs that are anchored in glycosylphosphatidylinositol (GPI), and within group eight MMP-23 is included, is membrane bound, has an N-terminal signal (SA) that targets the cell membrane, and therefore in a type II transmembrane MMP, and is characterized by a single domain of cysteine (CA) and immunoglobulin (Ig) [11]. This is shown in **Figure 1** [11].

In mammalian, MMPs are inhibited by four metalloproteinase tissue inhibitors (TIMPs), which are endogenous regulators of MMP family proteins, whose function is to determine the influence of ECM, cell adhesion molecules, cytokines, chemokines and growth factors. TIMPs are formed by an amino-terminal (N-terminal) domain, which is the inhibition domain that binds to the active site of MMPs, and a subdomain C. The capacity of these TIMPs to inhibit MMPs is due to the interaction in the N-terminal domain that binds within the cleft of the active site of the target MMP. The C-domain has two parallel β strands that are connected by an α-helix to two anti-parallel β strands. This structure provides the ability of TIMPs to interact with the hemopexin domain of some MMP [12].

There are four TIMP family members: TIMP-1, -2, -3 and TIMP-4; each of its N and C domains, in their final position, possess six cysteine residues, which constitute three disulfide domains. The N-terminal region is assembled into the catalytic domain of MMPs where the action of MMP will be inhibited; in the case of the C region, it binds to the proformas of a domain called hemopexin C—in its terminal position for the case of the MMP-2,9 and thus binds to a pro-enzyme complex inhibitor. For TIMP-2, this binds specifically to the surface of the cell with TIM-1MMP and pro MMP-2, this to carry out the activation of the

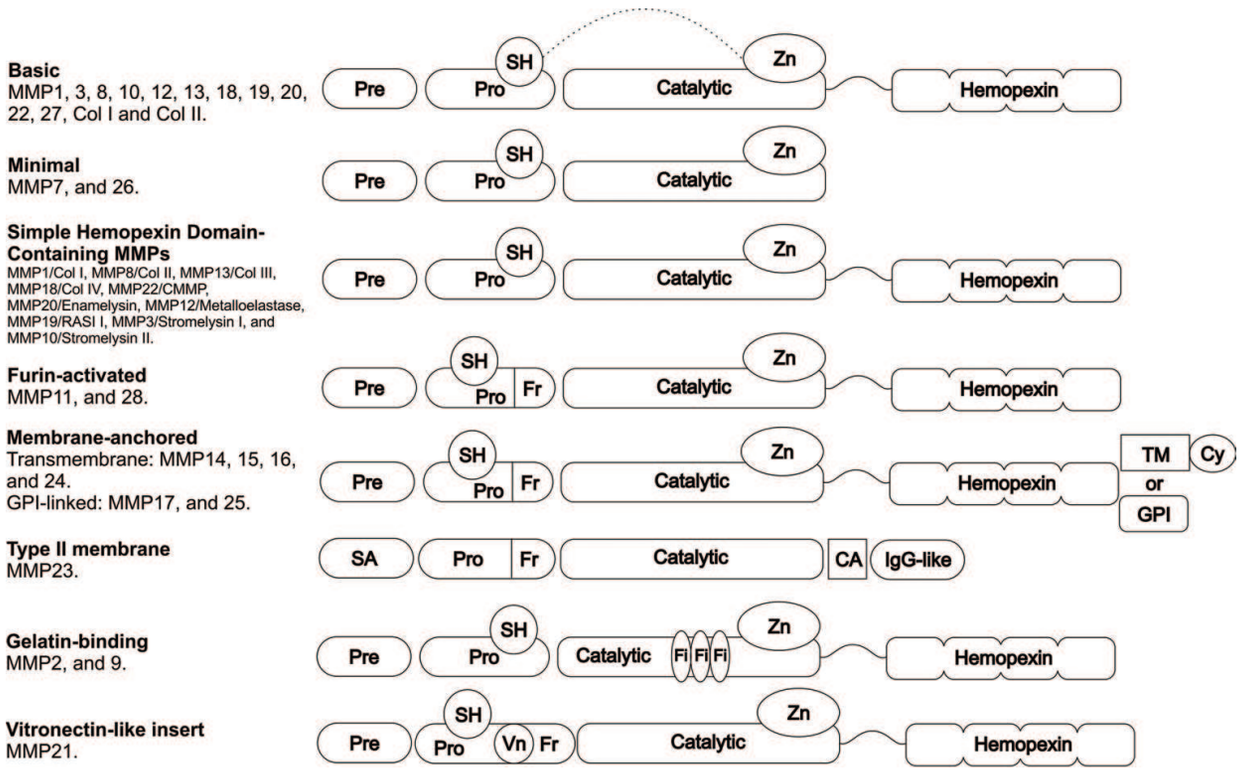

Figure 1. Domain structure of MMP groups. All human MMPs show the signal peptide, the pro-domain and the catalytic domain. Pre = pre-domain (contains the signal peptide) and Pro = pro-domain, which contains cysteine sequence that complexes Zn^{2+} in the zymogen form. The catalytic region contains the center domain. Fr = furin cleavage, Fi = fibronectin repeat, Vn = vitronectin-like insert, Cy = cytosolic, CA = cysteine array, Hemopexin = hemopexin domain, IgG-like = Ig-like domain, TM = transmembrane domain and GPI = glycosylphosphatidylinositol anchor. The hemopexin domain is linked to the catalytic center by a hinge region.

pro-MMP-2 in a simple way [13]; as consequently, TIMP-2 is an inhibitor that also functions as an activator of MMPs. The four TIMPs can inactivate the already active MMPs, but they will not do so with the same effectiveness. MMP-1,3,7 and 9 are inhibited by TIMP-1, in the case of TIMP-2 inactive to MMP-2. TIMP-3 is inactivating MMP-2,9 but similarly to the ADAM group, finally TIMP-4 inactivates MT1-MMP and MMP-2. Therefore, in regard to the function of TIMPs is the regular proteolysis activity and in those functions related to the activities of the MMPs [14]. The role of TIMP1 is expressed in mammalian tissues, specifically in reproductive organs; TIMP2 is constitutively expressed in most tissues, but not inducible by growth factors and TIMP3 is expressed in tissues as a matrix protein. TIMP4 is expressed relatively in heart, ovary, pancreas, colon and testes [12], where they observed the specific expression constitutively or inducible, which is regulated at transcriptional level by cytokines and growth factors [15]. It has been proposed to have a relevant role in processes including cell proliferation, adhesion and migration and/or apoptosis by cutting bioactive molecules that modulate these processes [16]. MMPs modulate biological processes during pathophysiological events, such as skeletal formation, angiogenesis, cell migration, inflammation, wound healing, coagulation, pulmonary and cardiovascular diseases, arthritis and cancer. They have been identified in human degrading components of ECM, cellular receptors and cytokines [17].

2.2. Regulation of MMP activity

In normal physiological conditions, the activity of MMPs is accurately regulated at four levels. (1) *Transcription*. The transcription of MMPs is induced by various exogenous signals, including cytokines, growth factors, chemical agents, physical stress and oncogenic cellular transformation, and also by cell-matrix and cell-cell interactions. The genes that control MMPs respond to extracellular signals (MMP-1, MMP-13, MMP-3, MMP-10, MMP-7, MMP-12 and MMP-9), which possess an AP-1 (activator of the protein-1) in the promoter proximal to the position −70 of the site of initiation of the transcription [18, 19]. The promoter regions of the MMP-2 and MMP-11 genes do not contain an AP-1 element [20]. The promoter region of membrane type 1-matrix metalloproteinase (MT1-MMP) gene lacks of AP-1 element, but it contains Sp-1 binding site which is essential for MT1-MMP transcription [21]. (2) *Activation of precursor zymogens.* For the regulation of MMPs by a zymogen, a biochemical change is required to turn an inactive enzyme into an active enzyme. Propeptides that maintain MMPs in their zymogen form (proMMP) can be activated by proteinases or *in vitro* by chemical agents. Proteolysis is initiated in the exposed region between the first and second propellants of a propeptide, following the specificity of the region followed by the sequence in each MMP. After the initiation of proteolysis, a part of the propeptide is separated; this unbalances the rest of the propeptide, including the cysteine-zinc interaction and allows the intermolecular process that is carried out by activated MMP mediators [22]. Plasmin is generated from the plasminogen by means of the tissue plasminogen activator, which is bound to fibrin and to a urokinase activator attached to the cellular receptor. Plasminogen being the plasminogen enhancer of the urokinase is bound to the membrane, generating pro-MMP activation such as proMMP-1, 3,7,9,10,13 and ECM movement [23]. (3) *Interaction with specific components of ECM*. The location, action and specificity of the MMPs generate an association with the components of the NDE. One of the functions of MMPs may be the destruction of ECM in tissues. The ECM has the function of storing active biological molecules as they are growth factors [11]. For example, the degradation of type I collagen by collagenase is associated with osteoclast activation and keratinocyte migration during re-epithelialization [24]. (4) *Inhibition by TIMPs.* TIMPs are specific inhibitors that bind MMPs in a 1:1 stoichiometry and their expression is regulated during development and tissue remodeling. Under pathological conditions associated with unbalanced MMP activities, changes of TIMP levels are considered to be important because they directly affect the level of MMP activity. TIMPs are specific inhibitors that bind MMPs in a 1:1 stoichiometry; their expression is regulated during development and tissue remodeling. Under pathological conditions associated with unbalanced MMP activities, changes of TIMP levels are considered to be important because they directly affect the level of MMP activity. The proteolytic activity of MMPs is inhibited specifically by TIMPs and by nonspecific proteinase inhibitors, such as a 1-proteinase inhibitor and α2-macroglobulin. TIMPs are the major endogenous regulators of MMP activity in tissue, which are expressed by different cell types, including fibroblasts, keratinocytes, endothelial cells and osteoblasts. As inhibitors of MMPs, TIMPs maintain the balance between the ECM deposition and degradation in physiological and pathological processes [22].

2.3. Wound repair

Wound repair is a physiological event, in which tissue injury results in a repair process that finally leads to restoration of structure and function of the tissue [25]. During wound healing, the degradation of the components of the ECM by MMP is necessary to remove and rearrange the provisional matrices and allow cell migration [26]; thus, basal keratinocytes are the predominant source of MMP. Cutaneous wound repair can be divided into three overlapping phases: (i) formation of fibrin clot followed by inflammation, (ii) re-epithelialization and granulation tissue formation and iii) matrix formation and remodeling [27].

2.3.1. Formation of fibrin clot followed by inflammation

The first step for wound repair is a fibrin clot formed through platelet aggregation and blood coagulation. The coagulation cascades are initiated by coagulation factors of the injured skin, this by means of the extrinsic system. The thrombocytes are activated to generate aggregation by means of exposed collagen, this being controlled by the intrinsic system. Following this, the injured vessels continue with a vasoconstriction of 5 or 10 minutes, being triggered by platelets; this to reduce blood loss and begin to fill the void of tissue that was generated by the wound through a compound clot cytokines and growth factors [28]. Vasoconstriction generates clots, followed by vasodilation, where thrombocytes invade the wound matrix on a provisional basis [27]. The formed clot contains fibrin molecules, fibronectin, vitronectin and thrombospondin, forming the provisional matrix as a scaffold structure for the migration of leukocytes, keratinocytes, fibroblasts and endothelial cells [29]. Platelets influence leukocyte infiltration; this is mediated by the synthesis of factors for chemotaxis. Platelets and leukocytes release cytokines and growth factors for activation of the inflammation process. The interleukins IL-1α, β, IL-6 and TNF-α are involved, such as FGF-b, IGF, TGF-β are involved in the process of collagen synthesis, factors such as FGF-B, VEGF subunit A, HIF-1 and TGF-β are involved for angiogenesis and for the EGF, FGF-b, IGF, TGF-α [30]. See **Figure 2**.

2.3.2. Proliferation and re-epithelialization

In the proliferation phase, the main focus of the healing process is to cover the wound surface, to form granulation tissue and to restore the vascular network. After to tissue injury, platelets are recruited to the injury site to stop the bleeding. Platelets also release platelet-derived growth factor (PDGF) that initiates the migration of neutrophils and macrophages, in addition to causing the synthesis of growth factors and related cytokines in wound healing [31]. This factor is also involved in the stimulation of collagenase and in fibroblastic cell of human skin, with MMP-8 being more frequent in tissue damage [18]. The MMP-1,2,3,9 are synthesized by platelets; the function of MMP-1,2 aid in the balance of platelet adhesion and the conglomeration thereof [27]. In the inflammatory process, cells such as neutrophils are involved in the wound to protect infections and generate the synthesis and stimulation of MMP-8, being necessary for wound debridement and division of damaged type I collagen; the MMP-9 also participates by separating the collagen types that also participate (IV, V and X) [5].

Through the control of regulatory cytokines such as IFN-γ and TGF-β, the synthesis of collagen, fibronectin and other basic substances necessary for the healing of fibroblast wounds

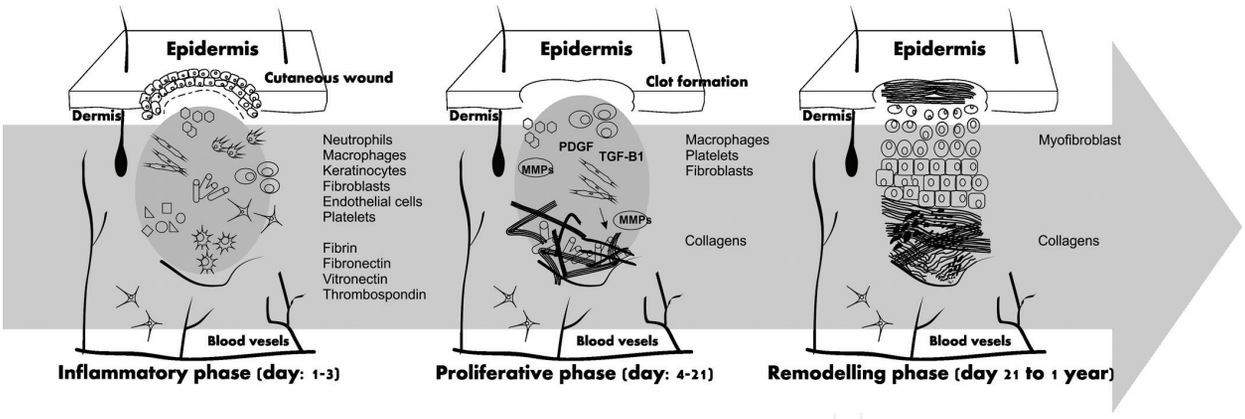

Figure 2. Wound repair phases. The wound repair phases involve: 1. Formation of the clot. The fibrin clot is being formed through platelet aggregation, coagulation cascades, fibrin molecules, fibronectin, vitronectin and thrombospondin to form a temporary scaffold for the initiation of leukocyte, keratinocyte, fibroblast and endothelial cell migration. 2. Proliferative phase. The platelets initiate the synthesis of MMPs 1, 2, 3, 9; MMP-1 and MMP-2 generate a balance in the adhesion of platelets and secrete PDGF initiating the migration of neutrophils, macrophages and growth factors. This generates the stimulation of different types of collagen, which are separated with the help of MMP-9. This stimulation of collagen is given by fibroblastic cells to begin the healing process and cover the surface of the wound. 3. Remodeling phase. In the remodeling of granulation tissue, there is an increase in the synthesis of collagen generating a decrease in fibroblasts. The keratinocytes initiate their migration to the clot through the granulation tissue to initiate tissue repair.

represents the basis for the new connective tissue matrix. Therefore, the migration of local fibroblasts along the fibrin network and the initiation of re-epithelialization from the wound edges, neovascularization and angiogenesis are activated by capillary sprouting [27]. This process is activated by signaling pathways of epithelial and non-epithelial cells at the wound edges, which release a myriad of different cytokines and growth factors such as EGF, KGF, IGF-1 and NGF [30].

In the process of re-epithelialization participate, the laminin is a component basal of the epithelium and plays roles in cell adhesion, migration, proliferation, differentiation and angiogenesis. There are 15 isoforms of laminin, of which laminin-5 is specific to epithelial cells. Laminin-5 has been shown to promote keratinocyte migration and induction of MMP-9; cell motility depends on MMP-9 activity, indicating that MMP-9 plays a role in re-epithelialization [32]. It is known that MMP-2 and MMP-14 cleave laminin-5 [33, 34] generating a fragment that binds to the epidermal growth factor receptor (EGF), which stimulates cell migration. The released FGF-2 from macrophages binds to heparan sulfate, which induces the growth of fibroblasts and endothelial cells [30]. Platelets and macrophages release vascular endothelial growth factor (VEGF), stimulating proliferation and migration of endothelial cells, as well as keratinocyte migration where are involved the MMP-1, MMP-2, MMP-9, and MMP-13 this plays a critical role in wound healing [35, 36].

2.3.3. Matrix formation and remodeling

Remodeling is the last phase of wound healing and occurs from day 21 to 1 year after injury. The collagen synthesis increases throughout the wound, whereas fibroblast proliferation decreases successively, adjusting a balance between synthesis and degradation

of ECM [37]. This shows a signal. It gives a retraction and reorganization of filaments in the intracellular tone towards cell migration, where the keratinocytes migrate into the fibrin clot by infiltrating the upper layers of the granulation tissue [38]. The onset of granulation tissue repair is stopped by apoptosis, since in an old wound it is characterized by the absence of vascular structures within it and by having only ECM and having absence of cells [39]. In the case of maturation of a wound, type III collagen is displaced by collagen type I [40]. In the early stages of the remodeling phase, the provisional wound matrix contains predominantly fibrin and fibronectin, which are subsequently replaced by proteoglycan and collagen type I and III molecules, increases the tensile strength of the scar matrix. Fibroblasts are stimulated to transform into myofibroblasts that contract the wound matrix. At the end of the remodeling stage, the high density of blood vessels and myofibroblasts decrease with apoptosis. At the end of the process, the wound is completely closed [41, 42].

2.4. Proteolysis in wound repair

The proteolytic degradation of ECM is necessary in many stages of wound repair, such as interim matrix degradation, angiogenesis, keratinocyte migration and remodeling of granulation tissue ECM [43]. MMP-28 and MMP-19 are found in keratinocytes in the basal strata and suprabasals of healthy skin in an *in vivo* model [44]; in addition, MMP-19 is also found in hair follicles, endothelial cells and in veins and arteries [45]. In some wounds, the basal membrane is destroyed; this lesion temporarily retains MMP-1 expression in migratory cells that are expressed in the dermis, such as keratinocytes, with absence of a basement membrane [46]. The synthesis of MMP-1 is paramount for the initiation of re-epithelialization, so keratinocytes bind with type I collagen. Native type I collagen is known to generate MMP-1 synthesis in cells in vitro, contrary to basement membrane proteins such as fibronectin or collagen type III that do not generate this synthesis of MMP-1 [47].

MMP-1 is important in the process of migration of keratinocytes into native type I collagen and its synthesis, which is being generated by $\alpha 2\beta 1$ integrin [24]. In humans, the $\alpha 2\beta 1$-MMP-1 complex is to function as a motor stimulating migration of keratinocytes on type I collagen during re-epithelialization. Subsequent to the initiation of re-epithelialization, a new basement membrane is generated; here, the expression of MMP-1 in epidermis is arrested by cellular junctions with basement membrane proteins [47]. The role of MMP-1 in the wound healing process has been demonstrated in murine models, where there has been a delay in total wound [48].

Collagenase-3 (MMP-13) has been found in human skin, and it is related to the role of wound healing in the dermis. MMP-13 is synthesized by fibroblasts in those human cutaneous fetal wounds [49]. MMP-1 and MMP-13 can also regulate the survival of fibroblasts during dermal wound healing (affecting fibroblasts), which is mediated by matrix shrinkage and matrix rigidity [50]. MMP-8 is expressed by neutrophils, being stored in the cellular granules and being secreted to the outside by the activation thereof. In excessive skin wounds, the MMP-8

is overexpressed [52]. Some experimental models in MMP-13-deficient knockout mice demonstrate that there is a MMP-13 compensation for MMP-8, demonstrating a delay in wound healing due to impaired re-epithelialization, low level of infiltration of neutrophils and a persistent inflammatory syndrome [51, 52].

The stromelysins, MMP-3 and MMP-10, are expressed by epidermal cells during wound repair in human and mouse wounds. MMP-3 is expressed by the basal proliferating keratinocytes behind migrating cells, whereas MMP-10 migration occurs by keratinocyte leaf [53]. MMP-3 can destroy substrates of the ECM, basement membrane proteins, in addition to increasing the activity and availability of cytokines and growth factors such as FGF-b and HB-EGF [22]. This poses a role for MMP-3 in the organization of the new basement membrane and in the involvement of cell migration and proliferation. The remodeling of the basement membrane after re-epithelialization, and degradation of the fibrin containing the provisional matrix, MMP-9 may be involved in the final adjustment of the epidermal tissue after wound healing by remodeling the cell [54].

MMP-2, 9, MT1-MMP and MMP-19 are synthesized in endothelial cells [55, 56]. Within these, MMP-2 and 9 have key participation in physiological aspects such as those tumorigenic and angiogenic processes [57]. These MMPs are responsible for degrading components of the vascular basement membrane, which is essential for the generation of new blood vessels. These MMPs physiologically participate in the activation of growth factors and cytokines related to angiogenesis [30]. MT1-MMP when involved in the generation of blood vessels is related to fibrinolytic and collagenolytic activity to generate invasion of these new vessels by crossing fibrin barriers in the stroma of damaged tissue [58]. MMP-19 is involved in the proliferation of epithelial tissue, endothelial cells, fibroblast cells and microvascular cells in macrophages [59]. See **Figure 2**.

2.5. MMPs in chronic wounds

Chronic wounds are defined as wounds where healing is delayed due to one or more factors. Depending on the etiology, a wound is considered to be chronic if it is still present after 4–6 weeks [60]. Such wounds may from the outset show chronic features, for example leg ulcers, pressure ulcers (PUs), DFUs and amputation stumps, or may initially be acute in nature (such as surgical wounds and traumatic wounds) and become chronic after several weeks of stagnation due to the patient's general condition or inappropriate care; they may last for several months or years.

The MMPs implicated in the wound healing process are listed in **Table 1**.

Chronic wounds present higher levels of protease activity than acute wounds. This has been demonstrated through comparative trials analyzing MMP levels in different populations. Chronic wounds, including venous leg ulcers (VLUs) [40, 61, 62], DFUs [63, 64], PUs [65] dehiscent surgical wounds and acute wounds that have become chronic, were found to have elevated MMP activity.

Type of MMPs	Subgroup of MMPs	Metalloprotease
Soluble gelatinases	Gelatinases	MMP-2: Gelatinase-A
		MMP-9: Gelatinase-B
Archetypal MMPs	Collagenases	MMP-1: Collagenase-1, interstitial collagenase
		MMP-8: Collagenase-2, neutrophil collagenase
		MMP-13: Collagenase-3
	Metalloelastase	MMP-12
	Stromelysins	MMP-3: Stromelysin-1
		MMP-10: Stromelysin-2
		MMP-11: Stromelysin-3
Matrilysins	Matrilysins	MMP-7: Matrilysin
		MMP-26: Matrilysin-2

MMP, matrix metalloproteinase.

Table 1. The main MMPs is involved into the wound healing process.

There is evidence that associates DM to changes in the foot structure, including abnormalities in fiber structure and organization, increased tendon thickness, volume and a tendency of impairing biomechanical properties [66]. Interestingly, these alterations may represent features of the ECM, which is in a constant state of dynamic equilibrium between synthesis and degradation. Besides the relevance of MMPs in the ECM and their role in the pathophysiology, data linking these proteases to the development and progression of diabetic disorders are still scarce. It has been found strong expression of MMP-13 and MMP-3 in diabetic foot ulcer both in diabetic and healthy, whereas MMP-13 expression was upregulated and MMP-3 expression decreased in the diabetic ulcer healing model. Moreover, upregulation of MMP-9 and MMP-13 and increased enzymatic activity of MMP-9 in a model *in vitro* treated with high glucose concentration is found [67].

As a result, it was obtained that at high glucose concentrations collagen degradation is induced by overproduction of MMPs, which leads to a vulnerable tissue [68]. Adding the MMPs also to the process of generation of diabetic fibrosis, this being a pathology differentiated by the excess of MMPs in the ECM generating changes in the same. This is a result of an imbalance between MMPs and TIMPs activity tissue such as hyperglycemia, dyslipidemia and hypertension [69], but increased production of collagen and other ECM components may also be involved. In fact, recruitment of inflammatory cells have been connected to dysregulation of homeostasis fibroblasts followed by secretion of ECM proteins, which results in an increased turnover and remodeling of the ECM. Moreover, MMPs are able to increase release of TGF-β1, which results in fibroblast cell proliferation and collagen I

degradation [70]. Therefore, the relationship between MMP/TIMP may be associated with the development of diabetic ulcer as a consequence of poor regulation of ECM [69]. It is known that during DM there is no efficient wound healing; this being a consequence of the complications that occur in the metabolism and being a greater production of gelatinases in diabetes as a result of a period of inflammation [71]. MMPs are present in the degradation of the ECM, but also participate in the recovery of the trauma and promoting the renewal of the tissue. Likewise, the relationship with collagen is involved in the pathogenesis of the diabetic ulcer, implying poor tissue regeneration through a decrease in MMP-3 [71].

2.6. MMP levels in diabetic wound healing

Persistent hyperglycemia in the blood of diabetic patients induces the majority of the micro- and macrovascular complications associated with DM [20] and increases MMP activity directly or indirectly through oxidative stress or advanced glycation end products (AGEs) [72, 73]. An increased activity of MMPs may initiate the development of diabetic peripheral arterial disease. Hyperglycemia affects the regulation of MMP/TIMP and increases the activities of MMP-1, MMP-2 and MMP-9 in vascular cells, stimulating the degradation of the ECM and causing an imbalance in diabetes [74]. An increase in expression of MMP-2 and MMP-9 as well as protein expression of TIMP-1 may be a resulting factor in impaired wound healing and might provide an explanation for human arterial vasculature in type 2 DM [73].

The significantly higher levels of MMP in patients with metabolic syndrome as compared with normal individuals indicate that such patients may have high tendencies of developing other physiological problems [75]. The process of wound healing necessitates ECM degradation to be controlled; thus, an imbalance between ECM formation and the degradation process could lead to the development of chronic ulcers or fibrosis [76]. Cellular and biochemical imbalances, tissue damage, or other disease conditions may present varied effects in the healing process. This also upsets the proteases, cytokines, and growth factors leading to an absence or delay of wound closure preventing successful skin repair [72].

Enzyme activity affected by hyperglycemia disrupts the expression of MMPs in diabetes, and this generates an increased proteolytic environment provoked by an alteration in MMPs and TIMPs that affects patients with diabetic ulcers [77]. In healthy tissues, the levels of MMPs and TIMP are low; however, their synthesis and the activation of these are stimulated at the time of the remodeling of a tissue. In healthy skin, only the constitutive MMP-3, 7, 19, 28 and TIMP-1 expression has been documented [78]. Increased levels of MMP-1, MMP-8 and MMP-9 have been associated with a slow epithelial regeneration to heal wounds, with relatively low TIMP levels. MMP-9 degrades fibronectin into fragments, which further activates MMP, cell migration and proliferation. This provokes white blood cell infiltration, tissue damage and continuous inflammation. MMP-1, MMP-8 and MMP-9 are highly expressed in venous wounds in the absence of TIMPs [79, 80]. In addition, overexpression of MMP-9 and MMP-2 has been found in serum of patients with metabolic syndrome. The altered expression of MMPs may provoke pathogenesis in several tissues [75, 81]; the altered gene expression in MMP-9 is a cause of non-healing diabetic ulcers, being augmented in

diabetic patients and not found in healthy patients [52], and MMP-1, MMP-2, MMP-8 and MMP-9 were highly expressed in normal and chronic diabetic wounds with a decrease in TIMP-2 [63]. This could be due to high proteolytic surroundings promoting poor healing in diabetes. Similarly, there was an overexpression of MMP-1 and MMP-9, as well as TIMP-1, in keratinocytes derived from foot ulcers in diabetic type 1 patients, supporting the theory on the upregulation of MMPs and TIMPs in diabetic foot ulcers [78]. Increased expression of MMP-9, TNF-α and other growth factors in DFUs has been found and concluded that they could be linked with slow-to-heal ulcers in diabetics and therefore a target for new therapeutic management [71].

Ascertained that MMPs and TIMPs are elevated in chronic wounds; however, they may also play a role in determining the level of chronicity. Yadav et al. [75] elucidated that chronicity is associated with an increase mainly in MMP-9 and MMP-8 and elastase activity that may eventually alter collagen synthesis and the release of growth factor and cytokines into the site of injury [82].

2.7. MMPs, prediction and healing in DFUs

MMPs are associated with wound healing. Investigating its expression in chronic wounds helps to generate a better evaluation in the prognostic aspect for diabetic foot ulcers; in this way, this knowledge assists in the investigation of aspects for the inhibition of these. DFUs often fail to heal, and the mechanism is not well explained. Normal wound healing is a complex process involving a highly orchestrated cascade of events that include hemostasis, inflammation, proliferation, angiogenesis and remodeling. In each of these events, the ECM interacts with growth factors and cells. Delayed healing is characterized by an increase in MMPs and a decrease in the levels of TIMPs and growth factors (specifically transforming growth factor TGF-β). MMPs and TIMPs are synthesized by cells associated with wound healing, where their concentrations vary according to the stage of healing in which the wound is found [83, 84].

Investigations on DFU wounds are limited by appearance to obtain tissue biopsies. The wounds secrete liquid, which can be obtained in a non-invasive way for the patient, solving the problem a little for future investigations. The use of wound secretion is supported by previous investigations, where a high bacterial count has been demonstrated, and this of course resulting in poor wound healing [85]. High concentrations of MMP-9 have been demonstrated, giving a prediction that would lead to poor healing in DFU. Although the mechanism that generates the increase of MMP-9 is not yet known, it is associated with the inflammatory syndrome, since MMP-9 is being synthesized by neutrophils and macrophages [86]. It has been found in previous studies that the high bacterial count in the wound despite the absence of infection is indicative of poor wound healing [85]. Generating the hypothesis about a high bacterial count and high concentrations of MMP is also related to poor healing. It has also been demonstrated a statistically significant relationship between the MMP-1/TIMP-1 and the favorable healing [87]. MMP-1 is the main responsible for healing collagenase-related due to the benefit it brings to complete the proliferative phase; this has shown that degradation of collagen I is required for keratinocyte migration, which involves re-epidermization [24, 88]. Other studies of MMP have studied the role of MMP-2; however, it is not yet clear, due to variations in expression or concentration levels. This proposes the role of MMP-2 at least in chronic wounds, because MMP-2 is known to be synthesized by fibroblasts that are secreted in the proliferative phase where inflammation predominates [89, 90].

2.8. MMPs and topical and biologic therapies for DFU

It is reported that the dynamic changes on the content and activity of MMP-2 and/or TIMP-2, are secreted by fibroblasts majorly, play much essential parts in the normal healings, especially during the midterm and later phases, including accelerating revascularization, granulation tissues regeneration as well as the connective tissues reformation and safeguarding the normal dermis to some extent [91]. Owning to the decreased content and/or activity of growth factors and the disturbed balance of MMPs/TIMPs system, which results in excessive solvent activity and then reduced content or damaged structure of the growth factors and ECMs, the diabetic cutaneous ulcers are always poorly healed. MMP-2 is found to be excessively generated while TIMP-2 is deficiently secreted in diabetic chronic wounds, and the pathologic imbalance may bring about retarded progress of tissue regeneration and revascularization [86]. The efficacy of autologous platelet-rich gel (APG) on refractory wounds in the healing mechanism is recognized [92, 93] including upregulating the content of many growth factors and releasing antibacterial peptides [94]. Furthermore, in some basic researches, TGF-β1 has been reported to inhibit the generation of MMP-2 by depressing its genetic transcription and enhance that of TIMP-2 meanwhile [5]. In preliminary clinical studies, the local concentration of TGF-β1 increases after APG treatment [95]. This has been proven and reported, where APG treatment may suppress the expression of MMP-2 and promote that of TIMP-2 in the diabetic chronic refractory cutaneous wounds and furthermore decrease the ratio of the MMP-2/TIMP-2, and TGF-β1 may be related to these effects [96].

The photobiomodulation (PBM) is a noninvasive form of light therapy for wound healing, whereby several biological, chemical and cellular processes are stimulated to speed up healing; investigations carried out demonstrated PBM to enhance wound healing [97]. The biostimulatory effect of PBM in the near infrared (NIR) range modulates wound healing events in various cells. This generates an increased collagen activity twofold, increased MMP-2 activity, upregulated MMP-1 and TIMP-2 expression and down regulated MMP-2 and IL-1β [98]. These findings are of therapeutic importance in situations with depleted smooth cells, weakened ECM and increased pro-inflammatory markers as major pathological components. The PBM is able to alter the expression of MMPs in diabetic wounds and enhance collagen production; however, experiments done on human skin demonstrated variations in gene expression in the fibroblasts [99]. Yadav et al. (2014) found that PBM altered 49 genes involved in the ECM in vitro, with genes in a diabetic wounded cell model mostly downregulated, among which were MMP-1, MMP-2, MMP-8, MMP-12, MMP-14 and MMP-16 [75]. PBM is known for its stimulatory effects and promotes MMP activity and gene expression; hence maintaining a dynamic balance between the proteolytic activity and degradation could be a target for therapeutic advancement [99]. However, its effect on various matrix proteins still needs to be further understood.

3. Conclusions

In the course of the regeneration of a lesion, degradation in the formation of blood vessels is necessary, also so that there is adequate cell migration and a proteolysis of the ECM to obtain a remodeling of granulation tissue. Consequently, the degradation is under a precise and strict control, where the loss of homeostasis between ECM deposition and proteolysis results

in a significant failure in wound healing, as occurs in DFUs. MMPs play a significant role in tissue remodeling; their role in normal and abnormal wound healing is not well characterized. The MMPs are known for degradation of the ECM and have been shown to be upregulated in most pathologies; in the case of DFUs, they have been recognized as predicting and its expression may show alterations in the tissues, serum, plasma or fluid of wounds identify the mechanisms involved in wound healing and thereby to intervene proactively to prevent the normal wound from becoming chronic and later in diabetic ulcer or amputation and if this progress may lead to death.

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