Proteases and the Diabetic Foot Syndrome: Mechanisms and Therapeutic Implications

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The diabetic foot syndrome represents a major problem in the health care of diabetic patients. Understanding the molecular basis of this disease is an important step toward a rational treatment. Due to the systemic character of diabetes, disturbances in several basic cell functions appear to contribute to impaired wound healing. Many essential processes of normal wound healing are regulated in large part by growth factors and proteases, and changes of their expression and activity are relevant for the pathogenesis of the chronic wound. This review summarizes the current status of research on diabetic foot syndrome and describes new implications for the treatment of this syndrome.

The diabetic foot syndrome is clearly one of the most important complications of diabetes. It not only occurs as a typical complication in the late stages of diabetes but also in patients with newly diagnosed diabetes (1). Despite the postulations of the St. Vincent Declaration that within 5 years the amputation rate has to be reduced by 50%, there are ~30,000 amputations reported each year in Germany due to the diabetic foot syndrome (2–6). Greater success in reducing the diabetic foot syndrome can be achieved using structured diagnosis, classification, and therapy of diabetes (7–12). For example, chronically elevated blood glucose levels result in reduced leukocyte function and cell malnutrition, which contribute to a high rate of wound infection and associated healing problems (13,14). Due to the systemic effects of diabetes, not only do cellular abnormalities exist but interactions of growth factors and other mediators of wound healing are also impaired (15,16). Thus, understanding the cellular and molecular abnormalities that contribute to the diabetic foot syndrome will enable the rational development of treatments that will reduce the incidence and severity of this major complication of diabetes.

BIOLOGY OF NORMAL WOUND HEALING

— The physiological cellular response to tissue injury in the skin progresses through a sequence of phases that is structured with regard to both time and space and normally results in a nearly complete recovery of the anatomic and functional integrity of the injured area (Fig. 1). The phases of wound healing—hemostasis, inflammation (acute phase), proliferation (granulation and epithelization), and remodeling—partly overlap and are coordinated in large part by cytokines and growth factors (17–20).

In practical terms, wounds can be described as either acute or chronic with respect to healing. In chronic wounds, the duration of the wound healing processes is either much slower or actually static, which results in anatomic and functional restrictions (21). In general, wound healing depends on several factors, including the patient's age and physical condition, the location of the wound, the cause of the injury, and accompanying diseases such as diabetes or renal insufficiency, which all have a negative effect on wound healing processes.

The complex and structured dynamics of wound healing involve several populations of cells (thrombocytes or platelets, neutrophile granulocytes, macrophages, fibroblasts, and keratinocytes), soluble factors (cytokines and growth factors), and proteases (e.g., matrix metalloproteinases [MMPs], plasmin, and elastase). The initial phase of healing is hemostasis, which is initiated by the activation of the clotting cascade. The resulting fibrin clot entraps erythrocytes and platelets and blocks blood flow. The fibrin clot forms the provisional wound matrix, and numerous growth factors that are released from the platelets granules chemotactically attract neutrophils, fibroblasts, endothelial cells, and keratinocytes into the wound. These growth factors include platelet-derived growth factor (PDGF), platelet-derived angiogenic factor (PDAF), transforming growth factor-β (TGF-β), and epidermal growth factor (EGF). This initial release of growth factors from platelets is very important in initiating the following phases of wound healing (Table 1) (22).

Within 6 h after tissue injury, the inflammation phase starts. Neutrophil granulocytes are the first cells that appear in wounds. They control the contamination with bacteria and cleanse the wound from cell detritus. After ~48 h, the concentration of neutrophil granulocytes reaches its maximum. Monocytes begin infiltrating the wound site ~24 h after injury, attracted by chemotactic factors including complement factor 5α, degradation products of fibrin, and TGF-β. In response to cytokines in the wound, monocytes differentiate into wound mac-

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Abbreviations: EGF, epidermal growth factor; IL, interleukin; MMP, matrix metalloproteinase; MT-MMP, membrane-type MMP; PDAF, platelet-derived angiogenic factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloprotease; TNF-α, tumor necrosis factor-α.

rophages, which are necessary for wound repair. Inflammation and proliferation overlap in the process of wound healing. The proliferation phase of healing is primarily characterized by granulation tissue (15). MMPs take part in the structured development of granulation tissue by removing damaged matrix proteins, helping cells migrate into the wound, and developing new blood vessels.

About 2 days after injury, macrophages that emerged from monocytes start to express growth factors. These are the dominant types of cells during the 3rd and 4th day. Macrophages continue to release PDGF, macrophage angiogenesis factor, and TGF-β. Together, PDGF, macrophage angiogenesis factor, and angiotensin stimulate the formation of new blood vessels, generating the characteristic granulation tissue in the wound. EGF, keratinocyte growth factor, and PDGF stimulate epidermal cells to migrate, divide, and differentiate (keratinize), covering the granulation tissue with a cellular barrier to desiccation and infection (15,18,23–25).

The remodelling phase begins about the 7th day of wound healing and can continue for 6 months to a year. Early in the remodelling phase, the provisional wound matrix, which consists predominately of fibrin and fibronectin, is replaced with proteoglycan molecules and collagen molecules (type III, type I) that become cross-linked by enzymatic action, which greatly increases the tensile strength of the scar matrix. In addition, some fibroblasts are stimulated to transform into myofibroblasts that contract the wound matrix. In the final stages of remodelling, the high density of new blood vessels and myofibroblasts in the scar decrease as vascular endothelial cells and fibroblasts undergo programmed cell death (apoptosis), and the hypertrophic epidermal layer becomes thinner. These complex processes are regulated by the integrated actions of growth factors, cytokines, proteases, and extracellular matrix components. At the end of the wound healing process, the wound is completely closed. However, the repaired tissue does not completely regenerate the original tissue structure, and some level of functionality of the scar tissue is usually lost (15,22).

PATHOGENESIS OF WOUND HEALING IN CHRONIC WOUNDS

— Results of many studies have identified defects of wound healing in patients with diabetes that can be explained in large part by dysfunctional wound cells and by imbalances in key proteases, cytokines, and growth factors. In contrast to normal wound healing, the inflammatory reaction in poorly healing diabetic wounds appears prolonged, which generates a correspondingly intensified protease response, in particular MMPs and neutrophil elastase (Fig. 2). These inflammatory reactions are possibly the result of bacterial contamination and recurrent painless tissue trauma. Bacterial endotoxins, fragments of extracellular matrix, and cell detritus maintain this inflammation, which is evidenced by the large number of neutrophil granulocytes in the wound. The granulocytes also secrete proinflammatory cytokines, particularly tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β. Both of these cytokines are capable of directly stimulating the synthesis of MMPs. In addition, TNF-α stimulates its own secretion and that of IL-1β, which can contribute to a persistent inflammatory status (15,18, 26). Thus, normal wound healing requires a balanced interaction of growth factors, cytokines, proteases, and extracellular matrix. In chronic wounds, the high level of proteases in the wound site leads to a disrupted and uncoordinated wound healing process by degrading matrix proteins and growth factors that are

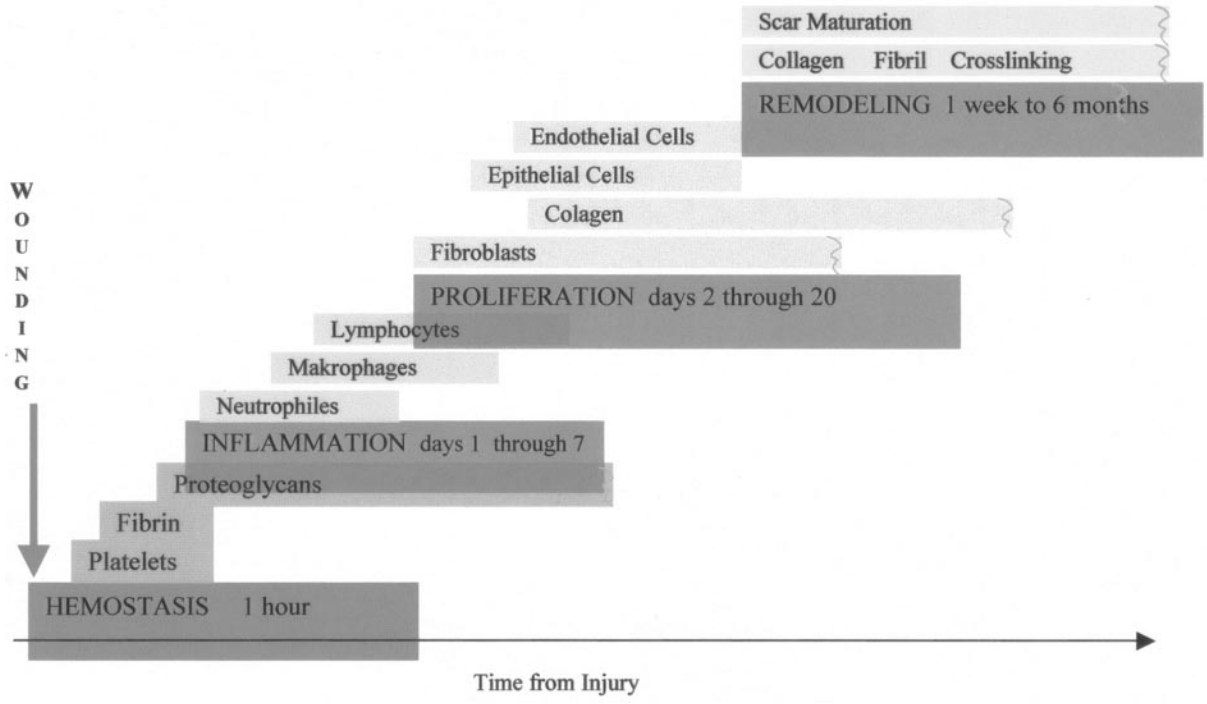

Figure 1—*Phases of normal wound healing.*

Table 1—*Major growth factor families*

Growth factor family	Cell source	Actions
TGF-β: TGF-β1, TGF-β2, and TGF-β3	Platelets	Fibroblast chemotaxis and activation
	Fibroblasts	ECM deposition
	Macrophages	Collagen synthesis
		TIMP synthesis
		MMP synthesis
		Reduces scarring
		Collagen
		Fibronectin
PDGF: PDGF-AA, PDGF-BB, and VEGF	Platelets	Activation of immune cells and fibroblasts
	Macrophages	ECM deposition
	Keratinocytes	Collagen synthesis
	Fibroblasts	TIMP synthesis
		MMP synthesis
		Angiogenesis
FGF: acidic FGF, basic FGF, and KGF	Macrophages	Angiogenesis
	Endothelial cells	Endothelial cell activation
	Fibroblasts	Keratinocyte proliferation and migration
		ECM deposition
IGF: IGF-I, IGF-II, and insulin	Liver	Keratinocyte proliferation
	Skeletal muscle	Fibroblast proliferation
	Fibroblasts	Endothelial cell activation
	Macrophages	Angiogenesis
	Neutrophils	Collagen synthesis
		ECM deposition
		Cell metabolism
EGF: EGF, HB-EGF, TGF-α, amphiregulin, and betacellulin	Keratinocytes	Keratinocyte proliferation and migration
	Macrophages	ECM deposition
CTGF	Fibroblasts	Mediates action of TGF-βs on collagen synthesis
	Endothelial cells	
	Epithelial cells	

Cytokines involved in wound healing	Cell source	Biological activity
Proinflammatory cytokines		
TNF-α	Macrophages	PMN margination and cytotoxicity, ± collagen synthesis, and provides metabolic substrate
IL-1	Macrophages	Fibroblast and keratinocyte chemotaxis and collagen synthesis
	Keratinocytes	
IL-2	T-cells	Increases fibroblast infiltration and metabolism
IL-6	Macrophages	Fibroblast proliferation and hepatic acute-phase protein synthesis
	PMNs	
	Fibroblasts	
IL-8	Macrophages	Macrophage and PMN chemotaxis and keratinocyte maturation
	Fibroblasts	
γ-Interferon	T-cells	Macrophage and PMN activation, retards collagen synthesis and cross-linking, and stimulates collagenase activity
	Macrophages	
Anti-inflammatory cytokines		
IL-4	T-cells	Inhibition of TNF, IL-1, and IL-6 production; fibroblast proliferation; and collagen synthesis
	Basophils	
	Mast cells	
IL-10	T-cells	Inhibition of TNF, IL-1, and IL-6 production and inhibits macrophage and PMN activation
	Macrophages	
	Keratinocytes	

CTGF, connective tissue growth factor; ECM, extracellular matrix; FGF, fibroblast growth factor; HB-EGF, heparin binding epidermal growth factor; KGF, keratinocyte growth factor; PMN, polymorphonuclear leukocyte; VEGF, vascular endothelial growth factor.

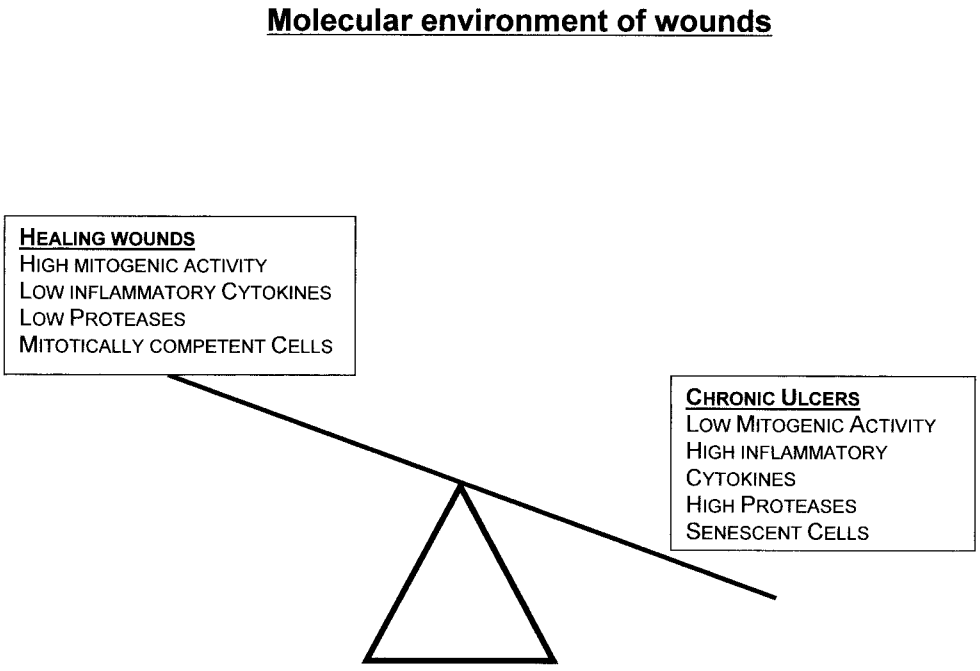

Figure 2—*Imbalances in the molecular environments of acute healing wounds and chronic nonhealing wounds.*

essential for healing (Fig. 3) (26,27). A recent study by Piaggesi et al. (28) found that pressure relief of neuropathic ulcers in 10 diabetic patients provided by total-contact casts significantly reduced ulcer size after 20 days of casting compared with patients with comparable lesions and glycemic control but without casts. Furthermore, the histopathological features of the two groups differed markedly. Patients without pressure relief showed a predominance of inflammatory elements as well as matrix alterations, vessel disruptions, inflammation, and debris. Ulcers in patients with total-contact casts showed a shift toward a reparative pattern with prevalence of newly formed capillaries and fibroblasts. These results indicate that pressure relief with a total-contact cast is associated with changes in the histology of neuropathic foot ulcers, indicating reduction of inflammatory and reactive components and acceleration of reparative processes. This supports the hypothesis proposed initially by Mast and Schultz (18) that repeated injury of tissue leads to prolonged inflammation, which causes elevated levels of proteases that degrade molecules that are essential to healing, eventually leading to a failure of the wound to heal.

CYTOKINES AND GROWTH FACTORS IN WOUND HEALING

— Cytokines and growth factors are small polypeptides that are secreted by different cell types and act as molecular signals that control cellular proliferation, differentiation, migration, and metabolism (Tables 1 and 2). They modulate the composition and turnover of various components of the extracellular matrix. In the first phase of wound healing, TNF-α, TGF-β, and PDGF appear to be particularly relevant (29,30). Multiple animal studies have reported that addition of exogenous growth factors are able to positively influence wound healing in acute and impaired wounds (29,31–33). Moreover, studies have found reduced concentrations of growth factors (PDGF, basic fibroblast growth factor, EGF, and TGF-β) in chronic wounds compared with acute wounds (34). In addition, Falanga et al. (35) reported that fluids collected from chronic venous ulcers interfered with cell proliferation. Also, Schultz et al. (36) found evidence that drainage fluid after mastectomy stimulated mitosis but exudates from chronic wounds inhibited cell proliferation. The results of these studies suggest that the activities of cytokines and growth factors that are essential for cell proliferation are absent or significantly reduced in chronic wounds.

A number of cytokines, such as IL-1, -2, -6, and -8, are upregulated during the process of acute wound healing (18). IL-1 plays an important role in the early phase of wound healing by recruiting leukocytes into the wound area (37). In skin wounds, IL-1 is predominantly produced by epithelial cells, and exogenously added IL-1 improved wound healing in several animal studies (38–40). Similarly, treatment of skin wounds in animals with IL-2 increased the number of lymphocytes in the wounds and resulted in better collagen production and mechanical consistency of the wounds, which demonstrates the positive influence of T-cells on wound healing (41,42).

In vitro, TGF-β stimulates several important functions of fibroblasts, including chemotactic migration, synthesis of extracellular matrix components (fibronectin and collagen), and contraction of matrix. Local application of TGF-β enhances collagen production and mechanical tensile strength of wounds in normal rats (43). In addition, injections of TGF-β (or basal fibroblast growth factor) into polyvinyl alcohol sponges implanted subcutaneously in normal rats or streptzotocin-induced diabetic rats significantly increased accumulation of granulation tissue and collagen (44).

PDGF is another key growth factor in wound healing. PDGF is secreted by macrophages, endothelial cells, fibroblasts, and megakaryocytes. It stimulates chemotactic migration of fibroblasts, smooth muscle cells and inflammatory cells, stimulates proliferation, and increases synthesis of collagen by fibroblasts (45,46).

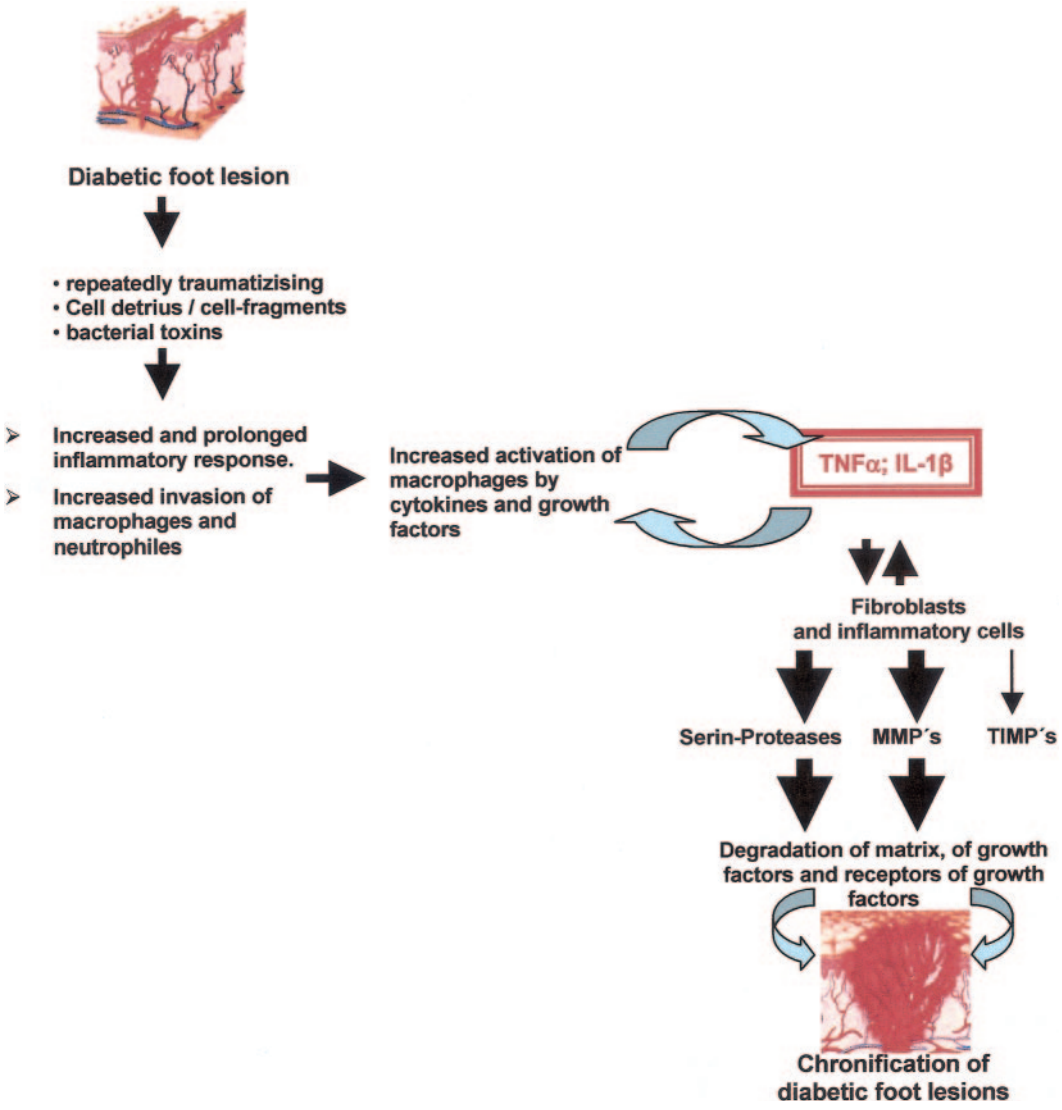

Figure 3—*Model of the molecular pathophysiology of chronic wounds.*

Local application of PDGF increases collagen production and angiogenesis in acute wounds of rats and normalizes healing processes in diabetic animals (47).

MMPS IN WOUND HEALING

— MMPs play essential roles in initial wound debridement as well as in the phases of angiogenesis, epithelialization, and remodelling of scar (48–53). The large family of MMPs contains about 20 different enzymes that can be grouped into several distinct subclasses (collagenases, gelatinases, stromelysins, and membrane-type MMPs [MT-MMPs]) based on the structure of the substrates that are cleaved and the structures of the MMPs (Table 3) (24,48,54). MMPs are broadly expressed by inflammatory cells, fibroblasts, endothelial cells, and keratinocytes at different times during wound healing. The control of expression into the wound area and the timed release of different MMPs is directly associated with a successful and well-structured wound healing (48,55). For example, the expression of MMP-1 is typically associated with the migration of keratinocytes (24,56,57). MMP-3 is needed for reconstruction of the new basement membrane. MMP-2 and MMP-9 are needed to remove denatured fibrillar collagen and for the proper development of granulation tissue (55,58,59).

Synthesis, activation, and inhibition of MMPs

The activity of MMPs is controlled on three levels. First, transcription is highly regulated by several cytokines, especially EGF, PDGF, IL-1, and TNF-α (60–65). While these factors primarily stimulate the production of MMPs, TGF-β is able to reduce the production of MMPs through inhibition of transcription (66–70). Second, MMPs are synthesized as inactive proenzymes that must be activated and released by proteases, including kallikrein, plasmin, or elastase (71). Third, MMP activities are regulated by inhibition by tissue inhibitors of metalloproteases (TIMPs) (48,72).

Different actions of MMPs

Collagenases. MMP-1, -3, -8, and -13 are the only subfamily of MMPs that are capable of rapidly cutting the intact triple helix of fibrillar collagens. Only after the collagenases make a single initial cut of fibrillar collagens can other MMPs, such

Table 2—*MMPs and TIMP*

Protein	Pseudonym	Substrates
MMP-1	Interstitial collagenase Fibroblast collagenase	Types I, II, III, VII, and X collagens
MMP-2	72-kDa gelatinase Gelatinase A Type IV collagenase	Types IV, V, VII, and X collagens
MMP-3	Stromelysin-1	Types III, IV, IX, and X collagens Types I, III, IV, and V gelatins Fibronectin, laminin and procollagenase
MMP-7	Matrilysin Uterine metalloproteinase	Types I, III, IV and V gelatins Casein, fibronectin and procollagenase
MMP-8	Neutrophil collagenase	Types I, II, and III collagens
MMP-9	92-kDa gelatinase Gelatinase B Type IV collagenase	Types IV and V collagens Types I and V gelatins
MMP-10	Stromelysin-2	Types III, IV, V, IX, and X collagens Types I, III, and IV gelatins Fibronectin, laminin, and procollagenase
MMP-11	Stromelysin-3	Not determined
MMP-12	Macrophage metalloelastase	Soluble and insoluble elastin
MT-MMP-1	Membrane-type MMP-1	Pro-MMP-2
MT-MMP-2	Membrane-type MMP-2	Not determined
TIMP-1	Tissue inhibitor of metalloproteinases-1	Collagenases
TIMP-2	Tissue inhibitor of metalloproteinases-2	Collagenases
TIMP-3	Tissue inhibitor of metalloproteinases-3	Collagenases

as the gelatinases, further degrade the collagen molecules. MMP-1 secretion by fibroblasts, migrating epidermal cells, and vascular endothelial cells begins to increase during the postacute phase of wound healing since the gene must be transcribed and the mRNA translated into protein (73,74). In contrast, preformed MMP-8 is released rapidly from storage granules of activated neutrophil granulocytes (57,75). MMP-1 and MMP-8 are additionally able to degrade elastin and types VII, VIII, and X collagen (53,74).

Gelatinases. MMP-2 (72-kDa gelatinase A) and MMP-9 (92-kDa gelatinase B) preferentially degest partially denatured fibrillar collagens (types I, I, and III) after collagenases make an initial cut that opens their extended triple helix structure (76). The gelatinase are also able to degrade nonfibrillar types of collagens, including IV, V, VII, and X collagen (74). MMP-2 is the most widespread of all MMPs, being expressed in skin fibroblasts, keratinocytes, vascular endothelial cells, and monocytes. MMP-2 is often made constitutively, but it is usually not activated unless conditions surrounding the cells change. In contrast, MMP-9 is not made constitutively, but its synthesis is induced in leukocytes, keratinocytes, monocytes, and macrophages as well as by various malignantly transformed cells (77).

Stromelysin. The Stromelysin subfamily of MMPs contains several members (MMP-3, -7, -10, -11, and -12). Due to their broad substrate specificity, this class of MMPs is especially connected with the degradation of proteoglycans, nonfibrillar collagens, and noncollagen components of basement membranes (collagen type IV, V, IX, and X, elastin, and fibronectin) (78,79). MMP-3 levels generally increase later in wound repair and may coincide with the initiation of wound contraction (73,80).

MT-MMPs. MT-MMPs are a unique subgroup of MMPs because they are bound to cell membranes by a hydrophobic segement. So far, four different types of MT-MMPs (MT1–MT4) have been found (24,81). Their functions include proteolytic activation of other pro-MMPs, including pro–MMP-2 and MMP-9 (82,83).

TIMPs. TIMP-1 and TIMP-2 bind noncovalently to the active form of MMPs and inhibit their activity. TIMP-1 can bind to all active MMPs but preferentially inhibits MMP-1. TIMP-2 is more effective in inhibiting MMP-2 than TIMP-1 (84,85–89).

ABNORMAL LEVELS OF MMPS IN CHRONIC WOUNDS

— A balance between proteases and their inhibitors is necessary for a correct wound healing, and several studies (90,91) have found elevated levels of proteases and reduced levels of inhibitors in chronic wounds (Fig. 2). Increased levels of MMP-2 and MMP-9 could be demonstrated in various chronic wound liquids (89). Increased levels of MMP-1 and MMP-8 were found in decubital ulcers (26). Similar results were obtained for MMP-13 in venous ulcer lesions (92). At the same time, reduced levels of TIMPs were found in chronic wound fluids (90,93). Ladewig et al. (56) demonstrated the ratio of MMP-9 to TIMP-1 as an important predictor for healing of chronic wounds, demonstrating an inverse correlation with the healing tendency of chronic pressure ulcers. Similar processes can be expected in nonhealing or badly healing diabetic foot lesions; first hints could be found in studies by Loots et al. (94), Dahn et al. (95), and Mansbridge et al. (96).

Our own data show higher concentrations of MMPs (MMP-2, -9, and -8) and reduced concentrations of inhibitors of MMPs (TIMP) in diabetic wounds compared with trauma lesions of a control group with normal glucose metabolism. In contrast to normal wound healing, an overexpression of these proteases seems to support a delayed wound healing and lead to a failure of wounds to heal. Additionally, there is evidence of an imbalance between MMPs and TIMPs that significantly contributes to the pathogenesis of nonhealing chronic lesions (97). First clinical studies (98–102) seem to confirm this concept and the clinical efficacy.

CLINICAL STUDIES WITH GROWTH FACTORS AND PROTEASE INHIBITORS

Local therapy with growth factors

Based on results of studies discussed above, which found low concentrations of several key growth factors, reduced mitogenic activity, and elevated levels of proteases in fluids collected from chronic wounds, it was reasonable to theorize that topical treatment of chronic wounds with exogenous growth factors would correct the deficiency of growth factors and promote healing (47). Numerous animal studies (29,31–33) supported the ability

of local application of various growth factors (PDGF, basic fibroflast growth factor, and TGF-β1) to promote healing of normal and impaired wound models. This led to one of the first clinical studies (103), performed almost 20 years ago, that showed a positive effect of locally applied autologous platelet extract on healing of human chronic wounds.

The use of standardized growth factor preparations produced by recombinant DNA technology was an attractive alternative to production of autologous platelet extracts by wound care providers because it bypassed the need for local technology infrastructure necessary to produce autologous platelet extract and the variability of growth factor activity between preparations. There are now multiple clinical studies (104–111) on the diabetic foot syndrome evaluating the use of recombinant PDGF (Regranex) that showed improvements in the probability of healing and reduction of healing time. Smiell et al. (112) reported that the percentage of patients with a fully healed wound after application of rhPDGF was much higher (39%) than of those treated with a placebo ($P < 0.007$). A very important clinical observation that came from the development of Regranex was that the wound bed needed to be properly debrided for the growth factor to have maximum benefit (113). This concept led to the formalization of the concept of wound bed preparation, which emphasizes the removal of barriers to healing and the integration of advanced technologies in wound care (114).

Local therapy with protease inhibitors

The other common characteristic of chronic wounds, besides reduced growth factor activity, is elevated protease activities. Thus, it is reasonable to theorize that local (or systemic) treatment of chronic wounds with protease inhibitor(s) would promote healing. This led to the simple approach of treating chronic diabetic foot ulcers with doxycycline, which is an antibiotic of the tetracycline family of molecules that has the unusual property of also being a competitive inhibitor of metalloproteases, including the MMPs and the TNF-α converting enzyme. Doxycycline can also reduce inflammation by reducing synthesis of nitric oxide (NO) (101,102, 115). Evaluation of a chemically tetracycline analogue in an animal study (116) also showed that local therapy reduced levels of MMP-8 and MMP-13 mRNA in dermal wounds of rats. Supporting this concept, an initial report (117) of a randomized controlled trial showed improved healing of chronic diabetic foot ulcers treated with a topical doxycycline gel.

Another approach to reducing protease activity in chronic wounds is to apply dressings that contain high concentrations of gelatin, which is a substrate for MMPs (98–100,118). In a clinical study, Cullen et al. (98) reported that elastase and plasmin activities in wound fluids were significantly reduced by a local therapy with the protease inhibitor dressing, Promogran. Further studies (99,100) reported a trend to more frequent and rapid healing of diabetic foot ulcers and venous ulcers with these protease inhibitors. It is theorized that the Promogran dressing improved healing by reducing the activites of MMPs (and perhaps serine proteases such as elastase) in the molecular environment of the wound. Further data will be needed to substantiate this theory. Another new dressing consisting of metal ions and citric acid (Dermax) was reported (119) to reduce reactive oxygen species and decrease MMP-2 production in vitro.

SUMMARY — The long duration of treatment as well as high costs to treat Wagner stage 2–4 of diabetic foot ulcers make it imperative to employ effective programs that prevent wounds from developing and accelerate healing rates once wounds occur (120). Most diabetic ulcerations can be prevented by educating and informing patients (120–127). Once a wound develops, ~70% of neuropathic foot lesions in diabetic patients can achieve healing by structured and stage-related therapy (off loading) and removing the barriers to natural healing by employing the concepts of wound bed preparation (debridement, control of infection/inflammation, proper moisture balance, and care of the epidermal edge) (128–131). When more advanced adjuvant therapies are needed to promote healing, the therapies employed should be based on correcting the molecular defects that have been identified in chronic wounds, such as increasing the levels of biologically active growth factors (106,132–135) and reducing elevated levels of proteases (22,95,98,101,120, 134,136–138). Advanced adjuvant therapies employing autologous platelet extracts, recombinant growth factors, protease inhibitors, dressings that reduce protease activities, and bioengineered skin substitutes are currently available. Future studies will need to evaluate if combinations of advanced adjuvant therapies are beneficial in especially hard to heal diabetic wounds.

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