

The Role of Matrix Metalloproteinases in Wound Healing

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The structure, classification, function, and regulation of matrix metalloproteinases in normal and abnormal wound healing is discussed. Results from key studies suggest that neutrophil-derived matrix metalloproteinase 8 (MMP-8) is the predominant collagenase present in normal healing wounds, and that overexpression and activation of this collagenase may be involved in the pathogenesis of nonhealing chronic leg ulcers. Excessive collagenolytic activity in these chronic wounds is possible because of the reduced levels of tissue inhibitor metalloproteinase 1 (TIMP-1). However, until recently, there have been no studies evaluating levels of matrix metalloproteinase or tissue inhibitors of metalloproteinase activity in chronic diabetic foot wounds. Improving basic knowledge and pharmaceutical intervention in this area ultimately may help clinicians identify and proactively intervene in an effort to prevent normal wounds from becoming chronic. This may prevent the high prevalence of morbidity associated with this significant health problem. (J Am Podiatr Med Assoc 92(1): 12-18, 2002)

The body's response to initial tissue injury is both complex and highly orchestrated (Fig. 1).¹ This response can be divided into initiation (clotting), inflammation, proliferation, and maturation.² The development and elaboration of a clot provides hemostasis and the foundation of what will later form the wound's extracellular matrix. This extracellular matrix exists primarily to facilitate cellular migration, adhesion, wound contraction, and epithelialization. Transformation, organization, and maintenance of the extracellular matrix depend on several highly controlled intracellular and extracellular processes. A chief agent of transformation and maintenance is the group of enzymes collectively known as matrix metalloproteinases.

Matrix metalloproteinases are enzymes belonging to the family of metalloendopeptidases that play a

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central role in wound healing.^{3,4} When first studied during World War II, extracellular matrix degradation was more important to the leather industry than it was to the biomedical industry. More than a generation later, developmental and structural biologists equated matrix metalloproteinase with potentially important biomedical processes. Amphibian experiments showed that this enzyme digested collagens, a major component of skin.

Tissue matrix metalloproteinases degrade several substances in the extracellular matrix, including cartilage, tendons, and fibrin, to facilitate the migration of cells, the deposition of new extracellular matrix, and the development of new tissue. At least four distinct subsets of enzymes exist within the matrix metalloproteinase family: collagenases, gelatinases, stromelysins, and membrane-type metalloproteinases. Sixteen matrix metalloproteinases have already been identified and characterized. Each of these enzymes has specificity for a different substrate.⁵ Thus, they are involved in any process that has to do with tissue reorganization, inflammation, and remodeling. In a nonpathologic state, the complex interaction between matrix metalloproteinases, other receptors,

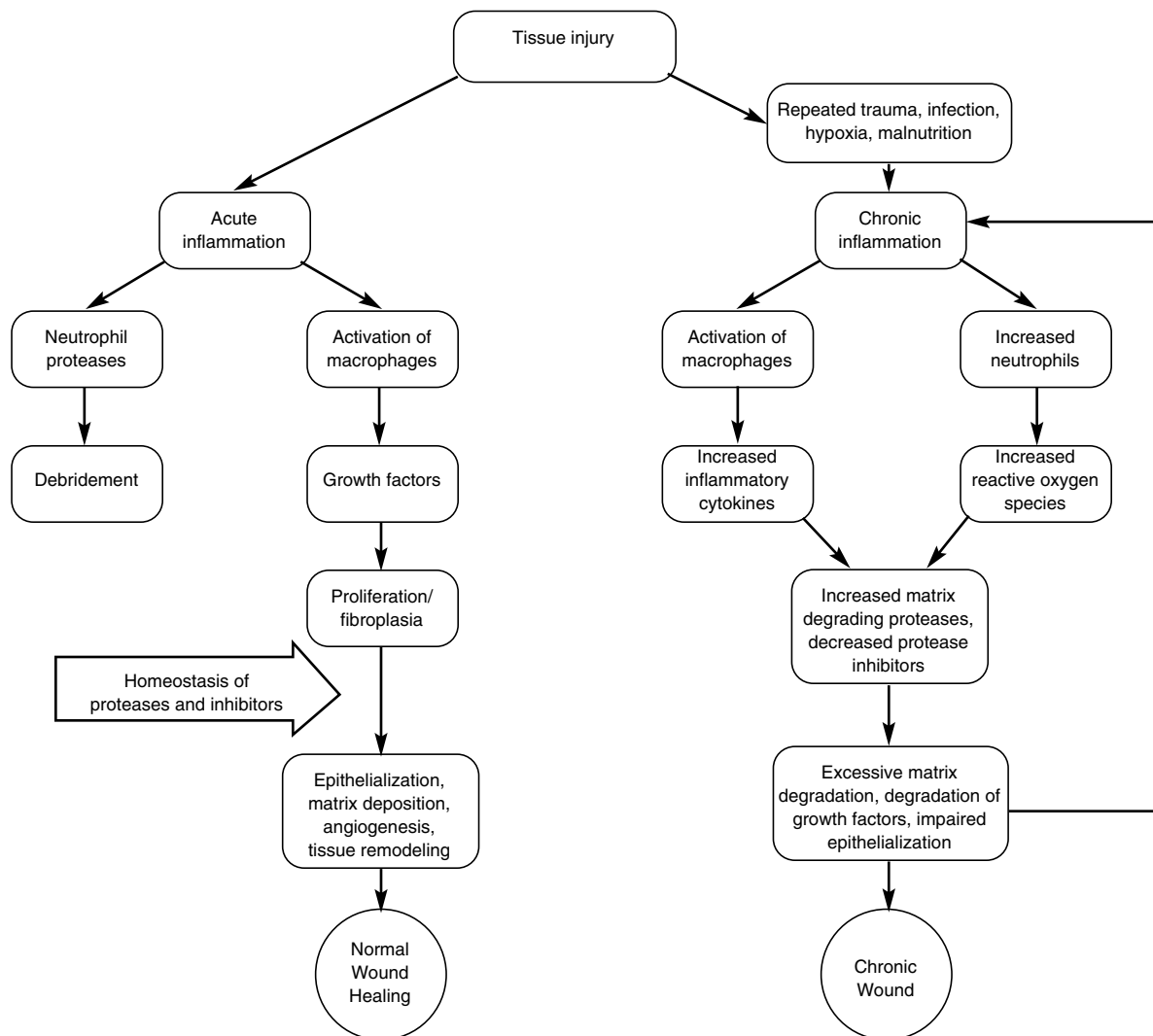


Figure 1. Pathway for normal and chronic wounds. Adapted from Nwomeh et al.¹

and circulating mediators in the extracellular matrix, results in remarkably efficient maintenance of the matrix. Failure of regulation of these matrix metalloproteinases has been correlated with numerous disease processes, including tumor growth,^{6, 7} arthritis,^{8, 9} atherosclerosis,^{7, 10, 11} emphysema,¹² and faulty wound healing.^{3, 4} This article focuses on the structure, classification, temporal activity, function, activation, and inhibition of the four classes of matrix metalloproteinases, with specific attention given to normal wound healing.

Structural and Functional Characteristics

The characteristics that make matrix metalloproteinases unique among enzymes can be divided into five

major areas: 1) presence of a zinc ion at the catalytic site; 2) secretion from the cell in an inactive form; 3) a grossly similar amino acid sequence; 4) specificity to degrade at least one component of the extracellular matrix; and 5) inhibition by tissue inhibitors of metalloproteinases.¹³

All matrix metalloproteinases are relatively similar in structure and possess several common domains. The first key domain is the signal peptide, which targets the molecule for secretion from the cell that produced it. This region of the molecule is cleaved before secretion and is not generally present in the inactive proenzyme. The second key domain is the propeptide domain. Cleavage of this domain activates the enzyme. The third region is the catalytic domain, which contains a subregion for zinc binding. The final domain is structurally similar to the molecule hemo-

pexin and is therefore referred to as the hemopexin domain. Differences in this domain modulate the individual substrate specificity of matrix metalloproteinases. This domain is present in all of the matrix metalloproteinases except matrix metalloproteinase 7 (MMP-7).

Characteristics by Subclass

Collagenase. MMP-1, MMP-8, and MMP-13 are the only enzymes in mammals with the capacity to cleave the triple helix of fibrillar collagen. Interstitial collagenase (MMP-1) appears to have preferential activity against type III collagen. Polymorphonuclear collagenase (MMP-8) has an affinity for type I collagen. MMP-13 appears to have the unique ability to cleave type I, type II, and type III collagen. MMP-13 has only been isolated in rats, arthritic cartilage extracellular matrix, chronic wounds, and fetal osseous tissue.³ While all collagenases have the unique ability to cleave fibrillar collagen, they are also secondarily active against other materials in the extracellular matrix. MMP-1 and MMP-8 can degrade gelatin and types VII, VIII, and X collagen.¹⁴ MMP-8 is stored in granules within the neutrophils and once activated can be released in seconds.¹⁵ MMP-1, on the other hand, must be transcribed by the appropriate gene. Thus, there is a significant gap (nearly 1/2 day) between transcription and secretion of this collagenase.¹⁴

The function of collagenase during normal wound healing has been relatively ill-defined because of methodological limitations in many studies. Most models have used burn wound blister fluid or post-operative (often postmastectomy) drainage collectant.^{16, 17} Recently, however, Nwomeh et al⁴ used tissue extracts from normal healing wounds by periodically collecting them under an occlusive bandage. That study, conducted on healthy human volunteers, indicated that levels of MMP-8 peaked at day 4 and persisted for about 1 week. MMP-1 levels were essentially not detectable until several days after wounding, and reached a peak approximately 1 week later than MMP-8. MMP-8 was more prevalent in the wound at its peak (nearly two orders of magnitude) as compared with the peak concentration of MMP-1.

In view of the above data, it may be possible to hypothesize regarding a specific mechanism of action. Within hours after wounding, white cells (mainly neutrophils) begin to infiltrate the wound during the inflammatory phase of healing. These neutrophils release numerous factors and proteins, including MMP-8. It may be postulated that higher concentrations of MMP-8 are required in the wound at this point compared with MMP-1 because of the large amount of

wound debridement and damaged type I collagen present (for which MMP-8 has an affinity). Later, in the proliferative phase of healing, type III collagen may be in greater abundance than type I, and the requirements for large-scale remodeling may be less extensive. This may explain the relatively lower concentration of MMP-1 collected and isolated in the Nwomeh et al⁴ study. MMP-1 is produced and secreted predominantly by cells prevalent in the postacute stages of wound healing (fibroblasts, endothelial cells).¹⁸ Certainly, lower collagenase activity in the presence of a stable, maturing matrix may allow for better keratinocyte migration as the normal wound moves toward epithelialization.

Gelatinases. While MMP-1, MMP-8, and MMP-13 have varying affinities for collagen types I and III, the gelatinases (MMP-2 and MMP-9) cleave other collagen types (IV, V, VII, and X), elastin, basement membranes, and denatured collagen.¹⁴ The gelatinases may also act synergistically with the collagenase family by further degrading types I, II, and III after they have been cleaved from the triple helix.¹⁹ MMP-2 and MMP-9 are secreted by different cells. MMP-2 is secreted by fibroblasts, and the molecularly larger MMP-9 is produced predominantly by leukocytes and perhaps also by keratinocytes.²⁰

Acute mastectomy and myoplasty wound fluids have shown high levels of MMP-2 and MMP-9 when compared with circulating plasma, with a somewhat higher concentration of MMP-9 than MMP-2.^{16, 21, 22} This would be consistent with an acute wound replete with inflammatory cells. As mentioned previously, MMP-9 is excreted from neutrophils while MMP-2 is generally fibroblast-derived.^{19, 23} In a wound excision/gel zymogram study of various extracellular matrix components, Arumugam et al¹⁸ observed that MMP-2 and MMP-9 levels persisted even after wound closure, suggesting that these matrix metalloproteinases probably play an important role in matrix (and possibly scar) remodeling. Furthermore, Salo et al²⁴ serially evaluated acute experimental wounds in the oral mucosa, demonstrating that MMP-2 remained stable during wound healing, while MMP-9 peaked between days 2 and 4. They hypothesized that MMP-9 was not only primarily expressed during inflammation, but perhaps it also played a role later in healing and was secreted by keratinocytes. Essentially, MMP-9 could participate in several key areas of wound healing, namely detaching anchored keratinocytes from the basement membrane and remodeling of the extracellular matrix, potentially enabling more efficient cellular migration. In contrast, Makela et al²⁵ evaluated wounded cell cultures and found that keratinocytes continued to grow and migrate when hete-

rocyclic carbonate-derived compounds inhibited MMP-9. When MMP-2 was inhibited by tetracycline analogs, there was a drastic reduction in the rate of keratinocyte growth. These authors hypothesized that MMP-2 plays a key role in detachment and promotion of keratinocyte migration along the extracellular matrix. Clearly, this is a complex area where differences in wound modeling and culture-specific characteristics may dramatically alter results, thereby creating potentially conflicting conclusions.

Stromelysins. Due to their broad base of substrate specificity, stromelysins (MMP-3, MMP-7, MMP-10, MMP-11, and MMP-12) play a varied role in degradation of the extracellular matrix. This class of matrix metalloproteinases has been associated with degradation of collagen types IV, V, IX, and X; elastin; fibronectin; and certain proteoglycans.^{26, 27} MMP-3 and MMP-10 can cleave the globular, but not the helical, type IV collagen. This form is found in the basement membrane of blood vessels.²⁸ Compared to other members of its family, MMP-7 shows a high affinity to elastin and also to entactin, a protein that bridges basement membrane collagen and laminin.²⁹

While the medical literature is replete with reports detailing the structural and, to an extent, the functional properties of stromelysins, there is little research on the specific temporal activity of these enzymes in normal human wound healing. In a longitudinal study of burn wounds, Young and Grinnell¹⁷ indicated that MMP-3 reached its peak level some time after day 4. In an excisional wound model, Arumugam et al¹⁸ found that MMP-3 was observed only on day 6, which coincided directly with the initiation of wound contraction. Therefore, the authors hypothesized that the secretion and subsequent activity of MMP-3 may play a role in clot dissolution during this period of normal wound healing, since stromelysins have the activity of the bulk of the materials found in the clot matrix.

Membrane-Type Matrix Metalloproteinases.

While members of this class of matrix metalloproteinases (MMP-14, MMP-15, MMP-16, and MMP-17) have certain unique structural characteristics, what differentiates them from all other membrane-type matrix metalloproteinases is that they are not secreted into the extracellular matrix. Rather, they exist on cell membranes and appear to function by binding to other matrix metalloproteinases and activating them or helping to localize their activity on that specific membrane. For instance, MMP-14 has been associated with binding and activation of gelatinases MMP-2 and MMP-9.³⁰ Additionally, MMP-15 and MMP-16 also bind to the gelatinases, but they do not appear to do so with the affinity of MMP-14.^{30, 31}

MMP-18 and MMP-20. MMP-18 and MMP-20 differ enough structurally and functionally from other classes of matrix metalloproteinases to preclude their inclusion in any one class.^{32, 33} MMP-18 shares 100% homology with MMP-19; therefore, it has been classified as the same enzyme.³² MMP-18 has been shown to have a modicum of activity against a synthetically designed stromelysin substrate.³² It may be considered to be functionally, if not structurally, related to the stromelysins. The function of MMP-20, however, appears to be isolated to the destruction of enamel proteins during tooth development.³⁴

Activation and Deactivation

Matrix metalloproteinase activity appears to be controlled at three basic levels. The first is at the gene level by transcriptional control. The second is at the molecular level by requiring factors to convert the proenzyme form to the active form. The third level is through tissue inhibitors of metalloproteinases. Activation, which is the regulation of tissue inhibitors of metalloproteinase, and the role of growth factors in the promotion and retardation of matrix metalloproteinase expression and activity, are briefly discussed below.

Matrix metalloproteinases are secreted as proenzymes. To become active, these structures require cleavage of the propeptide domain. All matrix metalloproteinases are either bound to the cell membrane or secreted into the plasma. The inactivity of matrix metalloproteinases is maintained by an interaction of a cysteine residue in the propeptide domain with the zinc ion, which is bound to the catalytic domain. Van Wart et al³⁵ identified a possible "cysteine switch" model for activation of matrix metalloproteinases. This switch is turned on when a specific enzyme cleaves a part of the propeptide domain of the matrix metalloproteinase. Once the switch is turned on, several other processes may occur that finally result in an activated enzyme. Trypsin, chymotrypsin, plasma kallikrein, plasmin, and some neutrophil enzymes have been implicated in this process.³⁶⁻³⁹

One of the most readily observable methods of regulation and inactivation of matrix metalloproteinases is through tissue inhibitors of metalloproteinases. Tissue inhibitors bind to matrix metalloproteinases and form stable complexes that are less biologically active against the extracellular matrix.^{40, 41} Ostensibly, this is accomplished by obscuring the catalytic region of the enzyme.⁴² They also exert their effect on inactive matrix metalloproteinases by slowing the process of activation.⁴³

Matrix metalloproteinases are induced by several

growth factors, including epidermal growth factor, platelet-derived growth factor, interleukin 1, and tumor necrosis factor alpha.⁴⁴ This is accomplished predominantly through gene induction. While these growth factors stimulate matrix metalloproteinase production, transforming growth factor beta has been shown to inhibit production of certain matrix metalloproteinases through transcription inhibition.^{45, 46} Somewhat similar inhibitory characteristics have been identified through the use of retinoids and glucocorticoids.⁴⁷

Matrix Metalloproteinases in Chronic Wounds

Chronic wounds contain high collagenase activity.^{4, 48} In a comparative study of acute and chronic human wounds, Nwomeh et al⁴ found distinct differences in collagenase patterns between these groups. Patterns of the collagenases in healing wounds indicated that MMP-8 appeared in significantly greater amounts than MMP-1. Chronic nonhealing ulcers were characterized by significantly higher levels of both MMP-1 and MMP-8, and by lower levels of tissue inhibitor of metalloproteinase 1 (TIMP-1), when compared with healing wounds. Levels of MMP-1 and MMP-8 varied greatly in chronic ulcers, although MMP-8 was always the predominant collagenase present in these wounds. In this study, collagenases were present almost exclusively in their inactive forms in healing wounds, whereas nonhealing ulcers possessed significant levels of the active forms of these enzymes. However, in a separate study of healing and nonhealing chronic leg ulcers, Harris et al⁴⁸ did not find statistically significant differences in collagenase activity between wounds that healed and those that did not. Reviewing the results from both of these studies, it can be postulated with some confidence that neutrophil-derived MMP-8 is the predominant collagenase present in normal healing wounds and that overexpression and activation of this collagenase may be involved in the pathogenesis of nonhealing chronic ulcers. In addition, excessive collagenolytic activity in chronic ulcers is made possible partly because of the reduced levels of the inhibitor TIMP-1.

Until recently, there have been no studies evaluating levels of matrix metalloproteinase activity in chronic diabetic foot wounds. In a study of biopsy specimens taken from diabetic foot ulcers, venous leg ulcers, healthy skin, and skin from patients with diabetes, Jude et al⁴⁹ found intense expression of MMP-8 and TIMP-2 in all chronic wounds, and MMP-9 was found to be particularly strong in venous wounds. MMP-1 and MMP-8 were expressed in nor-

mal skin and diabetic skin epidermal cells with no tissue inhibitor metalloproteinase staining noted in noninjured skin. The investigators concluded that matrix metalloproteinase and tissue inhibitor metalloproteinase expression appears elevated in chronic wounds. Furthermore, they may play a role in determining the chronicity of these wounds. Lobman et al⁵⁰ reported similar findings indicating that diabetic foot ulcers show increased expression of gelatinase (MMP-2) as compared to traumatic wounds.

While several advanced wound-healing modalities, such as exogenous growth factors and bioengineered tissue, show great promise in the treatment of chronic wounds, the very nature of the chronic wound environment may be hostile to optimal activity of these treatments. Trengove et al⁵¹ identified a significantly higher degradation of epidermal growth factor in chronic wounds and found that fluid from chronic wounds had 30-times greater matrix metalloproteinase activity when compared with acute wound fluid. Inhibiting excessive protease expression⁵² in these wounds may allow a prospective wound-healing treatment, whether it is a single growth factor or an entire bioengineered matrix, to reach its full therapeutic potential.

Conclusion

The structure, classification, function, and regulation of matrix metalloproteinases have been briefly discussed. Their role in normal and abnormal wound healing is not well characterized. However, any variance from the tightly orchestrated sequence of events that takes place during normal extracellular matrix maintenance may lead to a host of undesirable outcomes. Improving basic knowledge of this area will no doubt yield insight that will help the clinician to better identify the mechanisms involved in wound healing and thereby to intervene proactively to prevent the normal wound from becoming chronic. Ultimately, this will prevent the needlessly high prevalence of morbidity associated with this significant public health problem.

References

1. NWOMEH BC, YAGER DR, COHEN IK: Physiology of the chronic wound. *Clin Plast Surg* **25**: 341, 1998.
2. SUH DY, HUNT TK: Time line of wound healing. *Clin Podiatr Med Surg* **15**: 1, 1998.
3. NWOMEH BC, LIANG HX, DIEGELMANN RF, ET AL: Dynamics of the matrix metalloproteinases MMP-1 and MMP-8 in acute open human dermal wounds. *Wound Repair Regen* **6**: 127, 1998.
4. NWOMEH BC, LIANG HX, COHEN IK, ET AL: MMP-8 is the

- predominant collagenase in healing wounds and non-healing ulcers. *J Surg Res* **81**: 189, 1999.
5. WOESSNER JF, JR: The family of matrix metalloproteinases. *Ann N Y Acad Sci* **732**: 11, 1994.
 6. GASPARINI G: The rationale and future potential of angiogenesis inhibitors in neoplasia. *Drugs* **58**: 17, 1999.
 7. KUGLER A: Matrix metalloproteinases and their inhibitors. *Anticancer Res* **19**: 1589, 1999.
 8. TORTORELLA MD, BURN TC, PRATTA MA, ET AL: Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins [comments]. *Science* **284**: 1664, 1999.
 9. ABBASZADE I, LIU RQ, YANG F, ET AL: Cloning and characterization of ADAMTS11, an aggrecanase from the ADAMTS family. *J Biol Chem* **274**: 23443, 1999.
 10. ROUIS M, ADAMY C, DUVERGER N, ET AL: Adenovirus-mediated overexpression of tissue inhibitor of metalloproteinase-1 reduces atherosclerotic lesions in apolipoprotein E-deficient mice. *Circulation* **100**: 533, 1999.
 11. MOREAU M, BROCHERIOU I, PETIT L, ET AL: Interleukin-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human macrophages: relevance to stability of atherosclerotic plaque. *Circulation* **99**: 420, 1999.
 12. SHAPIRO SD: Elastolytic metalloproteinases produced by human mononuclear phagocytes: potential roles in destructive lung disease. *Am J Respir Crit Care Med* **150**: S160, 1994.
 13. BARAMOVA E, FOIDART JM: Matrix metalloproteinase family. *Cell Biol Int* **19**: 239, 1995.
 14. BIRKEDAL-HANSEN H: Proteolytic remodeling of extracellular matrix. *Curr Opin Cell Biol* **7**: 728, 1995.
 15. BORREGAARD N, KJELDSEN L, LOLLIKE K, ET AL: Granules and vesicles of human neutrophils: the role of endomembranes as source of plasma membrane proteins. *Eur J Haematol* **51**: 318, 1993.
 16. YAGER DR, ZHANG LY, LIANG HX, ET AL: Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. *J Invest Dermatol* **107**: 743, 1996.
 17. YOUNG PK, GRINNELL F: Metalloproteinase activation cascade after burn injury: a longitudinal analysis of the human wound environment. *J Invest Dermatol* **103**: 660, 1994.
 18. ARUMUGAM S, JANG YC, CHEN-JENSEN C, ET AL: Temporal activity of plasminogen activators and matrix metalloproteinases during cutaneous wound repair. *Surgery* **125**: 587, 1999.
 19. AGREN MS: Gelatinase activity during wound healing. *Br J Dermatol* **131**: 634, 1994.
 20. PILCHER BK, WANG M, QIN XJ, ET AL: Role of matrix metalloproteinases and their inhibition in cutaneous wound healing and allergic contact hypersensitivity. *Ann N Y Acad Sci* **878**: 12, 1999.
 21. BULLEN EC, LONGAKER MT, UPDIKE DL, ET AL: Tissue inhibitor of metalloproteinases-1 is decreased and activated gelatinases are increased in chronic wounds. *J Invest Dermatol* **104**: 236, 1995.
 22. WYSOCKI AB, STAIANO-COICO L, GRINNELL F: Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. *J Invest Dermatol* **101**: 64, 1993.
 23. AGREN MS, JORGENSEN LN, ANDERSEN M, ET AL: Matrix metalloproteinase 9 level predicts optimal collagen deposition during early wound repair in humans. *Br J Surg* **85**: 68, 1998. [Erratum. *Br J Surg* **85**: 715, 1998].
 24. SALO T, MAKELA M, KYLMANIEMI M, ET AL: Expression of matrix metalloproteinase-2 and -9 during early human wound healing. *Lab Invest* **70**: 176, 1994.
 25. MAKELA M, LARJAVA H, PIRILA E, ET AL: Matrix metalloproteinase 2 (gelatinase A) is related to migration of keratinocytes. *Exp Cell Res* **251**: 67, 1999.
 26. MURPHY GJ, MURPHY G, REYNOLDS JJ: The origin of matrix metalloproteinases and their familial relationships. *FEBS Lett* **289**: 4, 1991.
 27. QUANTIN B, MURPHY G, BREATHNACH R: Pump-1 cDNA codes for a protein with characteristics similar to those of classical collagenase family members. *Biochemistry* **28**: 5327, 1989.
 28. McDONNELL S, MATRISIAN LM: Stromelysin in tumor progression and metastasis. *Cancer Metastasis Rev* **9**: 305, 1990.
 29. SIRES UI, GRIFFIN GL, BROEKELMANN TJ, ET AL: Degradation of entactin by matrix metalloproteinases: susceptibility to matrilysin and identification of cleavage sites. *J Biol Chem* **268**: 2069, 1993.
 30. UENO H, NAKAMURA H, INOUE M, ET AL: Expression and tissue localization of membrane-types 1, 2, and 3 matrix metalloproteinases in human invasive breast carcinomas. *Cancer Res* **57**: 2055, 1997.
 31. TAKINO T, SATO H, SHINAGAWA A, ET AL: Identification of the second membrane-type matrix metalloproteinase (MT-MMP-2) gene from a human placenta cDNA library: MT-MMPs form a unique membrane-type subclass in the MMP family. *J Biol Chem* **270**: 23013, 1995.
 32. LLANO E, PENDAS AM, KNAUPER V, ET AL: Identification and structural and functional characterization of human enamelysin (MMP-20). *Biochemistry* **36**: 15101, 1997.
 33. PENDAS AM, KNAUPER V, PUENTE XS, ET AL: Identification and characterization of a novel human matrix metalloproteinase with unique structural characteristics, chromosomal location, and tissue distribution. *J Biol Chem* **272**: 4281, 1997.
 34. BARTLETT JD, SIMMER JP, XUE J, ET AL: Molecular cloning and mRNA tissue distribution of a novel matrix metalloproteinase isolated from porcine enamel organ. *Gene* **183**: 123, 1996.
 35. VAN WART HE, BIRKEDAL-HANSEN H: The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci USA* **87**: 5578, 1990.
 36. NAGASE H, SUZUKI K, ENGHILD JJ, ET AL: Stepwise activation mechanisms of the precursors of matrix metalloproteinases 1 (tissue collagenase) and 3 (stromelysin). *Biomed Biochim Acta* **50**: 749, 1991.
 37. NAGASE H, ENGHILD JJ, SUZUKI K, ET AL: Stepwise activation mechanisms of the precursor of matrix metalloproteinase 3 (stromelysin) by proteinases and (4-aminophenyl) mercuric acetate. *Biochemistry* **29**: 5783, 1990.
 38. SUZUKI K, ENGHILD JJ, MORODOMI T, ET AL: Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). *Biochemistry* **29**: 10261, 1990.
 39. SUZUKI K, NAGASE H, ITO A, ET AL: The role of matrix metalloproteinase 3 in the stepwise activation of human rheumatoid synovial procollagenase. *Biol Chem Hoppe Seyler* **371** (suppl): 305, 1990.

40. MURPHY G: Matrix metalloproteinases and their inhibitors. *Acta Orthop Scand Suppl* **266**: 55, 1995.
41. MURPHY G, WILLENBROCK F: Tissue inhibitors of matrix metalloendopeptidases. *Methods Enzymol* **248**: 496, 1995.
42. BIRKEDAL-HANSEN H: Matrix metalloproteinases. *Adv Dent Res* **9** (suppl 3): 16, 1995.
43. DECLERCK YA, YEAN TD, LU HS, ET AL: Inhibition of autolytic activation of interstitial procollagenase by recombinant metalloproteinase inhibitor MI/TIMP-2. *J Biol Chem* **266**: 3893, 1991.
44. MAUVIEL A: Cytokine regulation of metalloproteinase gene expression. *J Cell Biochem* **53**: 288, 1993.
45. KERR LD, MILLER DB, MATRISIAN LM: TGF-beta 1 inhibition of transin/stromelysin gene expression is mediated through a Fos binding sequence. *Cell* **61**: 267, 1990.
46. MAUVIEL A, CHUNG KY, AGARWAL A, ET AL: Cell-specific induction of distinct oncogenes of the Jun family is responsible for differential regulation of collagenase gene expression by transforming growth factor-beta in fibroblasts and keratinocytes. *J Biol Chem* **271**: 10917, 1996.
47. VINCENTI MP, COON CI, LEE O, ET AL: Regulation of collagenase gene expression by IL-1 beta requires transcriptional and post-transcriptional mechanisms. *Nucleic Acids Res* **22**: 4818, 1994.
48. HARRIS IR, YEE KC, WALTERS CE, ET AL: Cytokine and protease levels in healing and non-healing chronic venous leg ulcers. *Exp Dermatol* **4**: 342, 1995.
49. JUDE EB, ROGERS AA, OYIBO SO, ET AL: Matrix metalloproteinase and tissue inhibitor of metalloproteinase expression in diabetic and venous ulcers. *Diabetologia* **44** (suppl 1): 3, 2001.
50. LOBMAN R, AMBROSCH A, SCHULTZ G, ET AL: Expression of gelatinase (MMP-2) in diabetic and non-diabetic wounds. *Diabetologia* **44** (suppl 1): 4, 2001.
51. TRENGOVE NJ, STACEY MC, MACAULEY S, ET AL: Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. *Wound Repair Regen* **7**: 442, 1999.
52. EDWARDS JV, BOPP AF, BATISTE S, ET AL: Inhibition of elastase by a synthetic cotton-bound serine protease inhibitor: in vitro kinetics and inhibitor release. *Wound Repair Regen* **7**: 106, 1999.