We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Roles of Matrix Metalloproteinases in Cutaneous Wound Healing

Trung T. Nguyen, Shahriar Mobashery and
Mayland Chang

Additional information is available at the end of the chapter

Abstract

Wound healing is a complex process that consists of hemostasis and inflammation, angiogenesis, re-epithelialization, and tissue remodeling. Matrix metalloproteinases (MMPs) play important roles in wound healing, and their dysregulation leads to prolonged inflammation and delayed wound healing. There are 24 MMPs in humans, and each MMP exists in three forms, of which only the active MMPs play a role in the pathology or repair of wounds. The current methodology does not distinguish between the three forms of MMPs, making it challenging to investigate the roles of MMPs in pathology and wound repair. We used a novel MMP-inhibitor-tethered affinity resin that binds only the active form of MMPs, from which we identified and quantified active MMP-8 and active MMP-9 in a murine diabetic model with delayed wound healing. We showed that up-regulation of active MMP-9 plays a detrimental role whereas active MMP-8 is involved in repairing the wound in diabetic mice. These studies identified MMP-9 as a novel target for therapeutic intervention in the treatment of chronic wounds. A selective inhibitor of MMP-9 that leaves MMP-8 unaffected would provide the most effective therapy and represents a promising strategy for therapeutic intervention in the treatment of diabetic foot ulcers.

Keywords: MMPs, chronic wounds, wound healing, selective MMP-9 inhibitor, MMP profiling

1. Introduction

Skin is one of the largest organs in humans. Its three main functions are protection against environmental damage, regulation of body temperature, and perception of environmental

change. The skin consists of two distinct layers of tissue, the epidermis and dermis. The epidermis is the outermost layer. The inner layer, dermis, provides cushioning and tensile strength for the skin through the support of the extracellular matrix (ECM) [1]. The ECM—a three-dimensional structure, where cutaneous cells and tissues are embedded—comprises approximately 300 proteins, including collagen, proteoglycans, and glycoproteins [2]. Injury to the skin would account for breaks in these protective layers, which become a cutaneous wound. The wound has to be repaired because of its critical role in prevention of infection, and the well-being of the tissue and the organism. Wounds that undergo a well-coordinated cascade of biochemical events in healing are called acute wounds. On the other hand, wounds that are recalcitrant to healing due to prolonged residency in one of the healing stages are called chronic wounds. For wound healing to progress, the ECM has to be remodeled properly, and endopeptidases such as matrix metalloproteinases (MMPs) contribute to this remodeling process. Whereas these subjects have been reviewed in excellent recent articles [3–6], the emphasis in this chapter focuses on the roles of the family of MMPs that are involved in the wound-healing process.
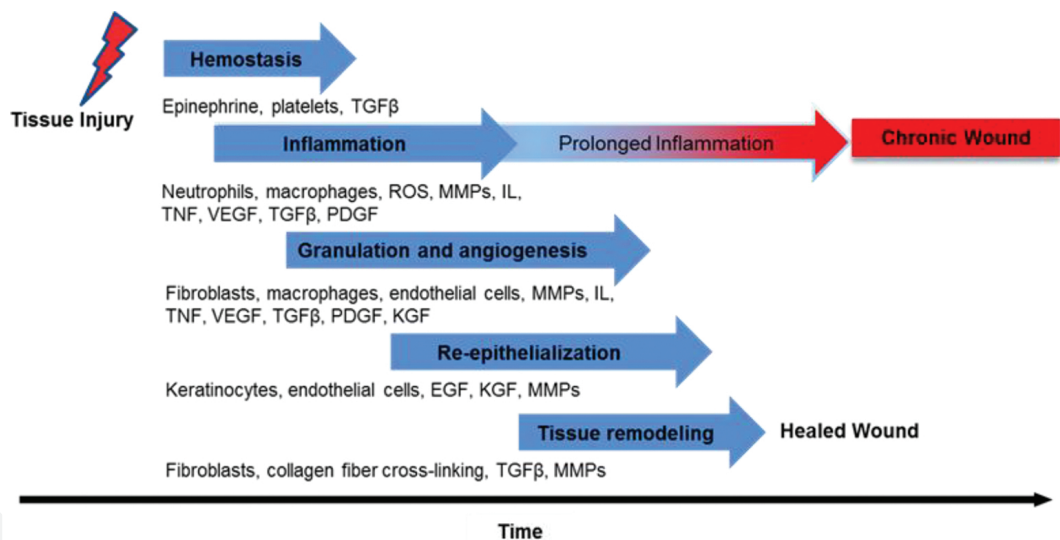

Figure 1. The phases of wound healing. After tissue injury, hemostasis and inflammation start immediately, in which cytokines, growth factors, and ROS are produced to recruit cells to the wound site. The next proliferative phases of wound healing include angiogenesis and re-epithelialization, where new tissue is formed by endothelial cells, fibroblasts, and keratinocytes. In diabetic wound healing, inflammation can be prolonged, causing the wounds to be chronic. The final phase is tissue remodeling. EGF (epidermal growth factor), IL (interleukin), KGF (keratinocyte growth factor), MMPs (matrix metalloproteinases), PDGF (platelet-derived growth factor), ROS (reactive oxygen species), TGF-β (transforming growth factor-beta), TNF (tumour necrosis factor), VEGF (vascular endothelial growth factor). Adapted from Schreml [22].

There are currently at least 24 known MMPs in humans [7]. Not all functions that these enzymes play in humans have been elucidated and concepts in their mechanistic roles in wound healing are emerging only recently. Yet, it is generally appreciated that MMPs play roles in each stage of wound healing, in large measure because of the need for restructuring of the ECM in the process of wound healing. The phases of wound healing consist of (1)

hemostasis and inflammation, (2) granulation and angiogenesis, (3) re-epithelialization, and (4) tissue remodeling and are depicted in **Figure 1**. All four phases of wound healing have to be coordinated and integrated properly in a timely and sequential manner for successful healing. The repair processes require the coordination of events involving various cells, the ECM components, growth factors, cytokines, and enzymes. Furthermore, it is increasingly evident that MMPs display a duality of functions in the physiology of the tissue and processes of pathology, as evidenced for chronic wounds, cancers, Parkinson's and Alzheimer's diseases [8–10]. As such, certain MMPs might have a beneficial effect in healing, yet others might exhibit detrimental effect as aberrations in the functions of these enzymes in disease development and progression. The differentiation of these functions—detrimental versus beneficial—has been a challenge. Yet, new tools and capabilities are becoming available to address exactly these issues in various diseases.

1.1. Stages of wound healing

Once injury to the skin takes place, the cutaneous wound immediately enters the first phase of hemostasis (**Figure 1**). The onset of blood vessel constriction prevents excessive bleeding, which is followed by the aggregation of platelets along the damaged endothelium to form a plug. A cascade of events ensues, which leads to the formation of a blood clot. The serine-proteinase thrombin cleaves fibrinogen into insoluble fibrin threads that are aggregated with platelets to create the clot. In addition to stopping the bleeding, the blood clot serves as a provisional matrix for cell migration [11]. The surrounding cells of a blood clot also release inflammatory cytokines and growth factors as signaling molecules to attract a variety of cells
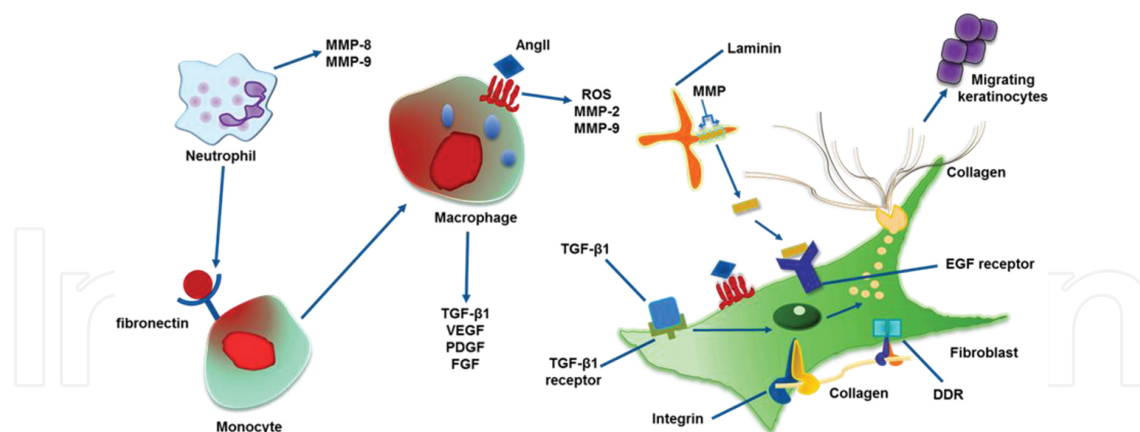

Figure 2. ECM–growth factor interactions and production of MMPs in wound healing. Monocytes migrate to the wound site and bind to fibronectin released by neutrophils. This interaction causes monocytes to differentiate into macrophages that secrete multiple growth factors. TGF-β1 binds to its receptor on fibroblasts and stimulates the cells to produce ECM components such as collagen, fibronectin, and hyaluronic acid. Neutrophils also produce MMP-8 and -9 in the wound. Binding of AngII to macrophages stimulates the cells to produce ROS and MMPs. MMPs can cleave laminin to release a fragment that binds EGF receptor on fibroblasts and stimulates migration and proliferation of keratinocytes. AngII (angiotensin II), ECM (extracellular matrix), DDR (discoidin domain receptor), EGF (epidermal growth factor), FGF (fibroblast growth factor), MMPs (matrix metalloproteinases), PDGF (platelet-derived growth factor), ROS (reactive oxygen species), TGF-β1 (transforming growth factor-beta 1), VEGF (vascular endothelial growth factor).

to the wound site to initiate the inflammatory phase. These cells include neutrophils, macrophages, and lymphocytes, which defend the site from infectious agents [12]. The earliest arrival of neutrophils takes place only a few hours after injury [13]. Neutrophils are responsible for releasing fibronectin, which has multifunctional roles, including a structural function due to its fibrillary composition, mediating interactions between ECM components and other cells, or serving as a bridge between cells [14, 15]. Fibronectin and fibrin act to provide provisional matrix that promotes cellular migration and adhesion, depending on the wound status. Also, during inflammation, fibronectin and other ECM protein fragments can attract monocytes, a type of white blood cells, to the wound site from the bloodstream. The interactions at the wound site cause monocytes to undergo differentiation into additional macrophages (**Figure 2**) [14]. Macrophages are stimulated by growth factors to produce reactive-oxygen species (ROS), MMPs, and multiple growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) (**Figure 2**) [16]. One factor that can stimulate macrophages is angiotensin II (AngII). The renin–angiotensin system (RAS) is a pathway to regulate angiotensin, a hormone peptide, to eventually produce the primary effector-AngII [17]. This effector is present in macrophages, neutrophils, fibroblasts, and endothelial cells of human skin [18]. With binding and stimulation of AngII, inflammatory cells such as macrophages generate ROS and MMPs that subsequently promote migration and proliferation of keratinocytes. This will be discussed in a later section of this chapter.

Among many growth factors and cytokines, TGF-βs play critical roles in regulating the development of the ECM. There are three isoforms (TGF-β1–3) in humans, with each playing distinct roles in regulating synthesis of the ECM components, and even cellular proliferation or cellular death [19, 20]. TGF-βs are produced in latent forms that need to be activated by cleavage of their pro-peptides, before exerting their activities on the ECM, which include stimulation of cellular production of ECM components [20]. The most well known is TGF-β1, which can control production and degradation of many constituents involved in wound healing [14]. Once TGF-β1 binds to its receptor, this interaction stimulates the synthesis of ECM components such as collagen, fibronectin, and hyaluronic acid in many types of cells, including fibroblasts [21]. Fibroblasts are cells that synthesize collagen and other constituents deposited on the ECM [14]. Besides monocytes/macrophages, fibroblasts also generate ROS, including peroxide anion, hydroxyl ion, and superoxide anion, which are important in defense against pathogenic microorganisms [22]. ROS, in turn, has the effect of stimulating the production of more cytokines that lead to increased production of proteinases such as MMPs to modify components of the ECM [22]. Dualities of functions reveal themselves in ROS as well. The function against the pathogens is beneficial, but high-level ROS can cause damage to the ECM components [22]. This fine balance for ROS could stimulate complex signal pathways that would lead to up-regulation of MMPs in the wounds. The enhanced presence of ROS and the attendant stimulated turnover of ECM components could cause tissue destruction and hinder the repair processes [23]. This duality of roles for ROS was observed in a murine wound model that documented severe damage to the endothelium in a background that lacked ROS-detoxifying enzymes [24]. In diabetic patients with chronic wounds, the production of ROS

has been found to exceed the antioxidant capacity, adding more oxidative stress to the wounds that subsequently increases MMP levels by 60-fold over those in acute wounds [12].

Granulation and angiogenesis take place in the next phase of wound repair, which is also known as the proliferative phase (**Figure 1**). Granulation tissue is defined as a matrix of collagen, with microscopic blood vessels that are newly formed from preexisting blood vessels in a process called angiogenesis. New blood capillaries supply oxygen to the wound tissue, which is critical for the healing process. Granulation, in the form of a red or pink soft tissue, forms on the surface of the wound. Macrophages are tasked with initiating this phase by degrading the blood clots and by producing a variety of cytokines and chemokines to attract fibroblasts to enter the wound site [22]. The population of fibroblasts at the wound site will expand by both migration and proliferation through dynamic interaction with growth factors and the ECM. This is mediated by integrins, a set of receptors for fibroblast, which consist of an extracellular domain that binds to the ECM, and an intracellular portion that associates with the cytoskeleton for biochemical signaling [25]. Integrins and discoidin domain receptor 2 (DDR2), another receptor for fibroblasts, bind to type I collagen within the ECM. This inter-action stimulates the production of MMP-2 to promote migration of fibroblasts to the wound site through the basement membrane during ECM remodeling [26], as indicated in **Figure 2**. In addition, as mentioned earlier, TGF-β1 can stimulate proliferation of fibroblasts. The wound tissue is hypoxic and would require a supply of oxygen for the demands of the biochemical processes of wound healing [22]. Hypoxia stimulates macrophages, keratinocytes, fibroblasts, and endothelial cells to produce more VEGF, which is a cytokine associated with angiogenesis [22]. The enhanced expression of VEGF causes endothelial cells at the wound bed to migrate, proliferate, and form new blood vessels into the wounds to supply oxygen during angiogen-esis [27]. In addition, VEGF has been shown to increase expression of the collagen-binding integrin in the dermal microvasculature [28]. These bindings with integrin help cells adhere to the ECM and promote additional growth factor expression. Rossiter et al. have reported that deletion of keratinocyte-specific VEGF impaired angiogenesis and delayed wound healing in a murine model [29]. Other researchers have shown that overexpression of VEGF can lead to enhanced wound healing in murine excisional wounds [30]. Another receptor that plays an important role in cellular migration during angiogenesis is epidermal growth factor (EGF) receptors, which can bind to EGF and laminin to enhance fibroblast migration [31, 32]. Laminin, which is a fibrous constituent of the basement membrane, plays important roles in cell adhesion, migration, and proliferation [33]. At the wound site, cleavage of laminin-332 (also referred to as laminin-5) by MMPs would generate a fragmented laminin peptide that binds to the EGF receptor and enhances the cellular motility of proliferating keratinocytes [34], demonstrated in **Figure 2**. In addition to its role in angiogenesis, fibroblasts are responsible for the production and deposition of immature collagen (type III collagen), which is essential in providing more strength for the wound ECM [22].

The formation of granulation tissue in the last phase provides a support matrix for epithe-lial cells to migrate across and to cover the wound surface in a process known as re-epi-thelialization (**Figure 1**). This stage of wound healing mainly involves keratinocytes, which are a predominant cell type in the epidermis of the skin [35]. In fact, to cover the

wound surface with a new layer of epithelium, the keratinocytes at the wound edge need to undergo migration, differentiation, and proliferation. In the basal layer of epidermis, keratinocytes have differentiating characteristics, in which they are able to change into a longer and flatter shape before they begin migration [35]. The migrating keratinocytes need to loosen their adhesion to each other before moving away from the wound edge toward the wound's central point to close the open area [35]. It is interesting to note that these cells need to establish adhesion to the new ECM around them via integrins, but at the same time they develop actin filaments to support cellular migration through a wound matrix of necrotic material, clots, and even bacteria [36, 37]. During this process, the continued enhanced expression of MMPs, released by a variety of cells (macrophages, keratinocytes, endothelial cells, and fibroblasts) plays crucial roles in degrading substrates of the provisional wound matrix [38]. After the first layer of cells that cover the wound area, keratinocytes need to proliferate to have adequate depth of cells in the wound. The onset of proliferation is brought about by a variety of factors such as EGF, TGF-α, TGF-β, keratinocyte growth factor (KGF), and hepatocyte growth factor (HGF) [39]. The migration and proliferation of these cells require increased supply of oxygen in the wound bed [22]. It is important to note that in chronic wounds, the epidermis fails to re-epithelialize due to non-migratory keratinocytes, compared to acute wounds [35]. Following the robust proliferation and epithelial migration, wound healing enters its last phase of tissue remodeling that could potentially last in the order of years. Type III collagen prevalent during this last phase is gradually replaced by the more stable type I collagen [22]. The collagen fibers at the wound site are rearranged, cross-linked, and aligned to increase the wound's tensile strength [40]. The participation of proteinases is necessary to ensure ECM remodeling, which will bring back normality to the tissue.

In the repair of acute wounds, interactions between growth factors and the ECM occur in an orchestrated manner, where each phase is allowed to transition properly to the next, resulting in a healed wound. There are numerous factors that contribute to the impairment of wound healing in patients. Some are local factors that directly affect wound closure such as supply of oxygen, infection, venous sufficiency, and imbalance between proteinases or growth factors [12]. Others are underlying conditions that influence the overall health of a patient, including age, diseases, obesity, medications, and an immunocompromised system [12]. For instance, septic conditions have been shown to delay wound healing in mice [41]. In the case of patients affected by hypoproteinemia, their protein deficiency can impair wound healing by affecting capillary formation, cellular proliferation, collagen deposition, and wound remodeling [12]. The most detrimental disease is diabetes, where patients are more prone to develop non-healing ulcers or chronic wounds. In these wounds, the interactions between growth factors and the ECM are disrupted because of biochemical abnormalities of the ECM and aberrantly elevated activities of MMPs [14]. The imbalance between MMPs and their endogenous regulators can cause excessive degradative activities and critical loss of the newly reformed ECM in wound healing.

1.2. Matrix metalloproteinases (MMPs): structures and regulation

Matrix metalloproteinases are a group of 24 enzymes in humans—there are a total of 28 MMPs known to date, including enzymes from other organisms—which are expressed as zymogenic inactive proteins [7]. These enzymes are highly regulated and one level of regulation is exerted in their proteolytic activation by other proteinases, including by other MMPs [42, 43]. As the pro-domain of the zymogens are removed, the active sites become available for catalysis. Tissue inhibitors of matrix metalloproteinases (TIMPs) are protein inhibitors of these enzymes that form non-covalent complexes with the catalytic domain. The activation events and the inhibition by TIMPs account for various steps in the regulation process, which we will expand on in the following sections. These events are graphically depicted in **Figure 3** for MMP-2.
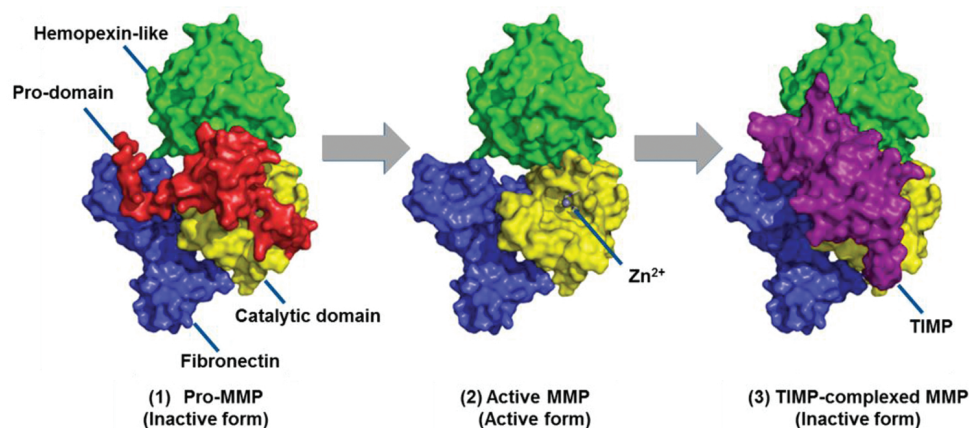

Figure 3. MMPs, as exemplified in this figure by MMP-2, exist in three forms: pro-MMPs (inactive), active MMPs, and TIMP-complexed MMPs (inactive). MMPs are first produced as latent pro-MMPs (1) with a pro-domain (shown in red) blocking the active site (shown in yellow). The removal of the pro-domain is required to activate MMPs by revealing the zinc ion in the catalytic site (2). Active MMPs are then able to cleave substrates. The activity of MMPs is regulated by interaction with TIMPs (shown in purple), which inactivate the MMPs (3).

MMPs are zinc-dependent endopeptidases. They are either secreted into the ECM or are membrane-anchored on the surface of the cell [9, 44, 45]. The most basic components of all MMPs consist of three domains: a signal sequence at the N-terminus, a pro-domain that caps the active site, and a catalytic domain, as depicted in **Figure 4**. This minimal domain organization is present in MMP-7 and MMP-26, also known as the matrilysins. The catalytic domain is characterized by the zinc-binding HExxHxxGxxH motif, containing three conserved histidines [46]. Several MMPs have an additional domain referred to as the hemopexin-like domain, which is linked at the C-terminus of the aforementioned basic sequence. The hemo-pexin-like domain is believed to play a role in substrate recognition. This organization of domains for MMPs is seen in MMP-3 and -10 (also known as stromelysin-1 and -2), MMP-1, -8, and -13 (also known as collagenases), MMP-12 (metalloelastase), MMP-20 (enamelysin), and MMP-22 and -27 [47] (**Figure 4**). MMP-2 and MMP-9 (or gelatinases) have more complicated structures by having fibronectin repeats inserted into the side of the catalytic sites [47]

(**Figure 4**). The membrane-bound MMPs have two types of membrane anchors. One is a transmembrane peptide domain and another is the GPI anchor (**Figure 4**). There are a few other variations, which are summarized in **Figure 4** graphically. The structural similarities among these MMPs are high. Certainly, individual domains are highly similar in both sequence and three-dimensional structures. As a consequence, these enzymes share significant overlap in their substrate preferences, which is likely a reflection of the fact that the functions of disparate MMPs in the physiology of the organism are critical and they exhibit some redundancy in their turnover of the substrates as a consequence. As it pertains to wound healing, important MMPs and their known substrates are listed in **Table 1**.
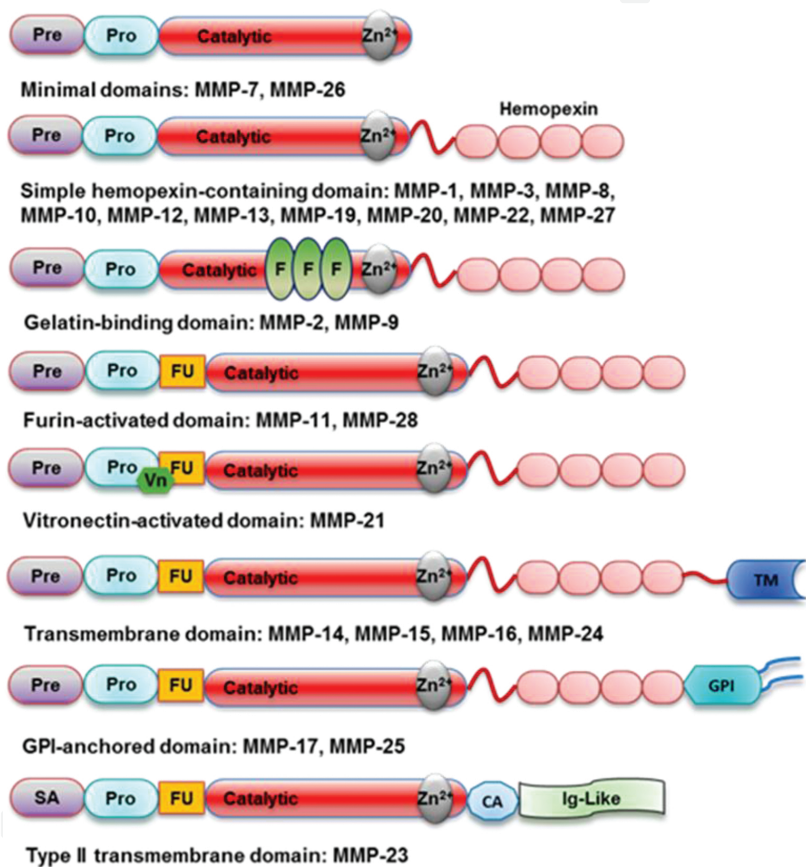

Figure 4. Structures of the MMP family. MMPs are divided into eight subgroups based on structural similarities. Pre: signal sequence, Pro: pro-peptide, Zn^{2+}: zinc-binding site, Catalytic: catalytic domain, F: repeats of fibronectin, Fu: furin-like serine proteinases, Vn: vitronectin-like insert, TM: transmembrane domain, GPI: glycosylphosphatidylinositol, SA: N-terminal signal anchor, CA: cysteine array, Ig-Like: immunoglobulin-like.

As indicated earlier, the functions of these enzymes are highly regulated. This regulation manifests itself at the transcriptional level as well as at the proteome level. Production of MMPs is stimulated in a variety of cells such as keratinocytes, fibroblasts, endothelial cells, and inflammatory cells during wound healing. These cells can be transcriptionally activated by a wide range of cytokines and growth factors including EGF, HGF, FGF, TGF-β, VEGF, PDGF, and KGF, as well as by interleukins and interferons [74]. Since there are many cytokines with

the ability to activate transcription to produce the zymogens, there are numerous signaling pathways implicated in the control of proteinase expression. These pathways include, for instance, mitogen-activated protein kinase (MAPK), or growth factor-dependent pathways of Smad, NF-kB, activation of focal adhesion kinase (FAK) by integrin activation, or Wnt cascade [11]. The highly regulated process is critical for the physiological roles. When the regulation goes awry, these enzymes cause pathological consequences. The pathological outcomes of MMP dysregulation have been the subject of many review articles [8, 10, 75, 76].

MMP	Preferred Substrates	Roles in Wound Healing	Cell culture	Human (H) or Mouse (M) wounds	Detection Method
MMP-1 (collagenase-1)	Collagen I, II, III, VII and X; aggrecan, serpins; alpha2-macroglobulin	• Promotes human keratinocyte migration on fibrillar collagen [38]	X		^{35}S-labeled antisense RNA probes [38]
		• Overexpression in keratinocytes delays re-epithelialization [38]	X		
		• Expressed by keratinocytes at their trailing membrane edge during wound healing [48]	X	H	Competitive ELISA [48]
		• Found to be elevated in diabetic foot ulcer patients [49]		H	
MMP-2 (gelatinase A)	Gelatin; collagen I, IV, V, VII, and X; laminin; aggrecan; fibronectin; tenascin	• Expressed by fibroblasts and endothelial cells in both mouse [50] and human acute wounds [51]		M H	ELISA and gelatin zymography [51]
		• Accelerates cell migration [34]	X		
		• Expressed in platelets, mediates platelet adhesion and aggregation [52]	X		
		• Keratinocyte migration [34]	X		
		• Activates MMP-9 [53]	X		
MMP-3 (stromelysin-1)	Collagen IV, V, IX, and X; fibronectin; elastin; gelatin;	• Expressed by basal keratinocytes in both human acute and chronic wounds [54]		H	^{35}S-labeled antisense RNA probes [54, 57]

MMP	Preferred Substrates	Roles in Wound Healing	Cell culture	Human (H) or Mouse (M) wounds	Detection Method
	aggrecan; nidogen; fibrillin; E-cadherin	• Affects wound contraction and delayed healing [55]		M	
		• Activates MMP-9 [56]	X		
MMP-7 (matrilysin)	Elastin; fibronectin; laminin; nidogen; collagen IV; tenascin: versican; α1-proteinase inhibitor; E-cadherin; tumour necrosis factor	• Required for re-epithelialization of mucosal wounds [58]		M	Immunohistochemistry [59]
		• Re-epithelialization of mucosal tissue is impaired in MMP-7 knockout mice [58]		M	
MMP-8 (collagenase-2)	Collagen I, II, and III; aggrecan, serpins; 2-MG	• Mainly expressed by neutrophils [3]			Western blot [62]
		• Promotes cutaneous diabetic wound healing [60]		M	MMP-inhibitor-tethered affinity resin [60]
		• Most prevalent collagenase in wounds [61]		H	
		• MMP-8 knockout mice show delayed wound closure [62]		M	
		• Found to be elevated in diabetic foot ulcer patients [49]		H	
		• Selective inhibition of MMP-8 delays murine diabetic wound healing [60]		M	*In-situ* zymography [63]
		• Topical application of active MMP-8 accelerates murine diabetic wound healing [63]		M	

MMP	Preferred Substrates	Roles in Wound Healing	Cell culture	Human (H) or Mouse (M) wounds	Detection Method

MMP	Preferred Substrates	Roles in Wound Healing	Cell culture	Human (H) or Mouse (M) wounds	Detection Method
MMP-9 (gelatinase B)	Gelatin; collagen I,III,IV, V and VII; aggrecan; elastin; fibrillin	• Hypoxia induces cell migration through increased expression of MMP-9 [64]		M	Immunohistochemistry and gelatin zymography [51]
		• Upregulation causes detrimental effects in murine diabetic wounds [60]		M	• MMP-inhibitor-tethered affinity resin [60]
		• Keratinocyte migration [65]	X		
		• Increased levels in wound fluid of diabetic foot ulcers, quantified by gelatin zymography [49]		H	
		• MMP-9 knockout diabetic mice have reduced re-epithelialization and delayed wound closure [63]		M	
		• Selective inhibition of MMP-9 accelerates diabetic wound healing [60, 63]		M	*In-situ* zymography [63]
MMP-10 (stromelysin-2)	Collagen IV, V, IX, and X; fibronectin; elastin; gelatin; laminin; aggrecan; nidogen; E-cadherin	• Expressed by epidermal cells three days post-wounding in human wounds [66]		H	^{35}S-labeled antisense RNA probes [57, 66]
		• Overexpression in keratinocytes resulted in normal wound healing but disorganized epithelium [67]		M	• RNA probes [67]
MMP-12 (metalloelastase)	Collagen IV; gelatin; fibronectin; laminin; vitronectin; elastin; fibrillin; apolipoprotein A; α1-proteinase inhibitor	• Expressed specifically in macrophages, but not expressed by epithelial cells [50]		M	RNA isolation and RNase protection analysis [50]
		• Potential regulator of angiogenesis due to ability to generate angiostatin [68]	X		Western blot [68]

MMP	Preferred Substrates	Roles in Wound Healing	Cell culture	Human (H) or Mouse (M) wounds	Detection Method
MMP-13 (collagnase-3)	Collagen I, II, III, IV, IX, X and XIV; gelatin; fibronectin; laminin; tenascin; aggrecan; fibrillin; serpins	• Promotes re-epithelialization indirectly by affecting wound contraction [69]	X		mRNA and immunohistochemistry [70]
		• Keratinocyte migration [70]		M	
		• MMP-13 knockout mice have reduced re-epithelialization and delayed wound closure [70]		M	
MMP-14 (MT1-MMP)	Collagen I,II, and III; gelatin; fibronectin; laminin; vitronectin; aggrecan; tenascin; nidogen; perlecan; fibrillin; α1-proteinase inhibitor, α2-macroglobulin	• Promotes keratinocyte migration and invasion [71]			^{35}S-labeled antisense RNA probes [72]
		• Involved in KGFR expression, and can regulate epithelial cell proliferation [72]		M	
		• Activates MMP-2 [73]	X		

Adapted from Martins and Caley [3].

Table 1. Mammalian MMPs: enzymatic substrates and roles in wound healing.

The complex orchestration of events that we outlined in Section 1.1 on wound healing involves important roles by MMPs. However, since MMPs are highly regulated at the proteome level, the transcriptional regulation is not the full picture. Yet, the transcriptional regulation of MMPs is the most studied, as the tools for it are readily available. For example, the increased transcription leads to higher translation to the inactive MMP zymogens, which have to experience proteolytic activation. This activation may only require disruption of the interaction between the active-site zinc ion and the conserved cysteine residue from the sequence …PRCGVPD… of the pro-domain to give rise to the active MMPs [3]. During physiological processes, pro-MMP activation can be achieved either by serine proteinases or by other MMPs [6]. In

particular, membrane-type MMPs have been shown to be capable of activating other pro-MMPs, both directly and indirectly. For instance, MMP-14 (or MT1-MMP) is involved in regulating activation of pro-MMP-9 in osteoclast migration [77]. Activation of MMPs by serine proteinases is regulated by inhibition of plasma proteinase inhibitors, including α1-proteinase and α2-macroglobulin or thrombospondin-1 and thrombospondin-2 [3]. The activity of MMPs is primarily regulated *in vivo* by endogenous tissue inhibitors of metalloproteinases (TIMPs) (**Figure 3**). In mammals, there are four TIMPs (TIMP-1, -2, -3, and -4) that bind specifically to inhibit MMPs [78]. The dysregulation such as imbalance between MMPs and TIMPs ratio leads to up-regulation of proteinase activity and damage to the ECM.

Method	Advantages	Limitations
mRNA and RT-PCR	Quick, simple, and inexpensive	Does not measure amount and activity of the proteinases
Western blot and immunohistochemistry	Simple, sensitive, and specific	Requires specific and expensive antibodies and does not distinguish between zymogen, active, and TIMP-complexed MMPs
Gelatin zymography	Inexpensive materials, semi-quantitative	Unable to distinguish between active and TIMP-complexed MMPs, low sensitivity
In-situ zymography	Identification of MMPs can be done in tissues	Limited to availability of fluorescent substrates, but not quantitative, and hard to discriminate between different MMPs, low sensitivity
Activity-based enzyme profiling	Specificity	Requires library of selective MMP-directed probes
TAPI-2 affinity resin	Identifies active MMPs	Starting materials are very expensive and requires user linking of TAPI to the resin
MMP inhibitor–tethered affinity resin	Identifies and quantifies active MMPs	Requires synthesis of MMP-inhibitor covalently attached to the resin
Adapted from Fisher and Mobashery [76].		

Table 2. Profiling methods for MMPs.

Once activated, the only MMP form that is not complexed by TIMPs would have catalytic competence. Hence, tools are needed for analysis at the protein level in the afflicted/diseased tissue. Many current methodologies to profile MMPs are limited because they are unable to detect active MMPs (summarized in **Table 2**). We have applied unique tools to this end in both diabetic and non-diabetic wounds. An MMP-inhibitor-tethered affinity resin that binds exclusively to the active forms of MMPs was used to fish out activated MMPs that exist in wound tissues [60]. Once bound, the active MMPs were digested with trypsin and the peptides

were analyzed by liquid chromatography—mass spectrometry/mass spectrometry (LC-MS/MS); the proteinases were identified from the peptides MS/MS data and a protein database search [60] (**Figure 5**). Subsequently, each identified active MMP was quantified using LC-MS/MS methods and custom-synthesized peptides. This analysis led to the discovery of active MMP-8 and MMP-9 in both diabetic and non-diabetic wounds from mice. The quantification revealed that MMP-9 was elevated at statistically significant levels, whereas levels of MMP-8 were slightly up-regulated after seven days from infliction of the wound [60].
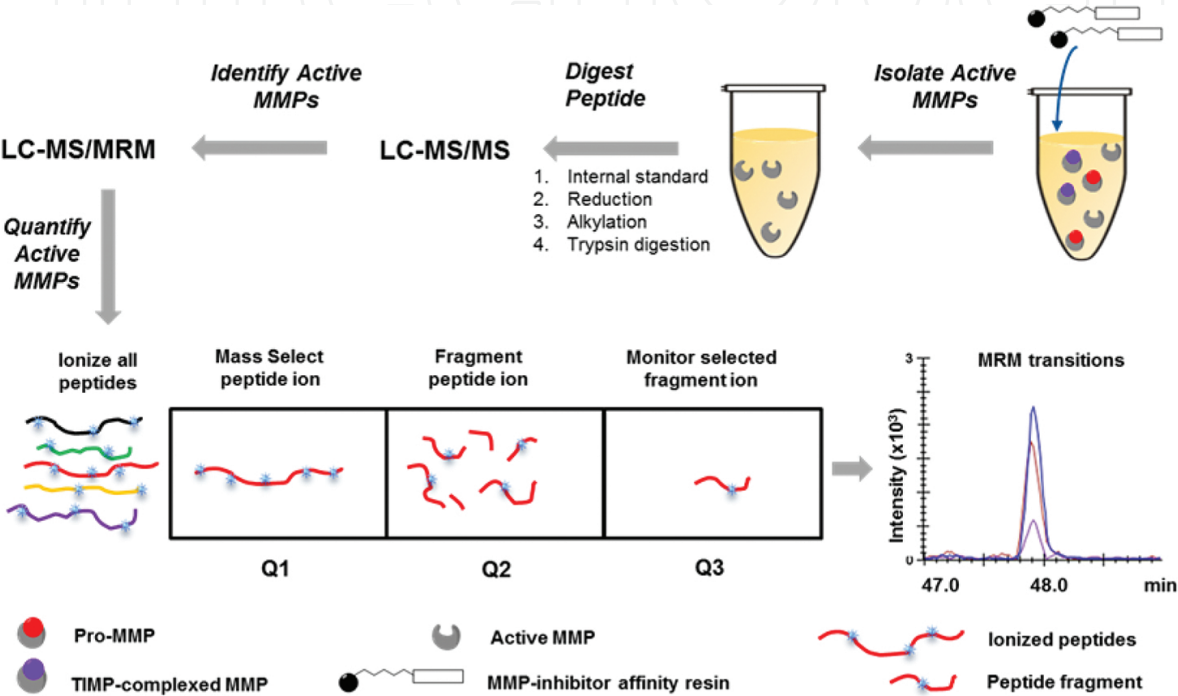

Figure 5. MMP-inhibitor-tethered affinity resin to identify and quantify active MMPs. Wound tissues are homogenized, and the homogenate is incubated with the MMP-inhibitor-tethered affinity resin, which binds only to active MMPs. The isolated active MMPs are reduced (to reduce disulfide bonds between the thiol groups of cysteine in MMPs), alkylated (to prevent reformation of disulfide bonds), and trypsin digested. The resulting peptides are analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and identified by a protein database. The identified MMPs are quantified using three peptides and three transitions per peptide using LC with multiple-reaction monitoring (MRM). In this highly specific quantitative MS method, the ionized peptide selected in the first quadrupole (Q1) generates to a pool of fragments in the second quadrupole (Q2), where the highest intensity fragment ion is selected for monitoring in the third quadrupole (Q3). This transition from peptide to fragment ion is monitored, and the area under this peak is integrated. Finally, the concentrations of active MMPs in wound samples are quantified by using peak area ratios relative to the internal standard and calibration curve regression parameters.

Whereas these studies were followed up by investigations of knockout mice as well, the knockout mice do not provide a superior opportunity for elucidation of the functions of MMPs in our opinion. Knockout MMP-9 mice, which survive the embryonic stage, were made diabetic to explore the role of the enzyme. We hasten to add that the compensatory activities of other MMPs in light of the overlapping profiles for the substrates create ambiguity in interpretation of the data. These compensatory activities will be present throughout the embryonic development up to the point in which the experiment is conducted with these mice. The more

superior approach, in our opinion, in elucidating the roles of the two enzymes (MMP-8 and MMP-9) is the use of selective pharmacological agents that afford total temporal control of abrogation of activity within the wounds in the time course of the experiments. Highly selective or specific inhibitors for the given enzyme are critical for the success of these studies. These investigations indeed revealed the duality of MMP functions, beneficial and detrimental, in diabetic wounds [60, 63]. It was documented that MMP-8 had a beneficial role in wound healing, as it might be the body's response to the healing process. On the other hand, MMP-9 was shown to serve a detrimental role in diabetic wound healing; hence, an aberration in the regulatory events in diabetic animals led to its formation with detrimental consequences. Indeed, pharmacological intervention by selective MMP-9 inhibitors with no activity toward MMP-8 would appear to be a promising approach to speed up healing of diabetic wounds. As the non-healing wounds remain open for a long period of time, they face the fatal threat of infections with methicillin-resistant *Staphylococcus aureus* [79, 80] that lead to amputations like in the case of diabetic foot infections [81]. There is a serious need to develop new approaches to facilitate healing in chronic wounds since current treatments have not been proven effective. The only FDA approved drug Regranex™ (becaplermin), a platelet-derived growth factor, is associated with malignancies and increased risk of death [82]. In addition, the effectiveness of negative-pressure wound therapy is still unclear, stem cell therapies do not clear the infection, or topical antibiotics, and antimicrobial dressings induce antibiotic resistance [81].

2. Detection of matrix metalloproteinases in tissue

As indicated above, MMPs are usually not detectable in normal adult tissues, but are up-regulated in disease. The tools available for assessment of MMP levels are quantification of mRNA, reverse transcription-polymerase chain reaction (RT-PCR), Western blotting and immunohistochemistry, gelatin zymography, *in-situ* gelatin zymography, activity-based enzyme profiling, and TAPI-2 resin [76]. However, these tools generally do not reveal whether the elevated levels of the MMPs that are being monitored are due to the inactive zymogenic forms, the active MMPs, or the MMPs in complex with TIMPs (inactive forms). Quantification of mRNA levels by Northern blot analysis and RT-PCR are limited in that these methods measure mRNA levels and not the amount and activity of the protein. Immunohistochemistry and Western blot require specific antibodies, which usually cannot distinguish between active and TIMP-inhibited MMPs, and might exhibit cross-reactivity. The sensitivity of zymography is not typically high, and this method also detects TIMP-complexed MMPs. *in-situ* zymography is limited by the availability of fluorescent proteinase substrates, which at present can be performed for MMP-1, -2, -3, -7, -8, -9, -12, -13, and -25. This method has limitations for quantitative determinations. Activity-based enzyme profiling of MMPs requires a library of selective MMP-directed probes [76]. A TAPI-2 affinity resin has been reported to identify active MMPs [76]. However, it is very expensive. With the exception of the TAPI-2 resin, the other methods do not identify and quantify the active forms of MMPs. A summary of advantages and limitations of these methods is given in **Table 2**. We add that another complication in these studies is that the active MMPs formed in diseased tissue might be present in minute quantities,

such that conventional detection methods at the proteome level might not be able to identify them. We reiterate that of the MMP forms, only the active MMPs in the absence of TIMP complexation would be able to perform its function in manifestation of the disease.

Expression of MMPs in normal uninjured skin is generally low. However, their activities are thought to be up-regulated when cutaneous wounds occur. For instance, low RNA expression levels of MMP-2 and MT1-MMP were reported in uninjured murine skin [50]. Once the cutaneous injury happens, up-regulation and expression of many MMPs have been reported, including collagenases (MMP-1 [83], MMP-8 [60, 61], and MMP-13 [70]), gelatinases (MMP-2 [51] and MMP-9 [51, 60]), stromelysins (MMP-3 and MMP-10 [54]), and other MMPs such as MMP-7 [58], MMP-12 [50], and MMP-14 [51]. However, it should be noted that most of these studies employed methods that do not distinguish between the active or inactive forms of MMPs, except the aforementioned TAPI-2 resin and the recent methodology that couples an MMP-inhibitor-tethered affinity resin with mass spectrometry, as mentioned in the previous section [60]. As such, observation of up-regulation or even the expression of a particular MMP does not necessarily imply a role for that MMP in wound healing. Parallel to MMPs, the expression of TIMPs is often increased in order to regulate the proteinase activities [84]. Hence, the biochemical imbalance that leads to aberrant consequence has to be the focus of research in elucidating the mechanistic basis of disease.

2.1. Beneficial roles of MMPs in wound repair

Collagenases have been implicated in wound healing for many years. As the name implies, these proteinases prefer to turn over various types of collagen (types I, II, and III), which is an important process in wound repair (**Table 1**). MMP-1, expressed by keratinocytes about a week after injury occurs, might facilitate keratinocyte migration when these cells come into contact with type I collagen in the early re-epithelization phase [83]. The interaction between keratinocytes at the wound edge and type I collagen in the matrix via the $\alpha2\beta1$ integrin receptor enhances the expression of MMP-1 [38]. MMP-1 cleaves type I collagen to generate cleaved fragments, which have been suggested to become a less adhesive binding ligand than the native protein and that loosens the matrix environment for cellular movements. Thus, the complex of MMP-1 and $\alpha2\beta1$ promotes migration of keratinocyte on type I collagen in the re-epithelialization phase, as shown in **Figure 6**. However, $\alpha2\beta1$ integrin-deficient mice still retain normal re-epithelialization, collagen deposition, and tensile strength, which indicate a possible compensatory mechanism by another integrin receptor [85]. Once the new basement membrane is established after re-epithelialization, the epidermal expression of MMP-1 is terminated by cellular contacts with proteins from the membrane. Specifically, the contact between keratinocytes with laminin-111 (previously called laminin-1) leads to the repression of MMP-1 in the presence of type I collagen [86]. Expression of MMP-1 has also been observed in fibroblasts during granulation and angiogenesis [83], where the enzyme might act to remodel the ECM of the wound [87]. Interestingly, overexpression of human MMP-1 in the epidermis of transgenic mice resulted in delayed wound closure; however, the genomic modification with human DNA in these animals may have resulted in unwanted phenotypes [88].
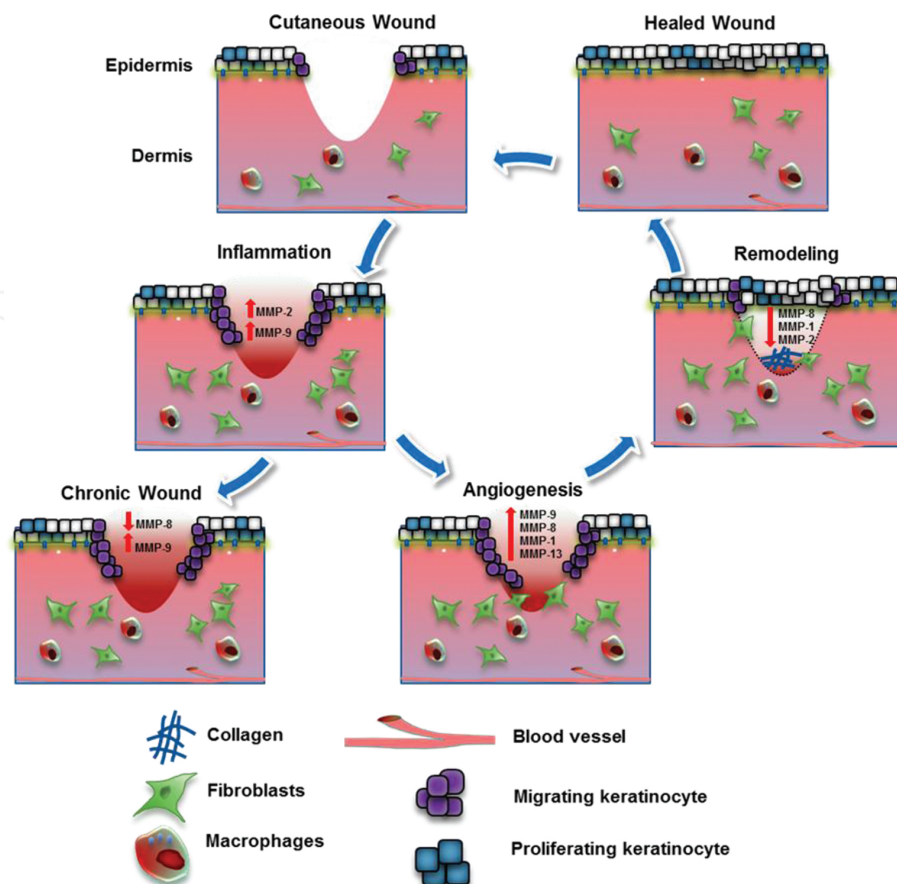
Figure 6. Involvement of MMPs in the wound-healing process. The healed or healthy skin, consisting of ECM and blood vessels, is populated by fibroblasts. Once the skin is damaged by a full-thickness injury, it becomes a cutaneous wound. In the early phase of inflammation, the wound is occupied with fibrin clot to seal the wound, and levels of MMP-2 and MMP-9 are increased. Fibroblasts and macrophages migrate into the wound site, where they are stimulated to release more MMPs to remodel the ECM. The inflammation phase is followed by angiogenesis, in which the up-regulation of a variety of MMPs (including MMP-1, MMP-8, MMP-9, and MMP-13) would stimulate epithelial cells (keratinocytes) to proliferate and migrate to re-epithelialize over the wound area. However, prolonged inflammation could cause the wounds to become chronic, as it has been observed in diabetic foot ulcers. During chronic wounds, the irregular up-regulation of MMP-9 has been associated with reduction of MMP-8 and plays a detrimental role in ECM remodeling. Tissue remodeling and expression of MMPs are attenuated when the epithelial cells proliferate and differentiate in order to reform the new epithelium. During this last phase of wound healing, fibroblasts can continue to remodel the underlying dermis over a period of several months.

Expression of human MMP-1 has shown similar patterns to expression of murine MMP-13 in an excisional wound healing model (**Figure 6**) [50, 89]. Both MMP-1 and MMP-13 may share roles in promoting the survival of fibroblasts while remodeling collagen deposition in the wound ECM [69]. Hattori *et al.* have shown that MMP-13 knockout mice had both delayed re-epithelialization and wound closure (**Table 3**) [70]. However, in another study, MMP-13 knockout mice showed normal efficiency of re-epithelialization, wound closure, inflammatory response, and unaltered remodeling of the wound matrix [90]. The inconclusive evidence on the role of MMP-13 in wound repair could in principle be explained by the redundancy in functions of proteinases that we mentioned earlier. There was up-regulated expression of MMP-8 (or collagenase-2) in these MMP-13-deficient animals [90], for example. The relevance

of MMP-8 in wound repair was unclear until this study indicated that there is enzymatic compensation by MMP-8 to facilitate normal wound healing [90]. There is mounting evidence that MMP-8 plays a beneficial role in repairing the wounds. As MMP-8 is expressed and secreted mostly by neutrophils, its expression peaks after four days in non-diabetic wounds, detected by ELISA [61]. In humans, MMP-8 is the most prevalent collagenase in cutaneous wounds, where it is required for debridement of the wound and for the removal of damaged type I collagen [61]. Yet, it is still challenging to conclude whether this is the active form of MMP-8 by the methods that were used. In mice, the exact levels of active MMP-8 were quantitatively determined by the use of an MMP-inhibitor-tethered affinity resin coupled with mass spectrometry [60]. In this study, Gooyit *et al*. also demonstrated that selective inhibition of MMP-8 caused delayed wound healing and incomplete re-epithelialization in diabetic mice [60]. In addition, MMP-8-deficient mice displayed a significant delay in wound healing, caused by a lag in neutrophil infiltration, persistent inflammation and impaired re-epithelialization (**Table 3**) [62]. However, MMP-8 knockout mice show compensation in MMP-9 [62], making it difficult to separate the roles of MMP-8 and MMP-9 in wound healing. Furthermore, topical application of active recombinant MMP-8 on murine diabetic wounds resulted in a significant acceleration in both wound healing and re-epithelialization [63]. These studies reveal the beneficial role of MMP-8 in wound healing, where the neutrophil-derived MMP-8 can facilitate repair processes by providing debridement for damaged proteins and paving a pathway for the formation of the provisional matrix for keratinocyte migration (**Figure 6**).

Gene	Modification	Wound phenotype	Reference
hmmp-1	Overexpression in keratinocytes	Delayed re-epithelialization	[88]
mmp-3	Knockout	Impaired wound contraction	[55]
mmp-8	Knockout	Delayed re-epithelialization, delayed arrival of and prolonged inflammation	[62]
mmp-9	Knockout	Enhanced re-epithelization, accelerated wound closure	[63, 64]
mmp-10	Overexpression in KCs	Unaltered wound closure, scattered epithelialization	[67]
mmp-13	Knockout	Delayed wound closure, and reduced re-epithelialization	[70]
mmp-14	Knockout	Unaltered wound closure over 7 days' but premature morbidity and mortality were observed in these mice	[92, 93]

Table 3. Metalloproteinase gene targeting in mice studies and wound phenotypes.

The underlying cause of diabetic complications leads to up-regulation of MMP-9 compared to MMP-8. For instance, biopsy samples from diabetic patients revealed only two-fold increase in MMP-8, but 14-fold increase in MMP-9 expression when compared to non-diabetic tissues

[49]. Although the application of recombinant MMP-8 accelerated healing of full-thickness wounds in diabetic mice, it might have a similar beneficial mechanism as the marketed drug Santyl®, which is indicated for debridement of chronic dermal ulcers and severely burned areas and contains collagenase derived from *Clostridium histolyticum*. Clinical evidence suggests that collagenase treatment expedites the removal of necrotic tissues and enhances keratinocyte migration [91]. When used after debridement, Santyl® promotes wound healing in patients with pressure ulcers, venous leg ulcers, diabetic ulcers, and severely burnt wounds [91]. However, excessive use of active recombinant MMP-8 may affect the formation of new ECM and may not be beneficial in wound healing. A dose-response study with active recombinant MMP-8 topically administered to wounds of diabetic mice showed that higher levels of this proteinase did not accelerate wound healing [63].

Gelatinases, MMP-2 and MMP-9, are also involved in wound repair. Early expression of both gelatinases is observed in platelets, where MMP-9 is involved in platelet production and MMP-2 mediates platelet adhesion and aggregation [52]. The early expression of gelatinases might contribute to degradation of gelatin matrix in biofilm produced by bacteria [94]. This degradation serves to weaken the attachment of bacterial biofilm to the wound site and might be a strategy in fighting infection [94]. In addition, gelatinases are able to digest various constituents of the wound matrix to initiate angiogenesis in the repair processes. After tissue injury, AngII, which plays roles in inflammation, cell proliferation, and migration, would stimulate macrophages and neutrophils to generate ROS and MMPs to promote cell adhesion and ECM formation [17]. Specifically, AngII has been shown to induce the expression of both gelatinases, MMP-2 [95] and MMP-9 [96, 97]. AngII has been demonstrated to promote angiogenesis via activation of VEGF and endothelial nitric oxide [98], whereas studies with AngII-type 1a receptor knockout mice or with inhibition of AngII receptor have resulted in delayed wound healing with reduced angiogenesis in animals [99]. Given the beneficial role of AngII in angiogenesis of wound healing, it is interesting to note that there is discrepancy in the outcomes of diseases treated with this factor's inhibitors. These inhibitors can either block AngII receptors or inhibit the enzyme that generates factor AngII in the RAS pathway. In cancer, AngII inhibitors have been shown to reduce the tumor-related VEGF expression, angiogenesis, and tumor size [16]. These drugs are also used to treat hypertension, in which dysregulation of RAS causes poor blood flow, inadequate supply of oxygen, and impaired wound healing [12]. When used in anti-hypertensive therapy, drugs such as losartan has been demonstrated to promote wound healing in diabetes-induced mice by improving vascular perfusion, without affecting VEGF expression [100]. To this extent, anti-hypertensive therapy appears to be beneficial for wound healing. Thus, there is a need for further research to elucidate the precise role of these inhibitors in diabetic patients with impaired wound healing.

Expression of MMP-2 is demonstrated to coincide with expression of laminin-332 (also referred to as laminin-5) during enhanced keratinocyte migration in wound healing [101]. Since both MMP-2 and MMP-9 can cleave the gamma-2 chain of laminin-322 [102], they result in a pro-migratory and EGF-like fragment that binds EGF receptor to trigger cell migration of kerati-nocytes at the wound matrix [34], demonstrated in **Figure 2**. This cleaved fragment has been found in both tumors and tissues that undergo remodeling, except for intact epidermis [34,

103, 104]. Interestingly, MMP-8 also cleaves laminin-332, which indicates the mechanistic redundancy of the roles of MMPs during wound healing [102]. Furthermore, the two gelatinases might contribute to angiogenesis possibly by activating cytokines such as TNF-α (tumor necrosis factor-alpha) [105] and VEGF [106, 107]. However, cleavage of laminin-332 by MMP-2 has only been shown in tumor cells and normal breast epithelial cells, but not in normal keratinocytes in wound repair. Some studies have implicated MMP-2 in cleaving the latency-associated peptide (LAP) of pro-TGF-β and latent TGF-β binding protein (LTBP) to release activated TGF-β to bind the ECM [108–110]. Another study has also indicated that the active form of MMP-2 can activate MMP-9 in cell culture [53]. Pro-MMP-2 itself needs to be activated, and it has been shown that this activation requires the cluster of MMP-14, pro-MMP-2, and αVβ3 integrin in a model of breast cancer cells [111]. It is important to note that active MMP-2 was not observed in wounds of diabetic and non-diabetic mice in the studies that used the MMP-inhibitor-tethered affinity resin, described earlier [60]. Therefore, the role of MMP-2 during wound healing has remained obscured with no *in vivo* verification to date. In fact, the study that implicated both active MMP-2 and MMP-9 in human wound healing used gelatin zymography as the tool [112]. However, this method lacks the ability to detect exclusively the active MMPs in the wound tissues, because the denaturation of the TIMP-MMP complex during electrophoresis could also result in an active MMP-2 band (**Table 2**). On the other hand, inhibition of MMP-9 activity with an antibody or MMP-9 ablation has delayed keratinocyte migration *in vitro*, which indicates the necessary involvement of MMP-9 during normal wound closure [65]. Others have also demonstrated *in vitro* that MMP-9 appears to promote keratinocyte migration [113]. Indeed, the study with the MMP-inhibitor-tethered affinity resin revealed that active MMP-9 was essentially undetectable in the intact skin, but it was expressed as early as one day after injury and remained up-regulated throughout the two weeks of study in non-diabetic and diabetic mice [60]. In the case of diabetic mice with delayed wound closure, the analysis showed up-regulation of active MMP-9, which could be detrimental to the repair process [60]. Besides promoting angiogenesis, gelatinases and other MMPs interestingly can inhibit angiogenesis by generating anti-angiogenic peptides from other precursor proteins. For instance, distinct proteinases such as MMP-3, -7, -9, -13, and -20 have been shown to generate active endostatin from human collagen XVIII [114] *in vitro*, whereas MMP-2, -3, -7, -9, and -12 are responsible for generating angiostatin from plasminogen [68, 115].

Expression of MMP-3 and MMP-10 (two stromelysins) has been found in epidermal cells during human and murine wound healing using RNA probes (**Table 1**). MMP-3 is expressed by the basal-proliferating keratinocytes, which are in contact with the intact basement membrane and close to the wound edge [54]. Expression of MMP-3 is also detected in fibroblasts during wound healing [11]. Research has shown that wound closure was delayed in non-diabetic MMP-3 knockout mice due to impaired wound contraction (**Table 3**) [55]. The implicated involvement of MMP-3 in normal wound healing may have resulted from demonstration that MMP-3 could activate MMP-9 [56], the gelatinase that plays roles in keratinocyte migration. However, MMP-2 could also trigger activation of MMP-9 [53], which corroborates the possibility of mechanistic compensation by other MMPs in physiology. Thus, the involvement of MMP-3 in the repair processes of wound healing still remains ambiguous. Nonetheless, it has been demonstrated that MMP-3 can activate several pro-MMPs, digest many ECM

components, and increase the availability, as well as the activities, of cytokines and growth factors [116]. These findings disclose roles for MMP-3 in cell migration and proliferation during wound repair.

Meanwhile, MMP-10 (stromelysin-2), is expressed with a different pattern even though both MMP-3 and MMP-10 can degrade several collagens and non-collagenous connective tissue substrates, including proteoglycans, gelatin, fibronectin, and laminin [117], as indicated in **Table 1**. Human MMP-10 is expressed by epidermal cells about three days post-wounding, where its regulation seems to depend on EGF, TGF-β, and TNF-α cytokines [66]. The role of MMP-10 in wound repair was investigated by overexpressing a constitutively active MMP-10 mutant in keratinocytes, which resulted in normal wound-healing architecture and normal wound-healing rate in these transgenic mice [67]. However, the epidermal histology was demonstrated to have a disorganized migrating epithelium, composed of degradation in the newly formed matrix via laminin-332, abnormal cell-to-cell contacts of keratinocytes, and finally an increased rate of apoptosis of keratinocytes [67]. These findings indicate that levels of MMP-10 require a tightly regulated expression to facilitate keratinocyte migration during wound healing. Although both stromelysins would appear to be players, more investigations are needed to ascertain the roles of active MMP-3 and MMP-10 in the physiology of wound repair.

In addition to gelatinases and collagenases, other MMPs might have roles in wound healing as well, even though the data are not conclusive. For instance, MMP-7 (matrilysin) has been shown to be expressed in injured epithelia of various mucosal tissues, including lung, kidney, cornea, and gut [58, 118–120], even though MMP-7 is not expressed in epidermal wounds. In the lungs, MMP-7 has been demonstrated to play a role in inducing epithelial migration by facilitating the shedding of syndecan-1, a transmembrane heparin sulfate proteoglycan [58]. In the same study, MMP-7 knockout mice displayed impaired re-epithelialization in the mucosal tissue [58]. Also in the lungs, MMP-7 has been shown to cleave E-cadherin in the process of facilitating cell migration away from the edge of the injured wound [118]. On the other hand, another study has shown that MMP-7 and MMP-13 are expressed at the invasive edge of tumors [121]. Another proteinase that might be important for the wound-repair process is MMP-12 (metalloelastase), which is expressed by macrophages surrounding blood vessels in acute murine excisional wounds [50]. Even though MMP-12 was not detected in either acute- or chronic-wound tissues in the presence of macrophages, this proteinase expression was found to be abundant in different human cutaneous granulomas [122]. In addition to its ability to degrade fibrinogen interfering with blood clotting [123], MMP-12 is a potential regulator of angiogenesis, since it was demonstrated to be most efficient at producing angiostatin [68]. Membrane-type MMPs might also be necessary for wound healing, more specifically MMP-14, which is the most extensively studied to date (**Table 1**). The pivotal role of MMP-14 in angiogenesis of wound healing may be attributed to the enzyme's fibrinolytic and collageno-lytic activity that is necessary for cell migration [71]. In addition, MMP-14 is needed for TIMP-2-mediated activation of pro-MMP-2, a process that is coordinated by two MMP-14 molecules and TIMP-2 [73]. Although MMP-14-deficient mice display abnormalities in bone develop-ment, impaired angiogenesis, and defective type I collagen [93, 124], wound closure in these

animals remains surprisingly unaffected (**Table 3**) [92]. However, MMP-14 has been demonstrated to regulate cell proliferation by altering the expression of the KGF receptor during wound healing in acute airway injury [72]. The overlapping functions of other membrane-type MMPs or other MMPs may compensate for the absence of MMP-14 in these animals, supporting the concept of proteinase redundancy among MMPs.

2.2. Roles of MMPs in the pathology of chronic wounds

Cutaneous injuries that are recalcitrant to healing will become chronic wounds. In addition to delayed wound closure, chronic wounds are characterized by excessive proteolysis, prolonged inflammation, and failure in re-epithelialization [125]. Although MMPs play important roles in restructuring the ECM and repairing the wounds, high levels of MMPs can be blamed for increased proteolysis that leads to excessive degradation of ECM constituents and disruptions of cell migration. These unwarranted events cause the wounds to enter a prolonged inflammation. ELISA was used to document 65-fold higher levels of MMP-1, twofold higher of MMP-8, and twofold lower of TIMP-2, whereas gelatin zymography showed 14-fold higher levels of MMP-9, and sixfold higher of MMP-2 in diabetic foot ulcers than in non-diabetic patients with acute wounds [49]. Up-regulation of MMPs hinders wound repair by degrading ECM components and growth factors excessively [126]. As the wounds stay open too long, the invading bacteria might also release bacterial proteinases to cause rapid degradation of growth factors [94]. In order to defend the wounds against the invading microbes, the body will secrete more ROS and inflammatory factors. High levels of ROS such as hydrogen peroxide cause tissue damage [22], and high levels of inflammatory factors can lead to elevated expression of MMPs, as discussed earlier. The delaying mechanism of this vicious cycle keeps the patient's wound in a chronic stage [127]. Most studies emphasize MMP-9 up-regulation, which is associated with poor wound healing in diabetic foot ulcers and chronic wounds (**Figure 6**). When high levels of exogenous MMP-9, parallel to human chronic wounds, was applied to non-diabetic mice, this treatment delayed wound healing of the animals [128]. In one study, up-regulated levels of MMP-9 were found in wound fluid from patients with unhealed diabetic foot ulcers when compared with healed ulcers, as determined by gelatin zymography [129]. Also, in this same study, the researchers found decreased levels of TGF-β1 and TIMP-1 using ELISA [129]. In another study of patients with diabetic foot ulcer, levels of MMP-9 were measured by Western blot with MMP-9 antibody and were higher in patients with high risk of developing foot ulcers [130]. Expression of this proteinase was detected in migrating epithelial cells by ELISA [50, 51] and in inflammatory cells including T cells and neutrophils by gelatin zymography [131, 132]. Nevertheless, it should be noted that increased levels of MMPs, specifically that of MMP-9, as determined by ELISA, Western blot or gelatin zymography do not necessarily imply that it is active or has any role in the pathology of chronic wounds. ELISA and Western blot depend on the specificity of the antibodies, which likely immunoreact with pro-MMPs, active MMPs, and TIMP-complexed MMPs. Similarly, the active MMP-2 and MMP-9 bands detected by gelatin zymography could be from the TIMP-complexed gelatinases that dissociate during electrophoresis [133]. Therefore, the expression of MMP-9 found in many studies cannot be established conclusively as active MMP-9, the only form of the proteinase that can modify substrates catalytically. Another common research

method is the use of MMP knockout animals, which may provide further insights into the roles of MMPs in wound healing (**Table 3**). However, the drawback of knockout animals is the possibility of mechanistic compensation by other MMPs in the absence of the ablated MMP, as discussed earlier. For instance, it has been shown that levels of MMP-9 are increased when MMP-2 or MMP-8 are ablated [62, 134]. Also, many MMPs share the same substrates, indicating the redundancy in the proteinase functions of MMPs [3].

We described earlier the MMP-inhibitor-tethered affinity resin that Gooyit *et al*. used to identify and accurately measure the levels of active MMP-9, which was found to be up-regulated in diabetic mice with delayed wound healing [60]. The dual roles of MMPs are exhibited in this case of MMP-9 up-regulation, which was demonstrated to be detrimental to diabetic wound repair by topical treatment with two distinct selective MMP-9 inhibitors (ND-322 and ND-336) [60, 63]. Inhibition of MMP-9 accelerated wound healing and promoted re-epithelialization. It has been shown that MMP-9 inhibits cell replication during epithelial migration; thus, MMP-9-deficient mice, both diabetic and non-diabetic, display a better rate of wound closure [63, 64]. Similar to MMP-2, MMP-9 can also activate pro-TGF-β and release it from LTBP [108, 109], while TGF-β has been shown to induce pro-MMP-9 in human skin [135]. Since TGF-β1 is a cytokine that elicits recruitment of inflammatory cells during wound healing [136], its up-regulation can regulate wound repair [137]. Interestingly, prolonged elevation in levels of inflammatory cytokines, such as TGF-β, can lead to a prolonged inflammation phase and consequentially delayed wound closure in diabetic mice [138]. However, it has also been shown that non-diabetic MMP-9 knockout mice had delayed wound closure [70]. The apparent conflicting role of MMP-9 may be explained by compensation of other MMPs, such as increased expression of MMP-3 and MMP-13 in MMP-9 knockout animals [139]. In addition, the redundancy of MMP substrates allow other MMP(s) to fulfill the same role, for instance MMP-1, MMP-2, MMP-9, and MMP-13 have a role in keratinocyte migration and can replace MMP-9 during normal wound healing [38, 70, 113, 140]. To date, topical application with a selective MMP-9 inhibitor, by itself or in combination with recombinant MMP-8, has shown therapeutic potential in accelerating murine diabetic wound healing [63]. These treatments improve diabetic wound healing by increasing angiogenesis and restoring levels of inflammatory cytokines, including TGF-β1 [63].

3. Conclusions

MMPs exist in three forms—pro-MMPs, active MMPs, and TIMP-complexed MMPs—of which only the active MMPs play a role in the pathology or repair of acute and chronic wounds. Current methodologies do not distinguish between the three forms of MMPs. Thus, the roles of MMPs in acute and chronic wounds are still not Qualitative and well-characterized quantitative profiling of only the active form of MMPs is necessary for investigating the critical roles of MMPs in remodeling the ECM during wound repair. We used a novel MMP-inhibitor-tethered affinity resin that binds only the active form of MMPs, from which we identified and quantified active MMP-8 and active MMP-9 in a murine diabetic model with delayed wound healing [60]. We showed that up-regulation of active MMP-9 plays a detrimental role whereas

active MMP-8 is involved in repairing the wound in diabetic mice [60, 63]. These studies identified MMP-9 as a novel target for therapeutic intervention in the treatment of chronic wounds. A selective inhibitor of MMP-9 that leaves MMP-8 unaffected would provide the most effective therapy and represents a promising strategy for therapeutic intervention in the treatment of diabetic foot ulcers.

Acknowledgements

Author details

Trung T. Nguyen, Shahriar Mobashery and Mayland Chang[*]

*Address all correspondence to: mchang@nd.edu

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana, USA

References

[1] Kirby GT, Mills SJ, Cowin AJ, Smith LE. Stem cells for cutaneous wound healing. Biomed Res Int. 2015;2015:285869.

[2] Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol. 2014;15(12):786–801.

[3] Martins VL, Caley M, O'Toole EA. Matrix metalloproteinases and epidermal wound repair. Cell Tissue Res. 2013;351(2):255–268.

[4] Rohani MG, Parks WC. Matrix remodeling by MMPs during wound repair. Matrix Biol. 2015;44–46:113–121.

[5] Salazar JJ, Ennis WJ, Koh TJ. Diabetes medications: impact on inflammation and wound healing. J Diabetes Complic. 2016;30(4):746–752.

[6] Caley MP, Martins VL, O'Toole EA. Metalloproteinases and wound healing. Adv Wound Care (New Rochelle). 2015;4(4):225–234.

[7]   Ugalde AP, Ordonez GR, Quiros PM, Puente XS, Lopez-Otin C. Metalloproteases and the degradome. In: Clark IM, Young DA, Rowan AD, editors. Matrix Metalloproteinase Protocols, vol. 622, 2nd ed. New York: Humana Press 2010. p. 3–29.

[8]   Brkic M, Balusu S, Libert C, Vandenbroucke RE. Friends or foes: matrix metalloproteinases and their multifaceted roles in neurodegenerative diseases. Mediat Inflamm. 2015;2015:620581.

[9]   Folgueras AR, Pendas AM, Sanchez LM, Lopez-Otin C. Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. Int J Dev Biol. 2004;48(5–6):411–424.

[10]   Chang M. Restructuring of the extracellular matrix in diabetic wounds and healing: a perspective. Pharmacol Res. 2016;107:243–248.

[11]   Toriseva M, Kahari VM. Proteinases in cutaneous wound healing. Cell Mol Life Sci. 2009;66(2):203–224.

[12]   Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010;89(3):219–229.

[13]   Egozi EI, Ferreira AM, Burns AL, Gamelli RL, Dipietro LA. Mast cells modulate the inflammatory but not the proliferative response in healing wounds. Wound Repair Regen. 2003;11(1):46–54.

[14]   Schultz GS, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing. Wound Repair Regen. 2009;17(2):153–162.

[15]   Clark RA. Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. J Invest Dermatol. 1990;94(Suppl 6):128S–134S.

[16]   George AJ, Thomas WG, Hannan RD. The renin-angiotensin system and cancer: old dog, new tricks. Nat Rev Cancer. 2010;10(11):745–759.

[17]   Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol. 2007;292(1):C82–C97.

[18]   Steckelings UM, Wollschlager T, Peters J, Henz BM, Hermes B, Artuc M. Human skin: source of and target organ for angiotensin II. Exp Dermatol. 2004;13(3):148–154.

[19]   Macri L, Silverstein D, Clark RA. Growth factor binding to the pericellular matrix and its importance in tissue engineering. Adv Drug Deliv Rev. 2007;59(13):1366–1381.

[20]   Hyytiainen M, Penttinen C, Keski-Oja J. Latent TGF-beta binding proteins: extracellular matrix association and roles in TGF-beta activation. Crit Rev Clin Lab Sci. 2004;41(3):233–264.

[21]   Roberts AB, Heine UI, Flanders KC, Sporn MB. Transforming growth factor-beta. Major role in regulation of extracellular matrix. Ann N Y Acad Sci. 1990;580:225–232.

[22]   Schreml S, Szeimies RM, Prantl L, Karrer S, Landthaler M, Babilas P. Oxygen in acute and chronic wound healing. Br J Dermatol. 2010;163(2):257–268.

[23] Gary Sibbald R, Woo KY. The biology of chronic foot ulcers in persons with diabetes. Diabetes Metab Res Rev. 2008;24(Suppl 1):S25–S30.

[24] Kumin A, Schafer M, Epp N, Bugnon P, Born-Berclaz C, Oxenius A, et al. Peroxiredoxin 6 is required for blood vessel integrity in wounded skin. J Cell Biol. 2007;179(4):747–760.

[25] Boudreau NJ, Jones PL. Extracellular matrix and integrin signalling: the shape of things to come. Biochem J. 1999;339(Pt 3):481–488.

[26] Olaso E, Labrador JP, Wang L, Ikeda K, Eng FJ, Klein R, et al. Discoidin domain receptor 2 regulates fibroblast proliferation and migration through the extracellular matrix in association with transcriptional activation of matrix metalloproteinase-2. J Biol Chem. 2002;277(5):3606–3613.

[27] Sen CK, Khanna S, Babior BM, Hunt TK, Ellison EC, Roy S. Oxidant-induced vascular endothelial growth factor expression in human keratinocytes and cutaneous wound healing. J Biol Chem. 2002;277(36):33284–33290.

[28] Senger DR, Claffey KP, Benes JE, Perruzzi CA, Sergiou AP, Detmar M. Angiogenesis promoted by vascular endothelial growth factor: regulation through alpha1beta1 and alpha2beta1 integrins. Proc Natl Acad Sci U S A. 1997;94(25):13612–13617.

[29] Rossiter H, Barresi C, Pammer J, Rendl M, Haigh J, Wagner EF, et al. Loss of vascular endothelial growth factor a activity in murine epidermal keratinocytes delays wound healing and inhibits tumor formation. Cancer Res. 2004;64(10):3508–3516.

[30] Hong YK, Lange-Asschenfeldt B, Velasco P, Hirakawa S, Kunstfeld R, Brown LF, et al. VEGF-A promotes tissue repair-associated lymphatic vessel formation via VEGFR-2 and the alpha1beta1 and alpha2beta1 integrins. FASEB J. 2004;18(10):1111–1113.

[31] Tran KT, Lamb P, Deng JS. Matrikines and matricryptins: Implications for cutaneous cancers and skin repair. J Dermatol Sci. 2005;40(1):11–20.

[32] Panayotou G, End P, Aumailley M, Timpl R, Engel J. Domains of laminin with growth-factor activity. Cell. 1989;56(1):93–101.

[33] Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. Dev Dyn. 2000;218(2):213–234.

[34] Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. Science. 1997;277(5323):225–228.

[35] Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, et al. Epithelialization in wound healing: a comprehensive review. Adv Wound Care (New Rochelle). 2014;3(7):445–464.

[36] Golliger JA, Paul DL. Wounding alters epidermal connexin expression and gap junction-mediated intercellular communication. Mol Biol Cell. 1995;6:10.

[37] Paladini RD, Takahashi K, Bravo NS, Coulombe PA. Onset of re-epithelialization after skin injury correlates with a reorganization of keratin filaments in wound edge keratinocytes: defining a potential role for keratin 16. J Cell Biol. 1996;132(3):17.

[38] Pilcher BK, Dumin JA, Sudbeck BD, Krane SM, Welgus HG, Parks CW. The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. J Cell Biol. 1997;137(6):12.

[39] Jacinto A, Martinez-Arias A, Martin P. Mechanisms of epithelial fusion and repair. Nat Cell Biol. 2001;3(5):E117–E123.

[40] Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37(5):1528–1542.

[41] Sommer K, Sander AL, Albig M, Weber R, Henrich D, Frank J, et al. Delayed wound repair in sepsis is associated with reduced local pro-inflammatory cytokine expression. PLoS One. 2013;8(9):e73992.

[42] Rosenblum G, Meroueh S, Toth M, Fisher JF, Fridman R, Mobashery S, et al. Molecular structures and dynamics of the stepwise activation mechanism of a matrix metallopro- teinase zymogen: challenging the cysteine switch dogma. J Am Chem Soc. 2007;129(44): 13566–13574.

[43] Kotra LP, Cross JB, Shimura Y, Fridman R, Schlegel HB, Mobashery S. Insight into the complex and dynamic process of activation of matrix metalloproteinases. J Am Chem Soc. 2001;123(13):3108–3113.

[44] Gomis-Ruth FX. Catalytic domain architecture of metzincin metalloproteases. J Biol Chem. 2009;284(23):15353–15357.

[45] Massova I, Kotra LP, Fridman R, Mobashery S. Matrix metalloproteinases: structures, evolution, and diversification. FASEB J. 1998;12(12):1075–1095.

[46] Lopez-Otin C, Overall CM. Protease degradomics: a new challenge for proteomics. Nat Rev Mol Cell Biol. 2002;3(7):509–519.

[47] Chen P, Parks WC. Role of matrix metalloproteinases in epithelial migration. J Cell Biochem. 2009;108(6):1233–1243.

[48] Saarialho-Kere UK, Kovacs SO, Pentland AP, Olerud JE, Welgus HG, Parks WC. Cell- matrix interactions modulate interstitial collagenase expression by human keratino- cytes actively involved in wound-healing. J Clin Invest. 1993;92(6):2858–2866.

[49] Lobmann R, Ambrosch A, Schultz G, Waldmann K, Schiweck S, Lehnert H. Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non- diabetic patients. Diabetologia. 2002;45(7):1011–1016.

[50] Madlener M, Parks WC, Werner S. Matrix metalloproteinases (MMPs) and their physiological inhibitors (TIMPs) are differentially expressed during excisional skin wound repair. Exp Cell Res. 1998;242(1):201–210.

[51] Mirastschijski U, Impola U, Jahkola T, Karlsmark T, Agren MS, Saarialho-Kere U. Ectopic localization of matrix metalloproteinase-9 in chronic cutaneous wounds. Hum Pathol. 2002;33(3):355–364.

[52] Jurasz P, Chung AW, Radomski A, Radomski MW. Nonremodeling properties of matrix metalloproteinases: the platelet connection. Circ Res. 2002;90(10):1041–1043.

[53] Fridman R, Toth M, Pena D, Mobashery S. Activation of progelatinase B (MMP-9) by gelatinase A (MMP-2). Cancer Res. 1995;55(12):2548–2555.

[54] Vaalamo M, Weckroth M, Puolakkainen P, Kere J, Saarinen P, Lauharanta J, et al. Patterns of matrix metalloproteinase and TIMP-1 expression in chronic and normally healing human cutaneous wounds. Br J Dermatol. 1996;135(1):52–59.

[55] Bullard KM, Lund L, Mudgett JS, Mellin TN, Hunt TK, Murphy B, et al. Impaired wound contraction in stromelysin-1-deficient mice. Ann Surg. 1999;230(2):260–265.

[56] Ogata Y, Enghild JJ, Nagase H. Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. J Biol Chem. 1992;267(6):3581–3584.

[57] Saarialho-Kere UK, Pentland AP, Birkedal-Hansen H, Parks WC, Welgus HG. Distinct populations of basal keratinocytes express stromelysin-1 and stromelysin-2 in chronic wounds. J Clin Invest. 1994;94(1):79–88.

[58] Chen P, Abacherli LE, Nadler ST, Wang Y, Li Q, Parks WC. MMP7 shedding of syndecan-1 facilitates re-epithelialization by affecting alpha(2)beta(1) integrin activation. PLoS One. 2009;4(8):e6565.

[59] Dunsmore SE, Saarialho-Kere UK, Roby JD, Wilson CL, Matrisian LM, Welgus HG, et al. Matrilysin expression and function in airway epithelium. J Clin Invest. 1998;102(7): 1321–1331.

[60] Gooyit M, Peng Z, Wolter WR, Pi H, Ding D, Hesek D, et al. A chemical biological strategy to facilitate diabetic wound healing. ACS Chem Biol. 2014;9(1):105–110.

[61] Nwomeh BC, Liang HX, Cohen IK, Yager DR. MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. J Surg Res. 1999;81(2):189–195.

[62] Gutierrez-Fernandez A, Inada M, Balbin M, Fueyo A, Pitiot AS, Astudillo A, et al. Increased inflammation delays wound healing in mice deficient in collagenase-2 (MMP-8). FASEB J. 2007;21(10):2580–2591.

[63] Gao M, Nguyen TT, Suckow MA, Wolter WR, Gooyit M, Mobashery S, et al. Acceleration of diabetic wound healing using a novel protease-anti-protease combination therapy. Proc Natl Acad Sci U S A. 2015;112(49):15226–15231.

[64] Mohan R, Chintala SK, Jung JC, Villar VV, McCabe F, Russo LA, et al. Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. J Biol Chem. 2002;277(3):2065–2072.

[65] Kyriakides TR, Wulsin D, Skokos EA, Fleckman P, Pirrone A, Shipley JM, et al. Mice that lack matrix metalloproteinase-9 display delayed wound healing associated with delayed reepithelization and disordered collagen fibrillogenesis. Matrix Biol. 2009;28(2):65–73.

[66] Rechardt O, Elomaa O, Vaalamo M, Paakkonen K, Jahkola T, Hook-Nikanne J, et al. Stromelysin-2 is upregulated during normal wound repair and is induced by cytokines. J Invest Dermatol. 2000;115(5):778–787.

[67] Krampert M, Bloch W, Sasaki T, Bugnon P, Rulicke T, Wolf E, et al. Activities of the matrix metalloproteinase stromelysin-2 (MMP-10) in matrix degradation and keratinocyte organization in wounded skin. Mol Biol Cell. 2004;15(12):5242–5254.

[68] Cornelius LA, Nehring LC, Harding E, Bolanowski M, Welgus HG, Kobayashi DK, et al. Matrix metalloproteinases generate angiostatin: effects on neovascularization. J Immunol. 1998;161(12):6845–6852.

[69] Toriseva MJ, Ala-aho R, Karvinen J, Baker AH, Marjomaki VS, Heino J, et al. Collagenase-3 (MMP-13) enhances remodeling of three-dimensional collagen and promotes survival of human skin fibroblasts. J Invest Dermatol. 2007;127(1): 49–59.

[70] Hattori N, Mochizuki S, Kishi K, Nakajima T, Takaishi H, D'Armiento J, et al. MMP-13 plays a role in keratinocyte migration, angiogenesis, and contraction in mouse skin wound healing. Am J Pathol. 2009;175(2):533–546.

[71] Seiki M. The cell surface: the stage for matrix metalloproteinase regulation of migration. Curr Opin Cell Biol. 2002;14(5):624–632.

[72] Atkinson JJ, Toennies HM, Holmbeck K, Senior RM. Membrane type 1 matrix metalloproteinase is necessary for distal airway epithelial repair and keratinocyte growth factor receptor expression after acute injury. Am J Physiol Lung Cell Mol Physiol. 2007;293(3):L600–L610.

[73] Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. J Biol Chem. 1995;270(10):5331–5338.

[74] Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. J Cell Physiol. 2007;211(1):19–26.

[75] Fisher JF, Mobashery S. Recent advances in MMP inhibitor design. Cancer Metastasis Rev. 2006;25(1):115–136.

[76] Fisher JF, Mobashery S. Mechanism-based profiling of MMPs. In: Clark IM, Young DA, Rowan AD, editors. Matrix Metalloproteinase Protocols, vol 622, 2nd ed. New York: Humana Press; 2010. p. 471–487.

[77] Chellaiah MA, Ma T. Membrane localization of membrane type 1 matrix metallopro-
      teinase by CD44 regulates the activation of pro-matrix metalloproteinase 9 in osteo-
      clasts. Biomed Res Int. 2013;2013:302392.

[78] Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions
      and therapeutic opportunities. J Cell Sci. 2002;115(Pt 19):3719–3727.

[79] Esposito S, Noviello S, Leone S. Epidemiology and microbiology of skin and soft tissue
      infections. Curr Opin Infect Dis. 2016;29(2):109–115.

[80] Bassetti M, Righi E, Carnelutti A. New therapeutic options for skin and soft tissue
      infections. Curr Opin Infect Dis. 2016;29(2):99–108.

[81] Uckay I, Gariani K, Dubois-Ferriere V, Suva D, Lipsky BA. Diabetic foot infections:
      recent literature and cornerstones of management. Curr Opin Infect Dis. 2016;29(2):
      145–152.

[82] Ziyadeh N, Fife D, Walker AM, Wilkinson GS, Seeger JD. A matched cohort study of
      the risk of cancer in users of becaplermin. Adv Skin Wound Care. 2011;24(1):31–39.

[83] Inoue M, Kratz G, Haegerstrand A, Stahlebackdahl M. Collagenase expression
      is rapidly induced in wound-edge keratinocytes after acute injury in human
      skin, persists during healing, and stops at reepithilialization. J Invest Dermatol.
      1995;104(4):479–483.

[84] Vaalamo M, Leivo T, Saarialho-Kere U. Differential expression of tissue inhibitors of
      metalloproteinases (TIMP-1,-2,-3, and-4) in normal and aberrant wound healing. Hum
      Pathol. 1999;30(7):795–802.

[85] Grenache DG, Zhang ZH, Wells LE, Santoro SA, Davidson JM, Zutter MM. Wound
      healing in the alpha 2 beta 1 integrin-deficient mouse: altered keratinocyte biology and
      dysregulated matrix metalloproteinase expression. J Invest Dermatol. 2007;127(2):455–
      466.

[86] Sudbeck BD, Pilcher BK, Welgus HG, Parks WC. Induction and repression of
      collagenase-1 by keratinocytes is controlled by distinct components of different
      extracellular matrix compartments. J Biol Chem. 1997;272(35):22103–22110.

[87] Pins GD, Collins-Pavao ME, Van De Water L, Yarmush ML, Morgan JR. Plasmin triggers
      rapid contraction and degradation of fibroblast-populated collagen lattices. J Invest
      Dermatol. 2000;114(4):647–653.

[88] Di Colandrea T, Wang LS, Wille J, D'Armiento J, Chada KK. Epidermal expression of
      collagenase delays wound-healing in transgenic mice. J Invest Dermatol. 1998;111(6):
      1029–1033.

[89] Ravanti L, Toriseva M, Penttinen R, Crombleholme T, Foschi M, Han J, et al. Expression
      of human collagenase-3 (MMP-13) by fetal skin fibroblasts is induced by transforming

growth factor beta via p38 mitogen-activated protein kinase. FASEB J. 2001;15(6):1098–1100.

[90] Hartenstein B, Dittrich BT, Stickens D, Heyer B, Vu TH, Teurich S, et al. Epidermal development and wound healing in matrix metalloproteinase 13-deficient mice. J Invest Dermatol. 2006;126(2):486–496.

[91] McCallon SK, Weir D, Lantis JC, 2nd. Optimizing wound bed preparation with collagenase enzymatic debridement. J Am Coll Clin Wound Spec. 2014;6(1–2): 14–23

[92] Mirastschijski U, Zhou ZJ, Rollman O, Tryggvason K, Agren MS. Wound healing in membrane-type-1 matrix metalloproteinase-deficient mice. J Invest Dermatol. 2004;123(3):600–602.

[93] Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, et al. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell. 1999;99(1):81–92.

[94] Gibson D, Cullen B, Legerstee R, Harding KG, Schultz G. MMPs made easy. Int Wound J. 2009;1(1).

[95] Arenas IA, Xu Y, Lopez-Jaramillo P, Davidge ST. Angiotensin II-induced MMP-2 release from endothelial cells is mediated by TNF-alpha. Am J Physiol Cell Physiol. 2004;286(4):C779–C784.

[96] Guo RW, Yang LX, Wang H, Liu B, Wang L. Angiotensin II induces matrix metalloproteinase-9 expression via a nuclear factor-kappaB-dependent pathway in vascular smooth muscle cells. Regul Pept. 2008;147(1–3):37–44.

[97] Kintscher U, Wakino S, Kim S, Fleck E, Hsueh WA, Law RE. Angiotensin II induces migration and Pyk2/paxillin phosphorylation of human monocytes. Hypertension. 2001;37(2 Pt 2):587–593.

[98] Tamarat R, Silvestre JS, Durie M, Levy BI. Angiotensin II angiogenic effect *in vivo* involves vascular endothelial growth factor- and inflammation-related pathways. Lab Invest. 2002;82(6):747–756.

[99] Kurosaka M, Suzuki T, Hosono K, Kamata Y, Fukamizu A, Kitasato H, et al. Reduced angiogenesis and delay in wound healing in angiotensin II type 1a receptor-deficient mice. Biomed Pharmacother. 2009;63(9):627–634.

[100] Kamber M, Papalazarou V, Rouni G, Papageorgopoulou E, Papalois A, Kostourou V. Angiotensin II inhibitor facilitates epidermal wound regeneration in diabetic mice. Front Physiol. 2015;6:170.

[101] Moses MA, Marikovsky M, Harper JW, Vogt P, Eriksson E, Klagsbrun M, et al. Temporal study of the activity of matrix metalloproteinases and their endogenous inhibitors during wound healing. J Cell Biochem. 1996;60(3):379–386.

[102] Mydel P, Shipley JM, Adair-Kirk TL, Kelley DG, Broekelmann TJ, Mecham RP, et al. Neutrophil elastase cleaves laminin-332 (laminin-5) generating peptides that are chemotactic for neutrophils. J Biol Chem. 2008;283(15):9513–9522.

[103] Koshikawa N, Giannelli G, Cirulli V, Miyazaki K, Quaranta V. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. J Cell Biol. 2000;148(3):615–624.

[104] Gilles C, Polette M, Coraux C, Tournier JM, Meneguzzi G, Munaut C, et al. Contribution of MT1-MMP and of human laminin-5 gamma2 chain degradation to mammary epithelial cell migration. J Cell Sci. 2001;114(Pt 16):2967–2976.

[105] Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003;83(3):835–870.

[106] Dean RA, Butler GS, Hamma-Kourbali Y, Delbe J, Brigstock DR, Courty J, et al. Identification of candidate angiogenic inhibitors processed by matrix metalloproteinase 2 (MMP-2) in cell-based proteomic screens: disruption of vascular endothelial growth factor (VEGF)/heparin affin regulatory peptide (pleiotrophin) and VEGF/Connective tissue growth factor angiogenic inhibitory complexes by MMP-2 proteolysis. Mol Cell Biol. 2007;27(24):8454–8465.

[107] Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol. 2000;2(10):737–744.

[108] Dallas SL, Rosser JL, Mundy GR, Bonewald LF. Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. J Biol Chem. 2002;277(24):21352–60.

[109] Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev. 2000;14(2):163–176.

[110] Imai K, Hiramatsu A, Fukushima D, Pierschbacher MD, Okada Y. Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-beta1 release. Biochem J. 1997;322 (Pt 3):809–814.

[111] Deryugina EI, Ratnikov B, Monosov E, Postnova TI, DiScipio R, Smith JW, et al. MT1-MMP initiates activation of pro-MMP-2 and integrin alphavbeta3 promotes maturation of MMP-2 in breast carcinoma cells. Exp Cell Res. 2001;263(2):209–223.

[112] Agren MS, Mirastschijski U, Karlsmark T, Saarialho-Kere UK. Topical synthetic inhibitor of matrix metalloproteinases delays epidermal regeneration of human wounds. Exp Dermatol. 2001;10(5):337–348.

[113] McCawley LJ, O'Brien P, Hudson LG. Epidermal growth factor (EGF)- and scatter factor/hepatocyte growth factor (SF/HGF)- mediated keratinocyte migration is coinci-

dent with induction of matrix metalloproteinase (MMP)-9. J Cell Physiol. 1998;176(2): 255–265.

[114]   Heljasvaara R, Nyberg P, Luostarinen J, Parikka M, Heikkila P, Rehn M, et al. Generation of biologically active endostatin fragments from human collagen XVIII by distinct matrix metalloproteases. Exp Cell Res. 2005;307(2):292–304.

[115]   O'Reilly MS, Wiederschain D, Stetler-Stevenson WG, Folkman J, Moses MA. Regulation of angiostatin production by matrix metalloproteinase-2 in a model of concomitant resistance. J Biol Chem. 1999;274(41):29568–29571.

[116]   Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92(8):827–839.

[117]   Murphy G, Cockett MI, Ward RV, Docherty AJ. Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan. A quantitative comparison of the activities of 95 kDa and 72 kDa gelatinases, stromelysins-1 and -2 and punctuated metalloproteinase (PUMP). Biochem J. 1991;277 (Pt 1):277–279.

[118]   McGuire JK, Li Q, Parks WC. Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium. Am J Pathol. 2003;162(6): 1831–1843.

[119]   Surendran K, Simon TC, Liapis H, McGuire JK. Matrilysin (MMP-7) expression in renal tubular damage: association with Wnt4. Kidney Int. 2004;65(6):2212–2222.

[120]   Kure T, Chang JH, Kato T, Hernandez-Quintela E, Ye H, Lu PC, et al. Corneal neovascularization after excimer keratectomy wounds in matrilysin-deficient mice. Invest Ophthalmol Vis Sci. 2003;44(1):137–144.

[121]   Kivisaari AK, Kallajoki M, Mirtti T, McGrath JA, Bauer JW, Weber F, et al. Transformation-specific matrix metalloproteinases (MMP)-7 and MMP-13 are expressed by tumour cells in epidermolysis bullosa-associated squamous cell carcinomas. Br J Dermatol. 2008;158(4):778–785.

[122]   Vaalamo M, Kariniemi AL, Shapiro SD, Saarialho-Kere U. Enhanced expression of human metalloelastase (MMP-12) in cutaneous granulomas and macrophage migration. J Invest Dermatol. 1999;112(4):499–505.

[123]   Hiller O, Lichte A, Oberpichler A, Kocourek A, Tschesche H. Matrix metalloproteinases collagenase-2, macrophage elastase, collagenase-3, and membrane type 1-matrix metalloproteinase impair clotting by degradation of fibrinogen and factor XII. J Biol Chem. 2000;275(42):33008–33013.

[124]   Zhou Z, Apte SS, Soininen R, Cao R, Baaklini GY, Rauser RW, et al. Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. Proc Natl Acad Sci U S A. 2000;97(8):4052–4057.

[125] Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. Impaired wound healing. Clin Dermatol. 2007;25(1):19–25.

[126] Wlaschek M, Peus D, Achterberg V, Meyer-Ingold W, Scharffetter-Kochanek K. Protease inhibitors protect growth factor activity in chronic wounds. Br J Dermatol. 1997;137(4): 646.

[127] Armstrong DG, Jude EB. The role of matrix metalloproteinases in wound healing. J Am Podiatr Med Assoc. 2002;92(1):12–18.

[128] Reiss MJ, Han YP, Garcia E, Goldberg M, Yu H, Garner WL. Matrix metallo-proteinase-9 delays wound healing in a murine wound model. Surgery. 2010;147(2): 295–302.

[129] Liu Y, Min D, Bolton T, Nube V, Twigg SM, Yue DK, et al. Increased matrix metallo-proteinase-9 predicts poor wound healing in diabetic foot ulcers. Diabetes Care. 2009;32(1):117–119.

[130] Dinh T, Tecilazich F, Kafanas A, Doupis J, Gnardellis C, Leal E, et al. Mechanisms involved in the development and healing of diabetic foot ulceration. Diabetes. 2012;61(11):2937–2947.

[131] Leppert D, Waubant E, Galardy R, Bunnett NW, Hauser SL. T cell gelatinases mediate basement membrane transmigration *in vitro*. J Immunol. 1995;154(9):4379–4389.

[132] Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM. Migration of eosinophils through basement membrane components *in vitro*: role of matrix metalloproteinase-9. Am J Respir Cell Mol Biol. 1997;17(4):519–528.

[133] Vandooren J, Geurts N, Martens E, Van den Steen PE, Opdenakker G. Zymography methods for visualizing hydrolytic enzymes. Nat Methods. 2013;10(3):211–220.

[134] Hsu JYC, McKeon R, Goussev S, Werb Z, Lee JU, Trivedi A, et al. Matrix metallopro-teinase-2 facilitates wound healing events that promote functional recovery after spinal cord injury. J Neurosci. 2006;26(39):9841–9850.

[135] Han YP, Tuan TL, Hughes M, Wu H, Garner WL. Transforming growth factor-beta - and tumor necrosis factor-alpha -mediated induction and proteolytic activation of MMP-9 in human skin. J Biol Chem. 2001;276(25):22341–22350.

[136] Crowe MJ, Doetschman T, Greenhalgh DG. Delayed wound healing in immunodefi-cient TGF-beta 1 knockout mice. J Invest Dermatol. 2000;115(1):3–11.

[137] Amendt C, Mann A, Schirmacher P, Blessing M. Resistance of keratinocytes to TGFbeta-mediated growth restriction and apoptosis induction accelerates re-epithelialization in skin wounds. J Cell Sci. 2002;115(Pt 10):2189–2198.

[138] Badr G. Camel whey protein enhances diabetic wound healing in a streptozotocin-induced diabetic mouse model: the critical role of beta-Defensin-1, -2 and -3. Lipids Health Dis. 2013;12:46.

[139] Ducharme A, Frantz S, Aikawa M, Rabkin E, Lindsey M, Rohde LE, et al. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. J Clin Invest. 2000;106(1):55–62.

[140] Makela M, Larjava H, Pirila E, Maisi P, Salo T, Sorsa T, et al. Matrix metalloproteinase 2 (gelatinase A) is related to migration of keratinocytes. Exp Cell Res. 1999;251(1):67–78.