Chapter

Matrix Metalloproteinases (MMPs) and Diabetic Foot: Pathophysiological Findings and Recent Developments in Their Inhibitors of Natural as well as Synthetic Origin

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Abstract

Management of diabetic foot remains a major challenge for healthcare system. Though wound healing is a multiphase process and involved multiple biomarkers that acts in stepwise manner, pathophysiology diabetic foot ulcers is still not much clear and need standardization. Matrix metalloproteinases (MMPs) are often linked with non-healing characteristic of diabetic foot ulcers. They play vital roles in various phases of healing process. Major functions are removal of damaged extracellular matrix in inflammatory phase, breakdown of capillary basement membrane prior to angiogenesis and facilitation in fibroblast migration during proliferation phase. For efficient healing, these enzymes are needed in certain amount only. Imbalance of these enzymes leads to excessive degradation which has been linked with the non-healing nature of diabetic ulcers. This chapter will shed light on the role of MMP's in various phases of wound healing and the inhibitors of MMP's from natural as well as synthetic origin. It would help researchers and physicians to the understand nature of diabetic foot more clearly and design of strategies for diabetic foot management.

Keywords: diabetic foot, matrix Metalloproteinases, MMP inhibitors, wound healing, inflammation

1. Introduction

Wound healing is a complex mechanism involves cascade of inter-related events, i.e., hemostasis, inflammation, proliferation and remodeling [1]. Various skin cells including epidermal, dermal, immune and endothelial cells are involved in initiating remodeling process. In wounded are, various signaling pathways and cellular mechanisms are observed to be active at same time which are responsible for ongoing the healing process. Moreover, various cellular events such as blood

clotting, fibroplasia, re-epithelization and matrix deposition along with neovascularization are also involved in the process [1–5]. Skin is the largest organ of human body and responsible to control thermoregulation, fluid imbalance and protection of other internal organs against microbes [6]. In wounds, this barrier gets disrupted and become prone to the microbial infections. The bacterial burden invades the layers beneath epidermis and also the deeper tissues associated with extracellular matrix (ECM) which worsens the wound state [7]. The matrix metalloproteinases (MMPs) are the zinc dependent proteolytic enzymes which were firstly discovered in the tadpoles having function of collagen degradation [8]. Total 24 MMPs have been reported so far having different substrate specificities and functions [9, 10]. The MMPs have been reported to be involved in cellular interactions, cell- matrix interactions by altering the levels of cytokines, growth factors and various biological fragments hidden in ECM [11–15]. MMPs indirectly modulates the cellular behavior by altering the cell surface receptors, junctional proteins and various cellular processes such as cell death and inflammation [16, 17]. MMPs play important role during microbial infection of wounds, the disrupted fragments of ECM possess antimicrobial activity which makes the MMPs to be the major component involved in healing process of wounds [18]. However, the bacteria itself are able to produce proteolytic enzymes which leads to the accumulation of degraded matrix components [19–21]. At the same time in some cases MMPs have been proven to be suitable candidate for gearing up the wound healing process [22, 23] but on other hands, several investigations reported the deregulation of these enzymes to be responsible for worsening the healing process and conversion of acute wounds to chronic wounds. This book chapter will focus on the various implications of MMPs in the chronic wounds along with their inhibitors of natural as well as synthetic origin.

2. Chronic wounds and infections

The disruption of skin barrier leads to increases susceptibility of bacterial strains to invade the wounds. The interaction of various bacteria/microbes has specificity with different matrix components turns to bacterial colonization. The bacterial colonization increases the bacterial burden in damaged wound site [24, 25]. This microbial colonization is the onset to the journey of an acute wound towards chronic wound [26, 27]. The minute to higher quantities of bacterial population is found in each and every acute wound known as contamination [28]. The quantity and the severity of these bacterial strains vary from wound to wound. If these bacterial population contains some pathogenic strains then there is a high risk of contamination turning into infection [29, 30]. Bacteria have ability to form biofilm with the help of self-secreting extracellular polymeric substances [31]. Biofilms involves the different layers of bacteria stick with each other to form thick films. These biofilms hinder the proper functioning of immune system of host [32]. The biofilms make the bacteria hard to evade from bacterial bed and delays the healing process [33]. The most prevalent bacteria found in the chronic wounds are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia coli* which prolongs the healing process [30, 34].

3. Chronic wounds and MMPs

The elevated level of proteolytic activity of MMPs is considered as the major factor responsible for impaired wound healing [35, 36]. The MMPs have capability to degrade ECM, non-ECM components, trans-membrane proteins, cell surface receptors and diminishes the function of cytokines and growth factors by

decreasing their level [37–43]. The tissue inhibitors of MMPs (TIMPs) are found to be decreased in chronic wounds that make the situation more worsen [44–47].

From the exudates of chronic wounds, it has been found that the proteolytic activity is surprisingly 116-fold higher than acute wounds. It marks the presence of MMPs in high levels [48, 49]. The plethora varies from wound to wound due to the specificity of different type of bacteria with ECM structural integrity [50, 51]. The *Staphylococcus aureus* has been found to increase the level of MMP-1 and -9 whereas *Pseudomonas aeruginosa* is known for raising the level of elastases [52, 53]. The bacterial pathogens not only target the host immune cells such as macrophages and neutrophils, but also attract the ECM matrix to release proteolytic enzymes which further mediates the release of MMPs. As in case of *Pseudomonas aeruginosa* the proteolytic enzyme thermolysin protease activates MMP-1, -8 and -9 [53]. Lipopolysaccharide derived serine proteinases activates pro-MMP-9 [54]. Different MMPs are upregulated specifically by various bacterial strains such as *Corynebacterium striatum* specifically gave rise to the level of MMP-2 and -9 and so as the other strains are specific for the modulation of MMPs [55–57].

4. MMPs and wounds

MMP family consists of 28 members, out of which 26 are expressed in humans and their homologs are found in birds, plants as well as algae also [58]. MMPs can be divided on the basis of similarity of protein fold- known an 'Clans' and on the basis of evolutionary relationships- called as 'Families'. The MMP class consists of 8 clans and almost 40 families. There are basically, two ways of classifying the MMPs, which can be described as following:

I. In accordance to the organization of the substrate specificity and homology:

   1. Collagenases (MMP-1, MMP-8, MMP-2)

   2. Gelatinases (MMP-2 and MMP-9)

   3. Stromelysins (MMP-3, MMP-10, MMP-12)

   4. Matrilysins (MMP-7, MMP-26)

   5. Membrane Type (MT) MMPs (MT-MMP-14, -15, -16, -17, -24, -25)

   6. Other MMPs (MMP-19, -20, -21, -22, -23, -27, -28)

II. In accordance to the structure of the MMPs:

   1. Archetypal MMP (type-1 collagenases)

   2. Martilysins: lacks the hemopexin domain

   3. Gelatinases: Comprised of three type II fibronectin domains

   4. MT-MMPs: Localized at the surface of cell membrane

Table 1 represents the classification of the MMPs based upon their substrate and targets. This classification provides wide range of information including their distribution in human body [59, 60].

S. no.	Type	Class	Substrates and targets	Distribution
1.	MMP-1	Collagenases	Collagen (I, II, III, VII, VIII, X), gelatin, aggrecan, nidogen, perlecan, proteoglycan link protein, serpins, tenascin C, Versican, casein, α1-antichymotrypsin, α1-antitrypsin, α1-proteinase inhibitor, IGF-BP-3 and -5, IL-1β, L-selectin, ovostatin, PAR-1, pro-TNF-α and SDF-1.	Endothelium, SMCs, fibroblasts, platelets, macrophages and varicose veins (interstitial/ fibroblast collagenase).
2.	MMP-2	Gelatinases	Collagen (I, II, III, IV, V, VII, X, XI), gelatin, aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, versican, active MMP-9 and MMP-13, FGF-R1, IGFBP-3 and -5, IL-1β, pro-TNF-α and TGF-β.	Endothelium, VSM, Adventitia, platelets, leukocytes, aortic aneurysm and varicose veins.
3.	MMP-3	Stromelysins	Collagen (II, III, IV, IX, X, XI), gelatin, aggrecan, decorin, elastin, fibronectin, laminin, nidogen, perlecan, proteoglycan, proteoglycan link protein, versican, casein, α1-antichymotrypsin, α1-proteinase inhibitor, antithrombin III, E-cadherin, fibrinogen, IGF-BP-3, L-selectin, ovostatin, pro-HB-EGF, pro-IL-1β, proMMP-1, -8, and -9, pro-TNF-α and SDF-1	Endothelium, intima, VSM, platelets, coronary artery disease, hypertension, varicose veins, synovial fibroblasts and tumor invasion.
4.	MMP-7	Matrilysins	Collagen (IV, X), gelatin, aggrecan, elastin, enactin, fibronectin, laminin, proteoglycan link protein, casein, β4 integrin, decorin, defensin, E-cadherin, Fas ligand, plasminogen, proMMP-2, -7, and -8, pro-TNF-α, syndecan and transferrin.	Endothelium, intima, VSM, uterus and varicose veins (PUMP).
5.	MMP-8	Collagenases	Collagen (I, II, III, V, VII, VIII, X), gelatin, aggrecan, elastin, fibronectin, laminin, Nidogen, α2-Antiplasmin and proMMP-8.	Macrophages and neutrophils (PMNL or neutrophil collagenase).
6.	MMP-9	Gelatinases	Collagen (IV, V, VII, X, XIV), gelatin, aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, versican, CXCL5, IL-1β, IL2-R, plasminogen, pro-TNF-α, SDF-1 and TGF-β.	Endothelium, VSM, adventitia, micro vessels, macrophages, aortic aneurysm and varicose veins.
7.	MMP-10	Stromelysins	Collagen (III, IV, V), gelatin, aggrecan, elastin, fibronectin, laminin, nidogen, Casein, proMMP-1, -8, and -10.	Atherosclerosis, uterus, preeclampsia, arthritis and carcinoma cells.
8.	MMP-11	Stromelysins	Aggrecan, fibronectin, laminin, α1-Antitrypsin, α1-proteinase inhibitor and IGF-BP-1.	Brain, uterus and angiogenesis.
9.	MMP-12	Other enzymes	Collagen IV, gelatin, elastin, fibronectin, laminin, casein and plasminogen.	SMCs, fibroblasts, macrophages and great saphenous vein.
10.	MMP-13	Collagenases	Collagen (I, II, III, IV), gelatin, aggrecan, fibronectin, laminin, perlecan, tenascin, casein, PAR-1, plasminogen activator 2, proMMP-9 and-13, and SDF-1.	SMCs, macrophages, varicose veins, pre-eclampsia and breast cancer.
11.	MMP-14	MT-MMP	Collagen (I, II, III), gelatin, aggrecan, elastin, fibrin, fibronectin, laminin, nidogen, perlecan, proteoglycan, tenascin, vitronectin, αvβ3 integrin, CD44, proMMP-2 and -13, pro-TNF-α, SDF-1, α1-proteinase inhibitor and tissue transglutaminase.	VSM, fibroblasts, platelets, brain, uterus and angiogenesis.

S. no.	Type	Class	Substrates and targets	Distribution
12.	MMP-15	MT-MMP	Collagen I, gelatin, aggrecan, fibronectin, laminin, nidogen, perlecan, tenascin, vitronectin, proMMP-2 and -13, and tissue transglutaminase	Fibroblasts, leukocytes and pre-eclampsia
13.	MMP-16	MT-MMP	Collagen I, Aggrecan, fibronectin, laminin, perlecan, vitronectin, Casein, proMMP-2 and -13	Leukocytes and angiogenesis.
14.	MMP-17	MT-MMP	Gelatin and fibrin	Brain and breast cancer.
15.	MMP-18	Collagenases	Collagen (I, II, III), gelatin and α1-Antitrypsin	Xenopus (amphibian, Xenopus collagenase), heart, lung and colon.
16.	MMP-19	Other enzymes	Collagen (I, IV), gelatin, aggrecan, fibronectin, laminin, nidogen, tenascin and casein.	Liver
17.	MMP-20	Other enzymes	Collagen (V), aggrecan, cartilage oligomeric protein and amelogenin	Tooth enamel
18.	MMP-21	Other enzymes	α1-Antitrypsin	Fibroblasts, macrophages and placenta
19.	MMP-22	Other enzymes	Gelatin	Chicken fibroblasts.
20.	MMP-23	Other enzymes	Gelatin	Ovary, testis, prostate and Other (type II) MT-MMP.
21.	MMP-24	MT-MMP	Gelatin, Chondroitin sulphate, dermatinsulfate, fibrin, fibronectin, N-cadherin and proMMP-2 and -13	Leukocytes, lung, pancreas, kidney, brain, astrocytoma and glioblastoma.
22.	MMP-25	MT-MMP	Collagen IV, gelatin, fibrin, fibronectin, proMMP-2 and α1-proteinase inhibitor	Leukocytes (leukolysin), anaplastic astrocytomas and glioblastomas.
23.	MMP-26	Matrilysins	Collagen IV, gelatin, fibrinogen, fibronectin, vitronectin, casein, β1-proteinase inhibitor, fibrin, fibronectin and proMMP-2.	Breast cancer and endometrial tumors.
24.	MMP-27	Other enzymes	—	Heart, leukocytes, macrophages, kidney, endometrium, menstruation, bone, osteoarthritis and breast cancer.
25.	MMP-28	Other enzymes	Casein	Skin and keratinocytes.

Table 1.
Distribution of MMPs in human body with their substrates and targets.

4.1 Collagenases

Collagenases are the enzymes known for their cleavage action on the bunch of extracellular components, i.e., Collagen, Aggrecan, Versican, Perlecan, etc., which are responsible for ECM accumulation. Collagenases activity has been found to be higher in chronic wounds with positively alleviated levels of MMP-1 and MMP-8 whereas the TIMP get downregulated. MMP-1 is known as collagenase-1. After the tissue rupturing, the integral proteins when coordinated with the keratinocytes alleviate the level of MMP-1. Furthermore, the MMP-1 degrades the ECM components and increases the turnover of proliferating cells at the other end of keratinocytes [61]. In the proliferation phase of wound healing the MMP-1 level is found to be high whereas the TIMPs are lower in the initial phases. On reaching the final phase of wound repair, i.e., the remodeling/re-epithelization the situation gets vice n versa. Some laminin isoforms of keratinocytes also regulate the MMPs level in various phases of wound repair [62]. In recent past several investigations report the dysregulation of MMP-1 in chronic wounds, i.e., even high level of MMP-1 is found in remodeling phase leads to damaged diabetic foot [63, 64]. The MMP/TIMP ratio is a crucial factor for repairing wounds [65]. The dermal ulcers also known as lipodermosclerosis are enriched with MMP-1 and MMP-2 associated with downregulation of TIMP-2 [66, 67]. Some immune cells stimulate the production of MMPs, i.e., collagenases and gelatinases [57, 68]. Among which the neutrophils derived MMP-8 (Collagenase-2) has been found to play an important role in pathophysiology of wounds. The upregulation of MMP-8 is majorly responsible for non-healing of wounds, i.e., for the state of chronic wounds [40, 69, 70]. On the contrary, MMP-8 has stronger affinity towards collagen-1 hence provide tensile strength to the wound tissues in the re-epithelization phase. Even in some reports MMP-8 is found to act as pro-enzyme in wound repair [71, 72]. Stromal cells derived MMP-13, collagenase-3 is reported to be highly expressed in wound site where as absence in the epidermis indicates its pivotal role in the formation of granulation tissue and extracellular matrix [63].

4.2 Gelatinases

Gelatinases, i.e., gelatinase A (MMP-2) and gelatinase B (MMP-9) have the broader specificity towards the substrates therefore leads to enhanced depletion of ECM components and retard the process of angiogenesis [6, 73]. The alleviated level of these MMPs has been found in the exudates of chronic wounds [46]. However, they possess broad specificity but an excellent substrate specificity exists between both the gelatinases. MMP-9 erodes the pro healing and other growth factors that leads to delayed in healing process however positively influence the inflammatory phase. The upregulation of MMP-9 degrades the specific biomarkers of wound healing, i.e., the vascular endothelial growth factor and dermatopontin and makes them non-functional. However MMP-2 stimulates the deterioration of laminin 332, enhance the keratinocytes migration and promotes the healing process [74–76]. The inflammatory cytokines (Interleukins; IL1-α, IL1-β, IL-2, IL-17, C reactive protein, Insulin like Growth Factors-1, Transforming Growth Factor-α) stimulates the release of protein called Neutrophil gelatinase associated lipocalin (NGAL). This NGAL activates the MMP-9 and makes the NGAL-MMP-9 complex which is considered as the underlying cause of slow healing in diabetic wounds. Diabetic wounds have been reported to be enriched with MMP-9, MMP-9-NGAL complex, NGAL and neutrophil. However, the situation gets opposite when given the insulin treatment [77–80].

4.3 Stromelysins and other MMPs

Stromelysin-1 and -2, i.e., MMP-3 and -10, respectively. MMP-3 along with collagenase-1 is found in distal end and stimulates the keratinocytes proliferation whereas MMP-10 is present in the starting edge of keratinocytes [60, 81]. MMP-3 regulates the migration of fibroblasts to the wound site resulting in wound contraction. On other hand, MMP-10 is responsible for the keratinocyte cell death and slow down the healing process. MMP-3 is a major activator of MMP-9 hence also contributing to inflammatory phase [82]. Other MMPs such as MMP-12, -7 and -14 are activated by stromal macrophages. MMP-7 interact with cyndecans and integrins to promote the skin regeneration in remodeling phase [83]. MMP-14 is majorly present in the fibroblasts on the wound bed. The level of MMP-12 gets naturally increased during inflammatory phase. These MMPs not only contribute in the cellular signaling pathways but also triggers the stimulation of other MMPs [22, 84–86].

5. MMPs in wound healing

5.1 Hemostasis

Followed by the tissue injury, the blood clotting and platelet aggregation is the former step in wound healing. The extrinsic and intrinsic system regulates the accumulation of platelets at wound site by means of coagulation factors and thrombocytes respectively [87]. The cytokines and other associated growth factors trigger the constriction of vessels which fills the voids in the wound area and lead to clot formation. The former step is followed by the vasodilation where the thrombocytes and fibroblasts like fibronectin, vitronectin and thrombospondin leads to form the provisional scaffold like wound matrix which allows the migration of keratinocytes, endothelial cells and leukocytes [88]. These platelets and leukocytes stimulate cytokines and growth factors which further assists the inflammatory process. The interleukins IL-1α, β, IL-6 and TNF-α are engaged in this process. Furthermore, the collagen synthesis is mediated by FGF-b, IGF, TGF-β and angiogenesis which get activated by FGF-B, VEGF subunit A, TGF-β and HIF-1 [89, 90]. Hemostasis is the initial phase in wound healing process and MMPs does not have any significant interference in this phase.

5.2 Proliferation and re-epithelization

The proliferation phase includes the granulation tissue to cover wound area by the strong network of vessels. Platelets are shifted to the injury site, to form the clot. Besides this, the platelets have another important function to stimulate the movement of neutrophils and macrophages to the wound site triggered by the release of platelets derived growth factors [91]. This factor is also engaged in mediating the collagenases the fibroblastic cells especially MMP-8 which have major role in tissue damage. MMP-8 are also released by neutrophils during the wound infection and assists the wound debridement and rearrangement of damaged collagen-I [92]. The synthesis of another MMPs such as MMP-1,2,3,9 are also driven by the platelets. MMP-1 and 2 has important function to control the adhesion of platelets and conglomeration [44, 88]. Moreover, MMP-9 filters the different collagen types and regulates the release of inflammatory cytokines such as IGN-γ and TGF-β. The collagen and fibroblast synthesis which in turn form the collective tissue network is also regulated by MMP-9. The new capillary formation at the wound site is also associated with movement of fibroblasts within the fibrin network which promotes angiogenesis and leads to neovascularization and re-epithelization [88]. The process of re-epithelization is also get started by the signaling

pathways regulated by the endothelial and non-endothelial cells which involve various cytokines such as EGF, KGF, IGF-1 and NGF [90]. The basic component of the endothelial cells known as laminin exists in various isoforms. Among which the laminin isoform-5 have pivotal role in induction of keratinocyte migration and MMP-9 activation. Cell movement is a major role of MMP-9 hence plays an important role in re-epithelization process [93]. Another MMPs such as MMP-14 and MMP-2 breaks laminin isoform 5 and release a factor which when interacts with the epidermal growth factor (EGF) turns up the movement of cells [94, 95]. One more factor FGF-2 released by macrophages when interacts with the heparin sulphate enhances the growth of endothelial and fibroblast cells. The vascular endothelial growth factor (VEGF) released by macrophages activates the cell migration and proliferation of keratinocytes and endothelial cells which include MMP-1, 2, 9 and 13 hence play a major role in wound healing [96, 97].

5.3 Matrix formation and remodeling

The final stage of wound healing is remodeling phase. It involves the upregulation of collagen turnover but decline in proliferation of fibroblast [98]. Moreover, the keratinocytes reach fibrin clot by crossing granulation tissue matrix [99]. The collagen-I replacement with collagen type III indicates the maturation of wound [100, 101]. However, in the early phase of remodeling phase fibronectin and fibroblasts are get displaced by collagen type I and III and proteoglycans which in turn enhances the tensile strength and integrity of wound matrix [102]. The level of myofibroblast and blood vessels get increased while reaching the end of this phase and high density of these two leads to the closure of wound [63, 103].

5.4 Proteolysis in wound repair

Many processes in wound healing such as keratinocyte migration, angiogenesis and re-epithelization are generally followed by the extra cellular matrix (ECM) degradation [104]. The MMPs are majorly involved in this proteolytic degradation. MMP-19 and 28 are present in keratinocytes of basal stratum and superbasals [105]. Moreover, MMP-19 is also found in the hair follicles, endothelial cells, arteries and veins [106]. MMP-1 expression is found to be upregulated in dermis part of the wounds where basal membrane is destroyed and promotes re-epithelization process and triggers the binding of keratinocytes with type-1 collagen [65]. Collagen type I is known to upregulate the level of MMP-1 whereas collagen type III and other basement proteins do not promote the MMP-1 synthesis. MMP-1 activates α1β2 integrin to synthesize collagen type-1 [16]. The MMP1- α1β2 complex enhances the migration of keratinocytes therefore boost up the re-epithelization process [107]. During the process of basement membrane formation followed by re-epithelization, MMP-1 expression gets knockdown by the cellular junctions of basal membrane proteins [16, 108]. Moreover, MMP-13 which is mainly present in the dermis along with MMP-1 regulates the fibroblast proliferation mediated by matrix shrinkage and matrix stiffness [109, 110]. MMP-8 stored in cellular granules are secreted when get activated by macrophages [111]. The overexpression of MMP-8 is found in the damaged wounds. MMP-13 downregulation is balanced by MMP-8 which slow down the healing process by improper infiltration of neutrophils, improper re-epithelization and constant inflammatory syndrome [111, 112]. As given in the classification section the stromelysins such as MMP-3 and MMP-10 are present in the epidermal cells i.e. proliferating keratinocytes. These MMPs especially MMP-3 has major role in disruption of fibrin containing provisional matrix and formation of new basal matrix after remodeling [113]. This process is majorly carried out by

cytokines and other growth factors such as FGF-b and HB-EGF [114]. Furthermore, the MMP-9 has also an important role in final phase that shaping the epidermal layer at the end during wound repair. In addition, MMP-2, -9, -19 and MT1-MMP are stored and released by the endothelial cells [115]. Among which MMP-2 and -9 have pivotal role in degradation of mature blood vessels and sprouting/growth of new blood vessels by activating angiogenesis related growth factors and cytokines [86, 89, 116, 117]. MT1-MMP possess proteolytic activity against mature collagen and fibrin by crossing the thick network of fibrin proteins in stroma of damaged tissues [118]. Whereas, MMP-19 is involved in growth process of endothelial cells, epithelial cells, fibroblast cells and small vasculature within macrophages [119].

The wounds that persist more than 4–6 weeks are generally recognized as chronic wounds [120]. Wounds such as venous leg ulcers [72, 121], diabetic foot ulcers [122, 123] and that caused due to pressure [66] are considered as chronic or delayed wounds. Some wounds which appears to be acute at initial stages but may turns to chronic one while reaching the final phase of healing are also categorized under chronic wounds. Main examples of these types of wounds are surgical wounds and traumatic wounds. These chronic wounds are specifically characterized by the altered levels on MMPs.

Abnormal structural integrity of fibrin network, increased tendon rigidity and altered volume and level of biochemical substances indicates the delayed and chronic wounds [124]. Proteolytic activity of MMPs has major impact on healing process of chronic wounds. Besides this MMP-3 and MMP-13 along with MMP-9 are actively found in the normal as well as diabetic foot ulcers. Where MMP-3 and MMP-9 have been upregulated, MMP-3 has been found to be knockdown in chronic wounds. The overexpression of MMP-13 and MMP-9 is associated with high glucose concentration at wound site [125]. The imbalance between MMP and TIMP level is a major cause of hyperglycemia, hyperlipidemia and hypertension during the condition of chronic wounds/diabetic ulcers [126, 127]. In the state of chronic wounds, the migration of inflammatory cells is followed by imbalanced fibroblast clotting which lead to secrete the ECM proteins. Meanwhile, MMPs have been found to increase the fibroblast proliferation and collagen degradation via TGF-β1 signaling [127, 128]. Higher production of gelatinases has been observed in the diabetic wounds. Conclusively MMPs in this state are associated with degradation of ECM components but at the same time are also responsible for the recovery of traumatic wounds by regenerating the capillary and blood vessels at the respective site [129].

6. Levels of MMPs in diabetic wounds

In the state of diabetic wounds, the glucose level is significantly higher [130]. Elevated levels of MMPs have been found in these wounds because of oxidative stress and end products of glycation which may lead to diabetic peripheral arterial disease [61, 131]. Degradation of ECM due to MMPs especially MMP-1, -2 and -9 turn these diabetic wounds to get more worse [132]. The mismatch between the extent of degradation and repairing of ECM is a critical factor to cause delay in wound healing process i.e. chronic wounds. Therefore, it necessitates the ECM components to be in controlled condition for boosting up the healing process [131]. Any other disease condition in diabetic ulcer may worsen the healing process due to imbalanced availability of cytokines and other growth factors needed for the healing of wounds [133, 134]. In each phase of diabetic wound repair, i.e., hemostasis, inflammation, proliferation and remodeling there has been altered expression of MMPs [135]. Epithelial remodeling is associated with raised levels of MMP-1, -8, -9 and downregulation of TIMPs. The fibronectin degradation is majorly carried out by MMP-9 which leads to cell migration

and proliferation [136]. MMP-1, -8 and -9 have been reported to be upregulated in the venous wounds due to absence of TIMP [73, 81]. Patients with metabolic syndrome have been found to be overly expressed with MMP-2 and -9 in their serum sample. The mutations in the gene expression of MMP-9 can also be a cause for delayed healing. Increased expression of MMP-9, TNF-α and other growth factors in diabetic foot ulcers has been found and concluded that they could be linked with slow-to-heal ulcers in diabetics and therefore a target for new therapeutic management [137].

7. Therapeutical targeting of MMPs

7.1 Synthetic approaches

MMPs has pivotal role in diabetic wounds hence they are the major target for the researchers. MMPs possess high resemblance in their structural morphology therefore it is difficult to target specifically one MMP at the time especially when they fall under same category such as gelatinase-A and gelatinase-B [138, 139]. Moreover, MMP-8 has been found to boost up healing in diabetic wounds in absence of MMP-9 and vice versa which necessitates the specific inhibition of MMP-9 without having any interaction with MMP-8. There are many broad spectrum MMP inhibitors (MMPI) which have been already investigated for the purpose but we need more selective therapeutics over the existing one [140]. Many small structural molecules have been discovered yet to target the same. In an investigation, it has been reported that the racemic mixture, i.e., (R, S)-ND-336 possess 55-fold more activity than the R or S isomer alone to target MMP-9 specifically than MMP-8 [74]. Moreover, based upon the Ki values the R isomer has been reported to be 10-fold more potent than the S isomer for selective inhibition of MMP-9 [141]. As it has been known that the synthetic molecule (R, S)-ND-336 falls under thiirane class, its structural ring gets unlatched and produce thiolate which gets interacted with the zinc ion within MMP-9 and inhibit its function. Reversal of the given process is very slow therefore it shows long lasting retention time. R-ND-336 has been investigated to be more effective than FDA approved drug becaplermin for the respective purpose [141]. Enhanced specificity for inhibition of MMPs can be obtained by using antibody approach. In the recent past, GS-5745 is an antibody being investigated for specific inhibition of MMP-9. It has dual mechanism to act on i.e. by interacting and hindering the active site of MMP-9 and another one is to cleave the MMP-3 zymogen which is involved in activation of MMP-9 [142–145]. Moreover, another two antibodies being investigated under the clinical trials are SSDS-3 and REGA-3G12 have been observed to possess selective inhibition against MMP-9 [146, 147]. Furthermore, the above antibodies have been more explored for the cancer targets hence there are many future possibilities to explore the wound healing potential of the above candidates [148]. Wound dressings are being commonly used for the purpose of healing and controlling the exudate secretion [149]. Many MMP inhibitors have been used to incorporate into these wound dressings. But these inhibitors are generally found to be non-specific, i.e., the broad-spectrum inhibitors. Most commonly used MMP-inhibitors in wound healing are bisphosphonates [150]. Another hypothesis involves the use of atelocollagen type I to be used along with 4-vinyl-benzyl chloride to specifically inhibit the MMPs present in wound exudates [151]. In addition, another clinical candidate GM-6001 exploited as wound dressing has broad spectrum activity but found to be less effecting in healing diabetic wounds than the therapeutics having specific inhibitors against MMPs [152]. RNA is a basic nucleotide to synthesize gene encoding MMPs [153]. So, the therapies being approached to inhibit RNA which in turn inhibit the MMPs at gene level have the high potential to heal the diabetic wounds than

other therapeutics [154]. But the major obstacle is to deliver the siRNA to the target site [155–157]. Therefore, to overcome this, the star shaped cationic polymer such as cyclodextrins have been reported to be used for the purpose as they have very low toxicity [158]. Furthermore, β-CD-(D3)7/MMP-9-siRNA has been found in an investigation to inhibit MMP-9 in the diabetic wounds where MMPs level is quite high due to TIMP knockdown which in turn promotes the healing process. These siRNAs are supposed to be taken by the fibroblasts on wound site [159, 160]. But when the siRNA was used alone in a further study it has been found to cause liver and kidney toxicity [161]. In addition, the miRNA-139 and miRNA-335 has been reported to possess an excellent potential in healing the diabetic wounds via inhibiting MMP-9 [162].

7.2 MMPs inhibitors of natural origin

Natural products have huge resource of biologically active molecules and provided large number of biologically active compounds to clinical practice for treatment of wide range of diseases and disorders. Considering capability of natural products in drug development, wide range of researchers across the globe has screened numerous constituents from natural sources for MMPs modulation activity. Major bioactive constituents (**Figure 1**) from natural sources with MMP modulation potential has been discussed below.

Withaferin A (3-azido Withaferin A) a naturally derived steroidal lactone from plant *Withania somnifera*, exhibits various pharmacological activities. *In vitro* studies revealed that withaferin A inhibit MMP-2 by up regulating the expression of pro-apoptotic protein par-4. Stimulation of par-4 by withaferin A enhances apoptosis by extrinsic pathway of apoptosis by activating FADD-caspase-8-caspase-3 in dose dependent manner [163]. Cantharidin is a natural compound obtained from *Mylabris phalerata* and showed remarkable inhibition of cell migration and invasiveness by suppressing MMP-2 and MMP-9 through affecting their upstream markers such as NF-κB, c-Jun and AP-1 in A375.S2 cells based wound healing and matrigel chamber invasion assays. Cantharidin exerts anti-cell migration and anti-invasiveness property by suppressing MMP-2 and MMP-9 via modulating their upstream markers such as NF-κB, c-Jun and AP-1. Cantharidin also down regulate the expression of NF-κB, p65 and proteins involved in PI3K-Akt (PI3K, ERK1/2, Rock1 and FAK) and MAPK (p38, ERK and JNK) signaling cascade [164]. Celastrol is another phytoconstituent of *Tripterygium wilfordii* having anti-proliferative activity. Celastrol inhibit metastasis and invasiveness of ovarian cancer cells (SKOV3 and OVCAR-3) by knockdown the expression of NF-κB/MMP-9. NF-κB modulates IκBα degradation, p65 translocation blocking and MMP-9 suppression [165]. Gallic acid has been proved to inhibit cell metastasis and invasiveness in PC-3 cells by degrading expressions of MMP-2 and MMP-9 otherwise they degrade the ECM and delayed wound healing. Gallic acid has been also observed to activate the TIMP-1 which is natural regulator of MMP-2 [166]. Ginsenoside Rd is obtained from *Panax ginseng* leaf which increases proliferation, migration and shows protective effect in human dermal fibroblast (HDFs) and keratinocyte progenitor cells which enhances healing of skin wound. Being a steroidal moiety it easily permeate to cell membrane and enhance healing of skin both in laser burn and excision wound by increasing expression of CREB, cAMP along with reducing expression of MMP-1 [167]. Lycorine a natural compound widely distributed in plant family Amaryllidaceae. Lycorine shows antimigratory effect in HepG2 cells by reducing the expression of MMP-2 and MMP-9. Lycorine increase polymerized F-actin by blocking the normal turnover of the actin cytoskeleton and a loss of depolymerized G-actin. The activation of ROCK1 in cells pre-treated with lycorine shows decrease in expression of cofilin, cyclinA, cyclin B1, cdc2, MMP-9 and MMP-2

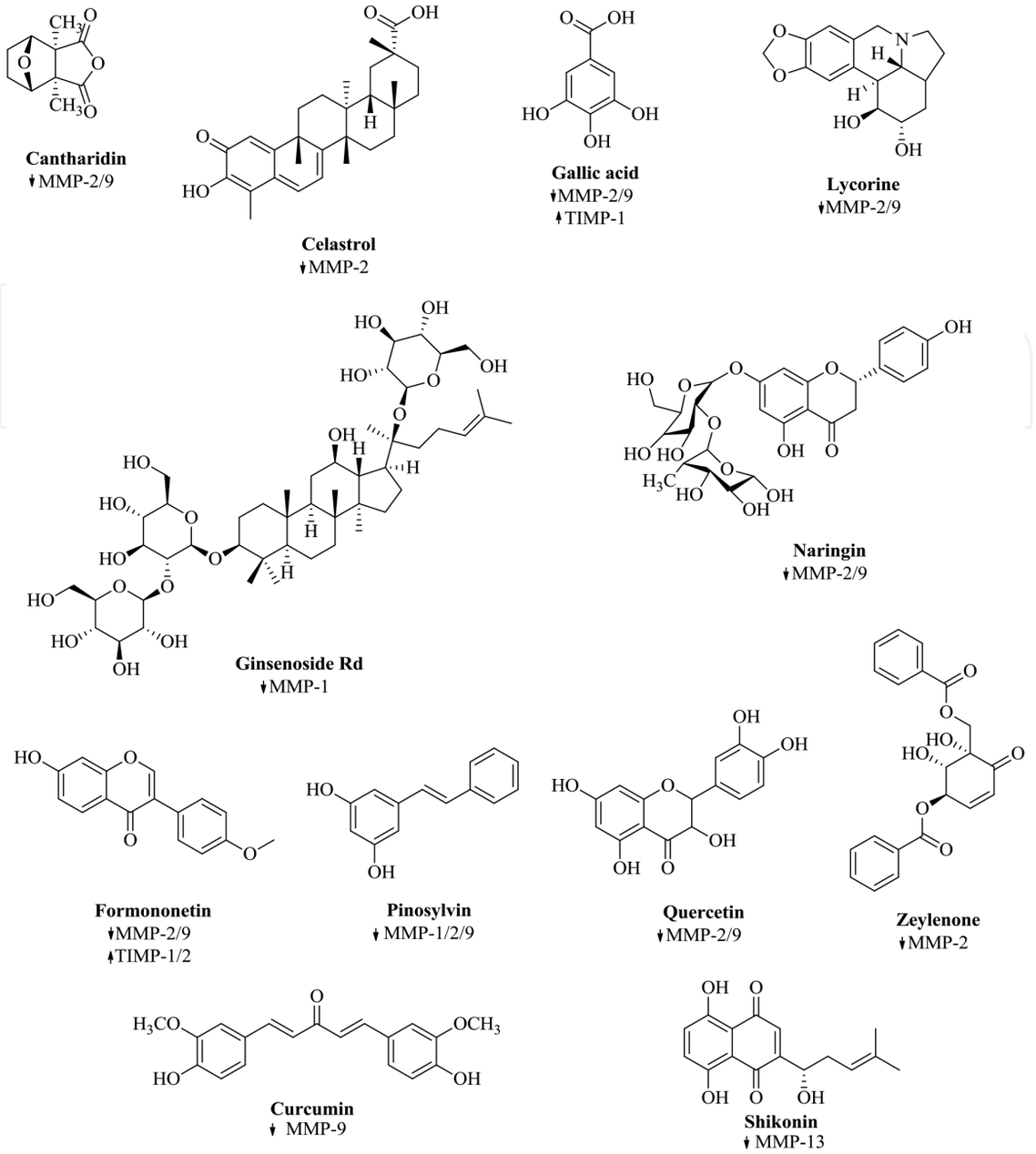

Figure 1.
Various MMP inhibitors of natural origin.

which shows lycorine inhibiting cell proliferation and migration in HepG2 cells via inhibition of ROCK1/cofilin-induced actin dynamics [168]. Naringin is a natural flavonoid present in citrus fruits. Treatment of naringin reduce the expression of p-ERK and p-JNK which are molecular markers involved in MAPK signaling pathway in turn reduce the expression of MMP-2 and MMP-9 [169]. Pinosylvin is a natural compound present in *Pinus* species. Pinosylvin downregulate the expressions of MMP-1, MMP-2 and MMP-9 in human fibrosarcoma HT1080 cells. Quercetin is another well-known inhibitor of MMP-2 and MMP-9 [170]. Platycodin D from *Platycodon grandiflorum* exhibit anti-invasive and antimetastatic activity in human breast cancer cells (MDA-MB-231). It inhibit cell invasion by down regulating the Mrna expression of MMP-9 [171]. Formononetin is a natural compound found in *Astragalus membranaceus, Trifolium pratense, Glycyrrhiza glabra* and *Pueraria lobata.* It shows inhibitory effect on the breast cancer cells progression, migration and invasiveness by suppressing the effect of MMP-2 and MMP-9 along with upregulating the expression of matrix metalloproteinase inhibitors such as TIMP-1 and TIMP-2 [172]. Zeylenone is natural

oxide with anticancer activity. In the present study, Zey is reported to have anti-cancer activity against prostate cancer. Zeylenone has been reported to suppress the expression of MMP-2, MMP-9 and upregulate the expression of TIMP-1 and collagen-1 in DU145 cells [173]. Curcumin is a natural compound extracted from plant *Curcuma longa* (Zingiberaceae). Curcumin possesses anti-oxidant, anti-inflammatory and anticancer activity and along with wound healing property. The mechanism of curcumin behind wound healing is the lowering the expression of TNF-α and increased proportion of α-SMA and collagen in fibroblast. Matrix metalloproteinase especially MMP-9 helpful in tissue migration and remodeling and someway helpful in normal wound healing. Curcumin treated cells negatively regulate the expression of MMP-9 and reduced the expression of TNF-α which positively modulate MMP-9. Curcumin also regulate the expression of NF-κB induce by TNF-α. Thus curcumin enhances wound healing activity by downregulating expression of MMP-9 and increase value of collagen by regulating expression of NF-κB induce by TNF-α in fibroblasts [174]. Shikonin is a natural component presents in *Lithospermum erythrorhizon* which exhibit decent wound healing property. Shikonin has been proved for its inhibitory effect on migration and invasiveness of U87 and U251cells by inhibiting the expression of MMP-2 and MMP-9 [175, 176].

8. Conclusion

From the ancient past wound healing has known to be a complicated topic as it involves many complex and unclear mechanisms. Moreover, wound healing process in diabetes like state get delay and more worsen. In the recent past various MMPs have been found to play a key role in the healing of diabetic foot. Structurally, it mainly has zinc on its active site. Furthermore, it is categorized in various types based upon the different substrate it cleaves, i.e., collagenases, gelatinases, stromelysins and various other MMPs. Also, the modulation of expression of various MMPs significantly alters the healing process. In addition, TGF-β has been reported to be the signaling pathway for MMPs to act upon for healing of chronic wounds. Moreover, different phases of wound repair involve alteration in level of various MMPs. Among various MMPs, MMP-9 has been widely discussed and investigated enzyme in the recent past and has also been considered as major culprit in altering the healing rate. The overexpression of various MMPs extends the time of healing or may devastate the condition. Therefore, various MMP inhibitors either of natural or synthetic origin have been explored for the purpose. Most of these candidates are under clinical trial and has proven to be very selective and effective for healing chronic wounds. Besides the wound healing MMPs possess therapeutic effectiveness for various other diseases. In future, there are various possibilities to explore and unlash various mechanisms of MMPs for chronic wound healing.

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Conflict of interest

The authors confirm that this chapter content has no conflicts of interest.

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