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Invited perspective

# Restructuring of the extracellular matrix in diabetic wounds and healing: A perspective

Mayland Chang

*Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, United States*

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ABSTRACT

Diabetic foot ulcers are a complication of diabetes for which treatment options are limited and not effective, resulting in 73,000 lower-limb amputations in the United States every year. Wound healing is a complex process with a highly orchestrated cascade of events, in which the extracellular matrix (ECM) interacts with growth factors and cells. Matrix metalloproteinases (MMPs) are involved in all wound healing events, in particular MMP-8 and MMP-9, whose physiological functions are to degrade damaged collagen type I and to facilitate keratinocyte migration and re-epithelialization, respectively. MMP substrate redundancy permits another MMP to substitute for MMP-9 during normal wound healing. Under the hypoxic and inflammatory environment of diabetic wounds, increased reactive oxygen species (ROS) and upregulation of MMP-9 results in wounds that are recalcitrant to healing. We have determined that MMP-8 plays a role in the body's response to wound healing and that MMP-9 is the pathological consequence of the disease with detrimental effects. Thus, selective inhibition of MMP-9, while leaving MMP-8 activity unaffected, is desirable. ND-336 has such inhibitory profile and is a promising strategy for treatment of diabetic foot ulcers.



Chronic wounds are a complication of diabetes that affect 900,000 people in the United States every year [1]. Approximately 25% of patients with diabetes develop foot ulcers over a lifetime [2]. Diabetic foot ulcers take a long time to heal and have a high rate of recurrence, 60.5% of patients develop an ulcer within 31.5 months [3]. The standard treatment for diabetic foot ulcers is debridement and off-loading. The only FDA approved drug for treatment of diabetic foot ulcers is becaplermin (Regranex$^{TM}$), a recombinant human platelet-derived growth factor (PDGF). However, becaplermin is associated with malignancies and higher risk of mortality [4]. Unfortunately, the current treatment options are not effective, resulting in 73,000 lower-limb amputations every year in the United States [5]. The median survival for people with diabetes after a lower-limb amputation is 5.8 months, with a mortality rate of 46% after one year and 79% after five years [6].

Normal wound healing is a complex process involving a highly orchestrated cascade of events that include hemostasis, inflammation, proliferation, angiogenesis, and remodeling. In each of these events the extracellular matrix (ECM) interacts with growth factors and cells (Fig. 1). The ECM gives structure and support to cells, as well as facilitates cell communication. The ECM is comprised of proteins, glycoproteins such as fibronectin and laminin, proteoglycans such as heparin sulfate, and polysaccharides such as hyaluronic acid. The main protein in the ECM is collagen type I, which forms fibers that provide tensile strength. Proteases, in particular matrix metalloproteinases (MMPs), are involved in all wound-healing events [7–13]. There are 23 MMPs in humans [14] characterized by the presence of the zinc ion at the active sites. These proteins are expressed as zymogens (proMMPs), requiring proteolytic removal of the prodomain for activation. MMP regulation occurs at different levels, including inhibition by tissue inhibitors of matrix metalloproteinases (TIMPs) [15].

After tissue injury, platelets are recruited to the injury site to stop the bleeding. Platelets also release PDGF, which promotes chemotaxis of neutrophils, macrophages, as well as stimulates production of growth factors and cytokines important in wound healing, including fibronectin, collagen, proteoglycans, and hyaluronic acid [16]. PDGF has also been shown to stimulate the production of collagenase in human skin fibroblasts [17]. MMP-8 is the most prevalent collagenase in wounds [18]. MMP-1, MMP-2, MMP-3, and MMP-9 are produced by platelets, where MMP-1 and MMP-2 mediate platelet adhesion and aggregation, while MMP-9 is involved in platelet production [7]. During inflammation, neutrophils infiltrate the wound to protect against infection and release MMP-8 [18], which is required for debridement of the wound and to cleave damaged collagen type I, as well as MMP-9, which

*E-mail address:* mchang@nd.edu

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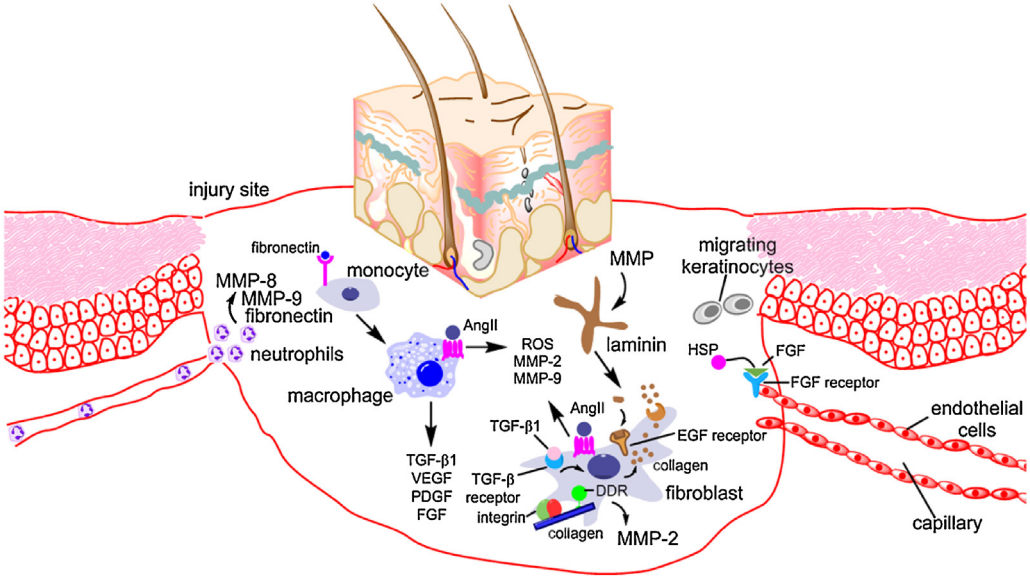


**Fig. 1.** Role of ECM in wound healing. During homeostasis, platelets are recruited to the injury site to stop the bleeding and secrete MMPs, as well as platelet-derived growth factor (PDGF). During the inflammatory stage of wound healing, neutrophils infiltrate the wound and excrete MMP-8, MMP-9, and fibronectin. MMP-8 is required for debridement of the wound and to cleave damaged collagen type I. Monocytes migrate from the bloodstream to the wound, binding to fibronectin and differentiating into macrophages, which secrete transforming growth factor (TGF)-β1, vascular endothelial growth factor (VEGF) that is critical for angiogenesis, PDGF, and fibroblast growth factor (FGF)-2. TGF-β1 stimulates the production of collagen and other ECM components including hyaluronic acid and fibronectin that form the new ECM. Angiotensin II (AngII) is stimulated under the hypoxic and inflammatory environment of the wound, releasing reactive oxygen species (ROS), as well as MMP-2 and MMP-9. Laminin is cleaved by MMP-2, MMP-9, and MMP-14; the resulting peptide binds to epidermal growth factor (EGF) receptor, which enhances fibroblast migration. The released FGF from macrophages binds to heparan sulfate proteoglycan (HSP) and to the FGF receptor on endothelial cells, inducing migration of keratinocytes, which is critical to wound re-epithelialization. Collagen binds to integrin and discoidin domain receptor (DDR) on fibroblasts stimulating the production of MMP-2, remodeling of the ECM, cell differentiation, and migration of fibroblasts.

cleaves collagen types IV, V, VII, and X [18]. Mice deficient in MMP-8 show increased inflammation and delayed wound healing, as well as increased transforming growth factor (TGF)-β1 signaling, suggesting that MMP-8 mitigates scarring [19]. The adhesive glycoprotein fibronectin present in blood plasma binds to monocytes at the wound site, which results in differentiation into macrophages. The macrophages secrete several growth factors, including TGF-β1, vascular endothelial growth factor (VEGF), PDGF, and fibroblast growth factor (FGF)-2. TGF-β1 binds to the TGF-β receptor in fibroblasts, stimulating the production of collagen and other ECM components—hyaluronic acid and fibronectin—to form the new ECM.

Angiotensin II (AngII) is the primary effector of the renin-angiotensin system; it is an octapeptide formed by the turnover of AngI by angiotensin-converting enzyme [20] and is present in human skin in macrophages, neutrophils, fibroblasts, and endothelial cells [21]. In the hypoxic and inflammatory environment after tissue injury, stimulation of AngII results in generation of reactive-oxygen species (ROS) from macrophages and neutrophils, promoting cell adhesion and ECM formation [20]. AngII has also been shown to induce the expression of MMP-2 [22] and MMP-9 [23].

Laminin, a component of the basal lamina of epithelia, plays roles in cell adhesion, migration, proliferation, differentiation, and angiogenesis [24]. There are 15 laminin isoforms, of which laminin-5 is specific to epithelial cells. MMP-2 and MMP-14 are known to cleave laminin-5 [25,26], generating a fragment that binds to epidermal growth factor (EGF) receptor, which stimulates cell migration [27]. Laminin-5 is also a substrate for MMP-9 [28]. Laminin-5 has been shown to promote keratinocyte migration and induction of MMP-9; cell motility is dependent on MMP-9 activity, indicating that MMP-9 plays a role in re-epithelialization [29]. The released FGF-2 from macrophages binds to heparan sulfate, which induces the growth of fibroblasts and endothelial cells [30]. Platelets and macrophages release VEGF, stimulating proliferation and migration of endothelial cells, as well as keratinocyte migration and collagen production from fibroblasts [31], in addition to stimulating angiogenesis that plays a critical role in wound healing. MMP-1, MMP-2, MMP-9, and MMP-13 have been shown to be involved in keratinocyte migration [32–35]. Fibrillar collagen binds to integrin and discoidin domain receptor (DDR) on fibroblasts stimulating the production of MMP-2, remodeling of the ECM, cell differentiation, and fibroblast migration [36].

Given the role of the ECM in wound healing, a strategy that has been pursued is to promote wound healing by treatment with growth factors. One such strategy is PDGF, a growth factor involved in healing, which to date is the only growth factor approved by the FDA for treatment of diabetic foot ulcers. PDGF is produced by macrophages, endothelial cells, fibroblast and keratinocytes, inducing angiogenesis [37] and production of fibronectin and hyaluronic acid [38]; it is a potent mitogen, promoting chemotactic recruitment and cell proliferation [39]. Becaplermin gel contains 0.01% of PDGF, which is produced by recombinant DNA technology in which the β-chain of human PDGF is incorporated into the yeast *Saccharomyces cerevisiae*. The efficacy of becaplermin has been evaluated in four clinical trials with 922 patients. In the first study, complete ulcer closure was achieved in 48% of patients in the becaplermin group (n = 61) compared to 25% in the placebo (n = 57, $p = 0.01$) [40]. The second study showed that healing rates were 49.5% in the 100 μg/g becaplermin (n = 123), 36.3% in the 30 μg/g becaplermin (n = 132), and 34.6% in the placebo groups (n = 127, $p = 0.007$) [41]. The third study showed that 44.1% of patients receiving becaplermin (n = 32) healed compared to 35.7% in the carboxymethylcellulose (n = 70), and 22% in the standard ulcer care groups (n = 68), however the study was statistically underpowered [42]. In the fourth study, 57.5% of ulcers treated with becaplermin healed in a mean healing time of 63 days [43]. While these clinical studies have shown that becaplermin significantly improves heal-