The role of extracellular matrix in the pathophysiology of diabetic wounds

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Abstract

Impaired healing leading to the formation of ulcerated wounds is a critical concern in patients with diabetes. Abnormalities in extracellular matrix (ECM) production and remodeling contribute to tissue dysfunction and delayed healing. Specifically, diabetes-induced changes in the expression and/or activity of structural proteins, ECM-modifying enzymes, proteoglycans, and matricellular proteins have been reported. In this review, we provide a summary of the key ECM molecules and associated changes in skin and diabetic wounds. Such information should allow for new insights in the understanding of impaired wound healing and lead to the development of ECM-based therapeutic strategies.

Introduction

Diabetes has become the seventh leading cause of death in the United States and the leading cause of amputation, blindness, and renal failure [1]. 30.3 million people in the United States suffer from diabetes, and the global burden of disease is estimated to exceed 693 million worldwide in 2045 [2]. Hyperglycemia-associated complications are the major cause of morbidity and mortality in diabetes patients making them a national and global health concern. One of the complications is the diabetic foot ulcer (DFU), which results from failure of the wound healing process. This can allow entrance for infectious agents that cause chronic infections and septicemia, leading to amputations and even death. 15–25% of diabetic patients will develop DFU at some point during their lifetime, and the 5-year mortality rate of DFU is 44% [3,4].

Besides impaired nerve sensitivity and peripheral arterial circulation, compromised extracellular matrix remodeling is a typical presentation of DFU as well as a major disease driver. In diabetes, ECM proteins undergo glycation-induced modification leading to the formation of advanced glycation end products (AGEs). The contribution of this mechanism to pathophysiology has been reviewed recently and will not be addressed here [5]. Additionally, ECM undergoes repair and remodeling when injured. During normal wound healing, broken fibrils are degraded by ECM enzymes such as matrix metalloproteinases (MMPs), a protein family that includes collagenases and gelatinases [6]. New ECM fibrils are also synthesized and modified to regenerate the network. Overall, normal ECM remodeling involves a balance between ECM degradation, production, and maturation. In defective wound healing such as DFU, the balance tends to yield more degraded, non-soluble fibrils, resulting in a disorganized ECM network (Fig. 1).

ECM production and remodeling are regulated by multiple molecules including structural proteins, enzymes, matricellular proteins, glycosaminoglycans, cytokines, and growth factors [7]. Cytokines and growth factors function more broadly to influence this process and play critical roles during the various phases of wound healing. Other regulators act more specifically to degrade or stabilize ECM structure and serve as a

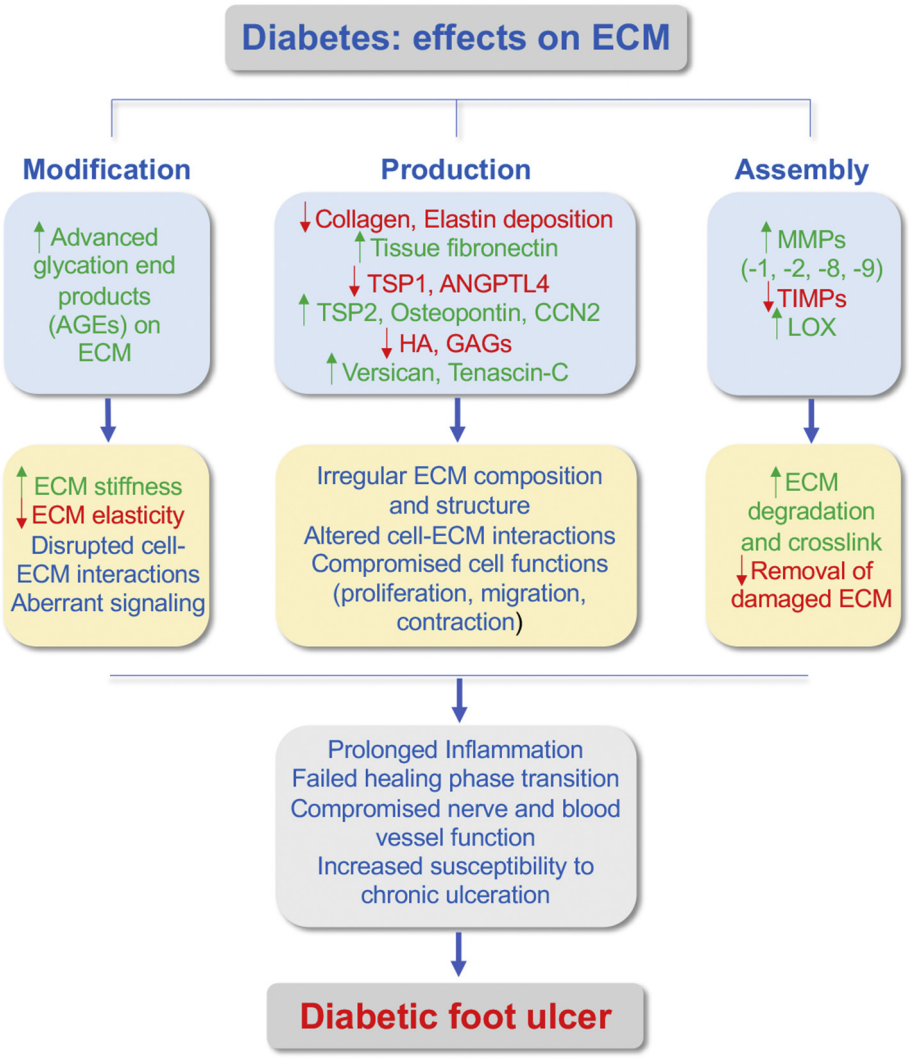

Fig. 1. Diabetes-induced changes in wound ECM.

conduits for communication between cells and ECM. Therefore, understanding the origins of cell and ECM dysfunction in diabetes could help elucidate the mechanisms of DFU. This review will discuss the major alterations of ECM molecules in diabetic wounds of humans and in experimental animal models. Foot ulcers occur in both type 1 and type 2 diabetic patients. Therefore, numerous animal models have been utilized to investigate compromised healing and evaluate experimental treatments. Mice and rats are the most common species used in such studies and include genetic, chemical, or metabolic interventions to induce diabetes. None of these fully recapitulate all aspects of the human disease but can allow for the investigation of specific cellular or molecular processes. Detailed reviews on the suitability of animals for the study of diabetes and diabetic wounds have been published [8,9]. In terms of wounding, the most common approach involves the introduction of full thickness excisional or incisional skin wounds. Of note, some investigators employ splints to minimize wound contraction because it is much greater in rodents in comparison to humans [10]. In terms of diabetes, rodents with genetic susceptibility to diabetes (mostly type 2) or animals treated with streptozotocin to destroy pancreatic islets (type 1) represent the majority of published studies.

Fibril-forming structural proteins

As the name indicates, structural proteins comprise ECM fibrils. Their production directly relates to ECM quantity and quality (Table 1).

Collagen

Based on their functions, collagen molecules can be divided into fibril forming collagens including Collagen Type I, III, and V, and non-fibril forming

Table 1. Fibril-forming structural proteins in diabetic wound healing.

Molecules	Function	Alterations in diabetic wounds	Possible contribution
Collagen	Major component of fibril	Overall decreased deposition [11,12]	Decreased skin thickness and integrity [13]
Fibrinogen	Forms the fibrin clot [14]	Increased (in plasma) [15]	Fibrin clots become denser and more porous [16]; increased level could be a diagnostic marker of DFU [15,17–19]
Fibronectin	Plasma fibronectin forms fibrin with fibrinogen; tissue fibronectin is required for collagen deposition [20,21]	Decreased plasma fibronectin; increased and prolonged expression of tissue fibronectin [22–24]	Improved wound healing displayed higher fibronectin [25,26]
Vitronectin	Temper the pro-fibrotic effect of fibronectin [27]	Increased (in diabetic patient plasma) [28]	Increased vitronectin could be a marker of metabolic syndrome [28]
Elastin	Define the rigidity and elasticity of the normal skin	Decreased expression [12]	Decreased skin elasticity [29]

collagens including Collagen Type IV–XIV [30]. As the major component, collagen constitutes 70% of skin ECM [31], and its expression, deposition, and remodeling are critical in repair. In the early proliferative phase of wound healing, collagen type III forms a provisional matrix with other ECM molecules, such as fibronectin and tenascin, to provide a substrate for cell activity. Subsequently, the composition of the ECM is dominated by collagen I and transforms to a more mature ECM during the remodeling phase. However, abnormal collagen metabolism in diabetic wounds prevents the progress of healing. When directly evaluated in a foreign body-induced collagen production model, collagen production was reduced in T1D patients [32]. Moreover, RT-PCR and two-photon microscopy analysis of dermal samples taken at the edge of DFUs revealed reduced collagen expression [11,12]. Increased levels of matrix metalloproteinases (MMPs) in DFUs also contribute to the degradation of collagen [11,32]. Reduced collagen is also due to the accumulation of advanced glycation endproducts (AGEs), and reductions in the levels of HSPs and PDGF (Reviewed in [33]). In contrast to wounds, levels of collagen I and III, both at the level of mRNA and protein, in intact skin were similar between healthy and diabetic patients [34]. In vitro studies have also shown alterations in collagen levels in diabetic conditions. Specifically, human skin fibroblasts displayed increased collagen production when cultured in media containing excess glucose [35]. Fibroblasts isolated from human diabetic foot ulcers also showed increased production of collagen I, when compared to fibroblasts isolated from healthy skin [36]. These observations highlight differences between the in vivo and in vitro conditions.

Animal models also showed alterations and the earliest studies in type I diabetic (T1D) and type II diabetic (T2D) rodent skin showed decreased collagen deposition [37,38]. Furthermore, numerous studies have shown decreased transforming growth factor -beta (TGF-β) expression in human diabetic wounds and experimental animal models (Reviewed in [33]).

Fibrin

Fibrin, derived from plasma fibrinogen, functions mainly in the early stage of wound healing. It forms a provisional clot matrix to stop bleeding and to provide a scaffold to support cell migration and proliferation, facilitating the transition from inflammation to repair [14]. As wounds close, the fibrin matrix contracted by fibroblasts is replaced by mature collagen fibrils during the matrix remodeling process [39]. Fibrinogen is an acute-phase protein, of which the synthesis could be activated by inflammation and interleukin-6 (IL-6). Chronic inflammation and increased IL-6 expression in DFU could be a stimulator for fibrinogen production by enhancing the transcription of three genes encoding the peptide chains of fibrinogen [40–42]. Moreover, hyperglucagonemia in type 2 diabetes might contribute to the increased production of plasma fibrinogen [43]. It has also been observed that fibrin clots isolated from T2D patients displayed a denser and less porous structure than those from healthy people [16], indicating a difference in fibrin matrix between diabetic and normal wound healing. Furthermore, the fibrinogen level is closely associated with glycemia control in diabetic patients, and could be used as a diagnostic marker for DFU [15,17–19].

Fibronectin

Fibronectin is a dimeric glycoprotein with abundant protein binding domains and participates in wound healing via its interaction with multiple cell types and proteins in the wound bed. Based on its spatial expression and distribution, it is divided into plasma or tissue fibronectin [44]. Plasma fibronectin can form a fibrin-fibronectin clot with plasma fibrinogen, which helps cells like keratinocytes migrate on and through the wound provisional matrix [20]. In diabetic

Table 2. ECM enzymes in diabetic wound healing.

Molecules	Subtypes	Function	Alterations in diabetic wounds	Possible contribution
MMPs and tissue inhibitor of MMPs (TIMPs)	MMP-1	Degrade collagen	Increased [54]	Result in a highly proteolytic environment, degrading ECM and growth factors, making the dermis more uneven and rough [34,54].
	MMP-8	Degrade collagen	Increased [54]	
	MMP-2	Degrade gelatin	Increased [54]	
	MMP-9	Degrade gelatin	Increased [54]	
	TIMPs	Inhibit the activity of MMPs	The ratio of MMPs/TIMPs is increased [55]	
LOX		Crosslink collagen fibrils	Increased (in diabetic patient skin) [56]	Produces more non-soluble fibril fragments, endows skin an aged appearance [34]

wound tissues it is degraded due to increased proteolytic activity. Overall, levels of plasma fibronectin were found to be reduced in diabetes [22]. In addition, there was more fibronectin degradation, observed as increased fibronectin fragments in DFU wound fluid [45–47]. Tissue fibronectin has a more complex role in ECM remodeling and it is required for the deposition of collagen I and III [21]. It also mediates the activity of lysyl oxidase (LOX), an enzyme that crosslinks collagen molecules. In diabetic patients, tissue fibronectin increases in skin but the significance of this increase is unclear [22,23]. Moreover, a study of chronic DFUs revealed prolonged presence of fibronectin, for over 12 mo, when compared to normal wounds [24]. Dysregulation of fibronectin expression, degradation, and clearance is thought to contribute to delayed healing by limiting progression to the remodeling phase [24,36]. Moreover, wound treatments that improve outcomes have been shown to involve increased fibronectin expression. For example, negative pressure wound therapy for a period of 7 days resulted in increased fibronectin mRNA and protein [26].

Experiments in diabetic animal models have also implicated fibronectin in wound repair. For example, treatment of excisional wounds in db/db mice with Notoginsenoside Ft1 accelerated healing by 5 days and was associated with increased expression of fibronectin mRNA [25]. Interestingly, fibroblasts isolated from db/db mice and human diabetic wounds display elevated expression of fibronectin suggesting inherent dysregulation [36]. Topical treatment with plasma FN improved diabetic wound healing in streptozoticin-treated rats and was associated with increases in infiltrating fibroblasts, TGF-β1, and hydroxyproline levels [48]. Similarly, fibronectin was shown to potentiate the effect of erythropoietin on the healing of excisional wounds in streptozoticin-treated mice [49]. Collectively, studies of DFUs and animal models suggest that the dysregulation of fibronectin production and degradation contribute to compromised healing. Moreover, strategies that either deliver or increase fibronectin production in wounds are associated with improved outcomes.

Vitronectin

Vitronectin mediates wound healing by counteracting the effect of fibronectin [27]. Specifically, it can reduce the exposed binding sites of fibronectin fibrils by adjusting their conformation. Thus, vitronectin could temper the pro-fibrotic effect of fibronectin. Likewise, vitronectin can indirectly regulate fibroblast proliferation by hiding the "growth promoting" regions in fibronectin. In diabetes, the plasma level of vitronectin is increased, which is proposed to be a marker to predict diabetes [28]. Additionally, glycation of vitronectin inhibits the activation of vascular endothelial growth factor (VEGF) receptor 2 by disturbing its phosphorylation and intracellular signaling, thus interfering with VEGF signaling in diabetes [50].

Elastin

Elastin defines the rigidity and elasticity of the normal skin. In skin repair, elastin fibers are proved to have a beneficial effect. Proteases could act on elastin to liberate the fiber-forming proteins, promoting fibroblast proliferation and collagen deposition, thus accelerating the healing process [51]. The elastin content in skin is low (2%) [52], and most studies focus on the fragmented elastin-derived peptide (EDP) rather than the overall expression level of elastin in diabetes. It is reported that glycated EDP has a higher expression level in serum and might be useful for monitoring vascular alterations in diabetes [53]. Furthermore, elastin is susceptible to more AGEs due to increased lysine composition. In diabetes, the glycation of elastin and collagen slackens the skin, leading to a sharp decrease in elasticity [29]. Two-photon microscopy analysis of the tissue at the edges of diabetic wounds revealed a significant loss of elastin throughout the dermis [12].

ECM enzymes

ECM enzymes are critical for remodeling and wound healing outcomes. Their dysregulation in DFU has been widely studied and many therapeutic agents are developed based on them (Table 2).

Matrix metalloproteinases and tissue inhibitor of MMPs

Matrix metalloproteinases (MMPs) are a family of proteases that could degrade collagen and gelatin [6]. Their expression in wound healing is tightly regulated. In the early phase, they act on the broken fibrils and promote inflammation. With phase transition, the expression level of MMPs gradually decreases, indicating successful wound healing progress. One hallmark of compromised diabetic wound healing is the chronic inflammation and highly proteolytic environment, which is contributed by the elevated expression levels of MMP-1, MMP-2, MMP-8, and MMP-9 [54]. The persistent high expression levels of MMPs are caused by hyperglycemia and hypoxia in diabetes [57]. Both conditions directly increase the production of MMPs or indirectly via the action of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and IL-6 [58]. Increased TNF-α upregulates MMPs transcription by suppressing the production of TGF-β which inhibits MMPs gene promoter activity [59–61]. IL-6, along with lipopolysaccharide, and high glucose could upregulate MMP-1 production via the extracellular signal-regulated kinase (ERK) and c-JunN-terminal kinase (JNK) pathway. As a consequence of increased expression of c-Jun, AP-1 is upregulated in high glucose, which is a key transcription factor for MMP-1 [62]. Furthermore, a genetic study revealed that the risk genotype of −1562 C>T polymorphism along with significant unmethylated CpG sites in MMP9 gene promoter might be the cause of abnormal MMP production in diabetic wounds [63]. As for tissue inhibitor of MMPs (TIMPs) in diabetes, previous work reported the expression level of TIMP-1 was decreased [55], while others reported that their expression level remained constant [34]. Lack of a concomitant increase will result in a greater ratio of MMPs to TIMPs, leading to more degraded, sparse, and disorganized ECM. It has also been suggested that the ratio of MMPs to TIMPs could be used as an indicator of good/bad wound healing in diabetic patients, helping to track disease progression [55]. It should be noted that fibril fragmentation could further impact ECM properties [64]. Consistent with this suggestion, the dermis layer in diabetics is rougher and more uneven compared to normal skin [34].

Lysyl oxidase

In contrast to MMPs, lysyl oxidase (LOX) crosslinks collagen fibers, granting them a strong resistance to degradation. Interestingly, the expression level of LOX in diabetic skin also increases [56]. But the phenomenon does not rebut the statement that ECM fibrils are degraded in diabetic wounds. Instead, it results in the presence of more non-solubilized and fragmented ECM fibrils due to the protective effect of increased crosslinks. Taken together, with more proteases and crosslinks, the collagen fibrils go through more degradation but also more crosslinking reactions, endowing the diabetic skin an aged appearance [34]. A genotypic study revealed that there was an association between LOX gene polymorphism G473A, G > A, and DFU. Specifically, a higher frequency of 'A' allele was found in diabetes. In the presence of 'AA' genotype in DFU,LOX transcription level was significantly increased [65].

Matricellular proteins

Matricellular proteins function predominantly as modulators of cell-ECM interactions [66]. They are usually expressed at low levels under normal condition, but are upregulated during wound healing to coordinate diverse cellular process [67] (Table 3).

Thrombospondins

Thrombospondins (TSPs) comprise a family of modular proteins that includes five members. According to their structure, they can be classified into

Table 3. Matricellular proteins in diabetic wound healing.

Molecules	Function	Alterations in diabetic wounds	Possible contribution
TSP-1	Associated with inflammation [68]	Decreased (in serum) [69]	Predicted: delayed wound healing
TSP-2	Regulates ECM assembly [70]	Increased (in both diabetic skin and diabetic wounded skin) [71]	Delayed wound healing [71]
Osteopontin	Maintain bone homeostasis;	Increased [75]	Exacerbated inflammation [75]
Osteonectin (SPARC)	influence fibroblast functions; link obesity with diabetes [72–74]	Increased (in serum and adipose tissue of diabetic patients) [76–78]	Making the tissue more fibrotic [79]
CCN2 (CTGF)	Has a positive relationship with collagen synthesis [80]	Increased (in serum) [81]	Increasing CCN2 is correlated to good wound healing [81]
Tenascin-c	Present in the edge of the wound to help recruit cells [82]	Prolonged expression [24]	Might be associated with delayed healing [24]
ANGPTL4	Involved in lipid and glucose metabolism; has a positive effect on keratinocyte migration and angiogenesis [83] [84]	Decreased (in diabetic mouse wounds) [85]	Topical application accelerates wound healing in diabetes [85]

two subgroups. Group A are homotrimers including TSP-1 and TSP-2. The remaining ones are homopentamers and belong to group B [86]. Although TSP1 and TSP2 share high sequence similarity and both have anti-angiogenic properties, their spatiotemporal expression patterns in animal models of wound healing differ [87]. TSP-1 first appears in the inflammation phase while TSP2 is primarily expressed in the remodeling phase, indicating that they have different roles. TSP1, primarily released by platelets and secreted by macrophages, is associated with inflammation [68]. On the contrary, TSP2, mainly produced by fibroblasts, plays a role in vascular regression and ECM assembly [70]. A recent study showed that TSP2 regulates LOX levels via a miR-29-dependent mechanism [88]. Studies have shown that suppressing or overexpressing TSP1 would exert a deleterious effect on wound healing [89], while depleting TSP2 improved wound healing [90]. Moreover, downregulation of TSP2 was correlated with the upregulation of MMPs and VEGF [91]. In human diabetic wounds, the expression level of TSP2 increased, and in vitro studies showed that this was due to the hexosamine pathway [71]. In contrast, the serum level of TSP1 in patients with DFU decreased, and is thought to be caused by reduced TGF-β [69].

Osteopontin and osteonectin

Osteopontin and Osteonectin are glycoproteins associated with bone homeostasis. They are also the very first ECM proteins described in adipose tissue, linking obesity and diabetes [72–74]. They are also implicated in the regulation of matrix turnover, the formation of dermal fibrosis, and fibroblast function [92–95]. In diabetic patients, the expression level of osteopontin and osteonectin in serum and adipose tissue both increase [76–78]. Moreover, the histological analysis of biopsy from DFU patients showed an increase of osteopontin [75]. It is suggested that the increased secretion of osteopontin by macrophages in diabetes is due to the upregulated pro-inflammatory cytokines, such as IL-6, TNF-α, and oxidized low-density lipoprotein [96]. In turn, the elevated osteopontin helps the recruitment of inflammatory cells, exacerbating chronic inflammation in diabetic wounds [75]. It is unclear how the levels of osteonectin change in DFU but its accumulation in diabetic adipose tissue promotes fibrosis [79].

CCN2

CCN2, also known as connective tissue growth factor (CTGF), is involved in fibroblast function and keratinocyte migration [97]. CCN2 shares a positive relationship with the synthesis of collagen I, collagen III, tissue inhibitor of MMPs, and basic fibroblast growth factor [80]. However, CCN2/CCN1 double knock-out mice did not display impaired wound healing, suggesting that CCN2 is dispensable for skin repair [98]. Nevertheless, hyperglycemia increased the production of CCN2 and it has been suggested that increasing its accumulation in wound fluid positively correlates with DFU healing rate [81]. Furthermore, treatment of diabetic wounds with CCN2 improved healing and was associated with a higher collagen IV expression in granulation tissue [99].

Tenascin-C

Tenascin-C is a glycoprotein that is usually expressed in fibrotic diseases [100]. It is expressed transiently and rapidly in response to injury or other disease-associate stress, and is regarded as a hallmark of inflammation [101]. In wound healing, it displays a unique distribution pattern in that it is primarily expressed at the edge of wounds [82]. It is thought that at this location, it facilitates fibroblast recruitment and wound contraction. In addition, its interaction with cells in the provisional matrix could limit remodeling and contraction [102]. As the healing progresses, proteases digest full-lengthTenascin-C into smaller fragments and these have been shown to inhibit fibroblast proliferation and prevent excess scar formation [103]. Despite its well described role in wound healing, Tenascin-C deficiency in mice was shown to have almost no effect in this process [104]. Immunostaining of human diabetic and venous ulcers revealed prolonged and higher expression of Tenascin-c in comparison to acute wounds [24]. This pattern of expression is also seen in other chronic inflammatory conditions, underscoring the association of diabetic wounds
Tenascin-C with inflammation [101].

Angiopoietin-like 4

Angiopoietin-like 4 (ANGPTL4) is important for lipid and glucose metabolism. Its reduction in diabetes relates to impaired glucose tolerance [83]. As a matricellular protein secreted by keratinocytes, it exerts a positive effect on migration, re-epithelialization, and angiogenesis [84]. It also coordinates cell-matrix interactions and can interact with vitronectin and fibronectin to slow their proteolytic degradation [105]. It was shown that its expression was compromised in diabetic wounds and topical application of recombinant ANGPTL4 accelerated wound closure [85]. Specifically, exogenous ANGPTL4 enhanced keratinocyte – endothelial – cell communication via an integrin/JAK/STAT3 – mediated iNOS upregulation pathway leading to improved angiogenesis and healing.

Proteoglycans and glycosaminoglycans

Proteoglycans (PGs) consist of core proteins and various glycosaminoglycans (GAGs) [106]. They not

Table 4. Proteoglycans (PGs) and glycosaminoglycans (GAGs) in diabetic wound healing.

Molecules	Function	Alterations in diabetic wounds	Possible contribution
Hyaluronan	Maintains tissue hydration and elastoviscosity; exerts biological functions in wound healing [108–110]	Decreased (in diabetic rat wounds and skin) [111]	Delayed wound healing. Treatment with exogenous HA or HA-mimics improves DFU [112–115].
Dermatan sulfate (DS)	Regulate growth factors [116]	Decreased (in diabetic rat wounds and skin) [111,117]	unknown
Chondroitin sulfate (CS)	Promote fibroblast migration [118]	Prolonged and elevated expression [24]	unknown
Heparan sulfate (HS)	Interact with chemokines to regulate cell recruitment [119,120]	Decreased but not significant (in diabetic rat wounds and skin) [111,117]	Treatment with exogenous HS or HS-mimics improves wound healing in diabetic rats [121].
Keratan sulfate (KS)	Recognize protein ligand, modulate cell motility [122]	Decreased (in diabetic rat skin) [117]	unknown
Decorin	Interact with multiple molecules to regulate ECM fibril structure, mechanical properties, and cytokine release [123–131]	Increased (in serum of diabetic patients) [132]	Predicted: decreased synthesis of collagen; contribute to the progression of T2D [133]
Biglycan		Increased (in skin of diabetic patients) [134]	Contribute to the progression of T2D [133]
Lumican	Retard the formation of fibrils and decrease skin thickness [135]	unknown	unknown
Fibromodulin		unknown	unknown
Dermatoponin	Activate the synthesis of fibronectin fibrils and promote keratinocyte migration [136,137]	Decreased (in T1D mouse skin) [138]	Predicted: abnormal fibronectin fibril synthesis
Versican	Work with HA to regulate fibroblast migration [109]	Prolonged expression [24]	unknown

only contribute to the structural integrity of ECM but also function as an active niche in ECM to interact with multiple molecules and cells [107]. Despite their critical role in ECM production and remodeling, few studies are available to depict their roles in impaired wound healing (Table 4).

Hyaluronan

Hyaluronan (HA), known for its role in maintaining tissue hydration and elastoviscosity, is a common and unique glycosaminoglycan in the ECM [110]. HA is a straight chain polymer with repeating units and varying chain lengths. In addition to modulating supramolecular assembly of proteoglycans in the ECM,HA exerts distinct biological effects depending on its molecular weight. High molecular weight (>500 KDa) HA is anti-angiogenetic and anti-inflammatory, and correlated with an increased production of Collagen III and increased TGF-β activity. In contrast, degraded HA products with a low molecular weight exert pro-inflammatory effects [109]. Such products also stimulate the production of Collagen I, and the proliferation and differentiation of fibroblasts and endothelial cells, but inhibit the proliferation of smooth muscle cells [108,139]. It has been suggested that elevated glucose levels stimulate the synthesis of high-molecular weight HA via the TSP1-TGF-β pathway but have no effect on the synthesis of low-molecular weight HA or HA degradation [140]. In addition, the increased UDP-*N*-acetylglucosamine (GlcNac) and O-GlcNacylation in hyperglycemia could stimulate the synthesis of HA by enhancing the transcription of HAS2, a HA synthase [141]. Studies on diabetic patients and animals claimed a positive correlation between diabetes and HA expression, and the increase of HA is related to diabetic nephropathy, angiopathy, intimal hyperplasia and inflammation [142–145]. Fibroblasts taken from the skin of patients with DFU also exhibited higher HA expression compared to diabetic patients without ulcers and healthy control [146]. However, in vivo observations in diabetic rats demonstrated that HA content decreased in diabetic wounds and skin [111,117]. The opposite results might be due to the highly proteolytic environment in diabetic wounds, underscoring the complexity and contribution of disrupted ECM. In addition, decreased HA correlated with delayed wound healing and treatment with exogenous HA and HA-mimics could improve DFU [112–115]. Furthermore the abundance of AGEs in diabetes could induce the fragmentation of high MWHA, leading to a pro-inflammatory response [147].

Sulfated GAGs

Dermatan sulfate (DS), chondroitin sulfate (CS), keratan sulfate (KS), and heparan sulfate (HS) are known as the sulfated GAGs that can form PGs when they attach to native protein cores [148]. However, they can also be found in the skin free of a protein core. In vitro findings suggested that free GAG chains forming weak interactions with surrounding molecules, mainly composed of CS (80%) and HS, could promote fibroblast migration [118]. Via these and additional interactions they could

influence wound healing. For instance, HS was shown to bind CXC chemokines and promote neutrophil and leukocyte recruitment [119,120]. It was also shown that CS and DS could regulate growth factors and modulate nitric oxide production [116]. In addition, KS could facilitate cell recognition of protein ligands and promote cell motility [122]. A study of human diabetic wounds showed that the expression of CS was higher and more persistent than in normal wounds [24]. In contrast, research on streptozotocin-induced diabetic rats showed that the GAG content in skin and wounds was decreased by 50–70% except for HS and this was attributed to altered biosynthesis due to effects effects of insulin-like growth factor [111,117]. Because sulfated GAGs have been implicated in other diabetic pathologies, such as nephropathy and retinopathy [149], it is reasonable to assume that their dysregulation contributes to DFU formation. Moreover, treatment with HS or HS-mimics improved wound healing in diabetic rats [121].

Decorin and biglycan

Decorin is a small proteoglycan with a core protein rich in leucine repeats [150]. Biglycan is also a small leucine-rich proteoglycan (SLRP), sharing 55% homology with decorin at the amino acid level [123,151]. Due to the amphipathic leucine-rich repeats, they are thought to interact with several proteins such as TGF-β, TNF-α, and collagen [124–126].. Both molecules play a critical role in collagen fiber structure, alignment, and mechanical properties. In decorin KO mice, collagen fibrils displayed irregular morphology and were larger in diameter [127]. These defects manifested in reduced tensile strength and fragile skin. Disruption of biglycan also evoked irregular fibril structure but diameters were smaller than control [128]. In addition, the mechanical properties of skin of biglycan KO mice were less compromised than decorin KO [129]. Interestingly, in the absence of decorin, a compensatory increased in biglycan expression was described [130]. Moreover, the phenotype of decorin and biglycan double KO mice was similar to single decorin KO mice, indicating a dominant role for the latter [150]. Following tissue stress or injury, ECM-bound decorin and biglycan are cleaved and their soluble forms function as signaling molecules by binding various cell surface receptors and mediating cellular activities. For example, biglycan can stimulate the synthesis and secretion of TNF-α as well as chemoattractants for macrophage and neutrophil recruitment via a TLR2/4-ERK-dependent pathway [126]. Likewise, decorin can also induce a pro-inflammatory environment via its direct interaction with TLR2/4 [125]. Overall, decorin can be a more potent regulator of cell functions, such as adhesion and proliferation, and this is achieved via its interactions with other ECM proteins including fibronectin and TSPs [123,131]. Differences between biglycan and decorin are also evident in the influence of their individual deficiencies on wound healing. Specifically, biglycan or decorin deficiency impairs initial and late repair phases, respectively [152]. In the context of diabetes, the expression of both molecules is increased in response to high glucose [132,134]. Because excess decorin can reduce the production of collagen I in fibroblasts in vitro [153], it is possible that its excess accumulation in diabetic skin might contribute to decreased collagen production. In addition, both decorin and biglycan are thought to play a role in the development of type II diabetes by facilitating the expansion of adipose mass [133].

Versican

Versican has a high affinity for HA [154]. HA-versican complexes are enriched in the provisional matrix and work synergically to regulate fibroblast contraction [109]. Although detailed information regarding the expression of versican is lacking, non-specific immunostaining of CS-PG in diabetic skin offers a hint that the deposition of versican exceeds the normal healing time frame [24]. Specifically, in normal human wounds, it is mainly found in dermis and its expression returns to the pre-wounding level 12 months after injury. In contrast, in diabetic foot ulcers, it is found at high levels in both dermis and basement membrane and its expression lasts >12 months. Clearly, the possible role of versican in diabetic wound healing should be investigated further.

Dermatopontin

Dermatopontin is a small SLRP, mainly found on the surface of collagen fibers [155]. It is enriched in the dermis and plays a critical role in re-epithelialization by promoting keratinocyte migration via a paracrine effect [136]. Moreover, dermatopontin could activate the synthesis of fibronectin fibrils in a dose-dependent manner [137]. Analyzing the skin of type I diabetic mice demonstrated reduced dermatopontin expression [138]. This suggests that its decrease could influence the formation of fibronectin fibrils leading to impaired cell migration in diabetic wounds.

Fibromodulin and lumican

Fibromodulin and lumican belong to another subfamily of SLRPs. They share 48% amino acid identity [156]. One in vitro study claimed that fibromodulin and lumican could bind to fibrillar collagen, retarding the formation of fibrils and decreasing the thickness of them [135]. But whether

and how their expression changes in diabetic wounds have not been investigated.

Conclusion

Wound healing is a highly dynamic and tightly regulated process, involving complex ECM-dependent molecular and cellular interactions. In diabetes, high glucose environment and other changes lead to alterations in ECM, such as decreased collagen deposition, increased production of MMPs, and the prolonged presence of abundant matricellular proteins. In turn, these molecular alterations negatively influence cell functions, worsening the pathological state of ECM, resulting in chronic wound healing. Nevertheless, there are still many unanswered questions regarding the role of many ECM molecules in the skin and wounds of diabetes patients. Importantly, the lack of detailed information on the molecular changes in DFU prevents the comprehensive understanding of impaired wound healing. However, rapid technological advances such as quantitative proteomics, tissue mass-spectrometry, two-photon microscopy, and cryoelectron microscopy should allow for in-depth analysis of ECM in this context. Such approaches could be applied to the analysis of human DFUs as well as wounds in animal models. Equally important, advances in gene modification techniques should accelerate the genetic dissection of this complicated process in experimental animals. Newly acquired knowledge should contribute to the development of more effective therapeutic strategies.

Abbreviations

ECM	extracellular matrix
DFU	diabetic foot ulcer
AGEs	advanced glycation end products
MMPs	matrix metalloproteinases
T1D	type 1 diabetes
T2D	type 2 diabetes
WT	wild type
IL-6	interleukin-6
EDP	elastin-derived peptide
TIMPs	tissue inhibitor of MMPs
TNF-α	tumor necrosis factor alpha
TGF-β	transforming growth factor beta
IL-1	interleukin-1
ERK	extracellular signal-regulated kinase
JNK	c-JunN-terminal kinase
LOX	lysyl oxidase
TSPs	thrombospondins
ANGPTL4	angiopoietin-like 4
PGs	proteoglycans
GAGs	glycosaminoglycans
HA	hyaluronan
GlcNac	UDP-*N*-acetylglucosamine
DS	dermatan sulfate
CS	chondroitin sulfate
KS	keratan sulfate
HS	heparan sulfate
SLRP	small leucine-rich proteoglycan
VEGF	vascular endothelial growth factor

Declaration of competing interest

There is no conflict of interest to declare.

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