

## Extracellular Matrix and Dermal Fibroblast Function in the Healing Wound

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**Significance:** Fibroblasts play a critical role in normal wound healing. Various extracellular matrix (ECM) components, including collagens, fibrin, fibronectin, proteoglycans, glycosaminoglycans, and matricellular proteins, can be considered potent protagonists of fibroblast survival, migration, and metabolism.

**Recent Advances:** Advances in tissue culture, tissue engineering, and ex vivo models have made the examination and precise measurements of ECM components in wound healing possible. Likewise, the development of specific transgenic animal models has created the opportunity to characterize the role of various ECM molecules in healing wounds. In addition, the recent characterization of new ECM molecules, including matricellular proteins, dermatopontin, and FACIT collagens (Fibril-Associated Collagens with Interrupted Triple helices), further demonstrates our cursory knowledge of the ECM in coordinated wound healing.

**Critical Issues:** The manipulation and augmentation of ECM components in the healing wound is emerging in patient care, as demonstrated by the use of acellular dermal matrices, tissue scaffolds, and wound dressings or topical products bearing ECM proteins such as collagen, hyaluronan (HA), or elastin. Once thought of as neutral structural proteins, these molecules are now known to directly influence many aspects of cellular wound healing.

**Future Directions:** The role that ECM molecules, such as CCN2, osteopontin, and secreted protein, acidic and rich in cysteine, play in signaling homing of fibroblast progenitor cells to sites of injury invites future research as we continue investigating the heterotopic origin of certain populations of fibroblasts in a healing wound. Likewise, research into differently sized fragments of the same polymeric ECM molecule is warranted as we learn that fragments of molecules such as HA and tenascin-C can have opposing effects on dermal fibroblasts.



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### SCOPE AND SIGNIFICANCE

THIS REVIEW HIGHLIGHTS translational research regarding the effect of the local extracellular matrix (ECM) on dermal fibroblasts. The modulation of ECM components in the healing wound is discussed, with some examples of current research related to the topic.

### CLINICAL RELEVANCE

Historically, the ECM has been misconstrued as relatively inert scaffolding, existing only to support cells. An initial insight into the role of ECM was expanded with the revelation that growth of most human cells, including skin cells, depends on cellular adhesion to the ECM through a mechanism

called anchorage dependence.<sup>1</sup> This gave greater credibility to the idea that the ECM is a vital participant in tissue activity and wound healing. Beyond simply anchoring cells, it is now known that the ECM is an active and complex tissue component which is capable of influencing cell survival, proliferation, and function.<sup>2</sup> Due to their ability to modify cellular properties in a healing wound, ECM components are attractive targets for the emerging clinical use of bioactive wound dressings, engineered tissues, and topical wound treatments (Table 1).

## TRANSLATIONAL RELEVANCE

Since dermal fibroblasts are so intimately linked to the ECM, as both an originator and a resident, the modulatory properties of the local ECM are perhaps most apparent in the functioning of dermal fibroblasts. The ECM is capable of influencing the migration, senescence, and gene expression of the fibroblast, and it is even capable of differentiating the fibroblast into entirely different cell types.<sup>3</sup> Since it is so poorly characterized on its own terms, in some sense the fibroblast is actually

**Table 1.** Selected list of clinically available extracellular matrix components for modulation of wound healing or scar treatment

Extracellular Matrix Component	Wound Dressings		Dermal Substitutes/Tissue Scaffolds		Soft Tissue Fillers	
Collagen I	BioBrane (p)	DermaSIL	Alloderm (h)	Glyaderm (h)	Primatrix (b)	Artefill (b)
	Biostep (p)	Fibracol (b)	Allomax (h)	GraftJacket (h)	Replifirm (h)	Cosmoderm (b)
	Biopad (e)	FortaFlex (p)	Apligraf (h,b)	ICX-SKN (h)	SIS (p)	Cosmoplast (b)
	Catrix (b)	FortaDerm	Biodesign (p)	Integra (b)	Strattice (p)	Cymetra (h)
	CellerateRX (b)	Medifil II (b)	Collamend (p)	Karoderm (h)	Surederm (h)	Dermalogen (h)
	Colactive AG (p)	Prisma Matrix	Dermagraft (h)	Matriderm (b)	Strattice (p)	Fascian (h)
	Collasorb (b)	Promogran Matrix (p)	DermaMatrix (h)	MatriStem (p)	Surgimend (b)	Zyderm (b)
	Collieva (p)	Puracol (b)	Endoform (o)	NeoForm (h)	TheraSkin (h)	Zyplast (b)
	DermaCol (p)	Skintemp (b)	EZ-Derm (p)	Oasis (p)	TransCyte (h,p)	
	DermADAPT	Stimulen	FlexHD (h)	OrCel (h,b)	Unite (e)	
	Dermagen		Fortaflex (p)	Permacol (p)	Veritas (b)	
			GammaGraft (h)	PermaDerm (b)	XenMatrix (p)	
	Collagen II	BioCell collagen		None		None
	Collagen III	None		Biodesign (p)	GraftJacket (h)	Primatrix (b)
			DermaMatrix (h)	ICX-SKN (h)	Surgimend (b)	
			Dermagraft (h)	Matriderm (b)	TheraSkin (h)	
			Endoform (o)	Oasis (p)	TransCyte (h,p)	
Collagen IV	None		Endoform (o)	GraftJacket (h)	Oasis (p)	None
			Dermagraft (h)	MatriStem (p)	TheraSkin (h)	
Collagen V	None		Matriderm (b)	TheraSkin (h)	TransCyte (h,p)	None
Collagen VI	None		Biodesign (p)	Oasis (p)	TheraSkin (h)	None
Collagen VII	None		GraftJacket (h)		MatriStem (p)	None
Collagen XIV	None		TheraSkin (h)			None
Fibrin	Crosseal (h)	Evicel (h)	BioSeed-S		ICX-SKN (h)	None
	Evarrest (h)	Tisseel (h)				
Fibronectin	None		Biodesign (p)	Oasis (p)	TransCyte (h,p)	None
			Endoform (o)			
Vitronectin	None		DermaMatrix (h)			None
Elastin	Elastatropin	Prolastil E-50	Alloderm (h)	DermaMatrix (h)	GraftJacket (h)	None
	TropolActive-F		Allomax (h)	Endoform (o)	Matriderm (b)	
			Dermagraft (h)	Glyaderm (h)	Oasis (p)	
Hyaluronan	BioCell collagen	Hyiodine	Biodesign (p)	Hyalograft	Laserskin	Belotero (es)
	HealSmart	Hylase	DermaMatrix (h)	Hyalomatrix GraftJacket (h)	Oasis (p)	Captique (es)
	Hyalofill	IPM Wound Gel	Endoform (o)			Hylaform (r)
	Hyalomatrix	RadiaPlexRx				Hydrelle (es)
						Juvederm (es)
						Restylane (es)
						Perlane (es)
Chondroitin sulfate	BioCell collagen	Dermagen	Biodesign (p)	Integra (b)	Oasis (p)	None
			GraftJacket (h)			
Decorin	None		Dermagraft (h)	Oasis (p)	TransCyte (h,p)	None
Versican	None			TransCyte (h,p)		None
SPARC	None		TransCyte (h,p)			None
Tenascin	None		GraftJacket (h)		TransCyte (h,p)	None

More than 30 dermal substitutes containing multiple ECM components are available clinically today. Numerous unlisted topical gels and powders featuring ECM components also exist.

b, bovine; e, equine; es, equine streptococci; h, human; o, ovine; p, porcine; r, rooster comb.

defined by its local ECM. Since the ECM sequesters and regulates exposure of the fibroblast to soluble cytokines, it is not possible to completely dissociate the role of these signaling molecules from that of the fibroblast's proteinacious matrix microenvironment.<sup>4</sup> However, this review focuses on the classic, macromolecular, insoluble components of the skin ECM, and the effect these structures have on the fibroblast. This review discusses the impact of various ECM components on wound-healing functions of dermal fibroblasts, including migration, proliferation, gene expression, and protein metabolism. As is the case with many aspects of wound healing, a balance should be struck between inadequate fibroblast activity, which may lead to chronic wound formation, and overly exuberant fibroblast activity, which can lead to fibrotic healing. Many components of the ECM are involved in regulating this equilibrium, and can potentially be manipulated to swing the balance one way or the other to improve overall wound healing.

## THE FIBROBLAST AND THE ECM

### Defining the fibroblast

Although it is the most prevalent cell in human dermis, and one of the most important architects of cutaneous wound healing, the fibroblast as a cell type remains relatively ill defined. The word "fibroblast" encompasses any stromal cell that does not express markers for a more specific mesenchymal lineage. This imprecise definition, and our impaired ability to track cellular changes due to a lack of distinguishing cell markers, lends to the misconception of the fibroblast as a homogenous and static cell. However, fibroblasts are an impressively heterogeneous and dynamic cell lineage. Fibroblast populations differ between human tissues, with significant alterations even apparent within a given tissue type. In skin dermis, for example, different dermal layers host diverse subpopulations of fibroblasts with both unique morphologies and physiologic functions.<sup>5-8</sup> Depending on their resident depth of dermis, fibroblasts express different quantities of collagen and ratios of collagen type I and III mRNA. Further, fibroblasts within the deeper dermis produce less collagenase mRNA than do fibroblasts in more superficial layers.<sup>9</sup>

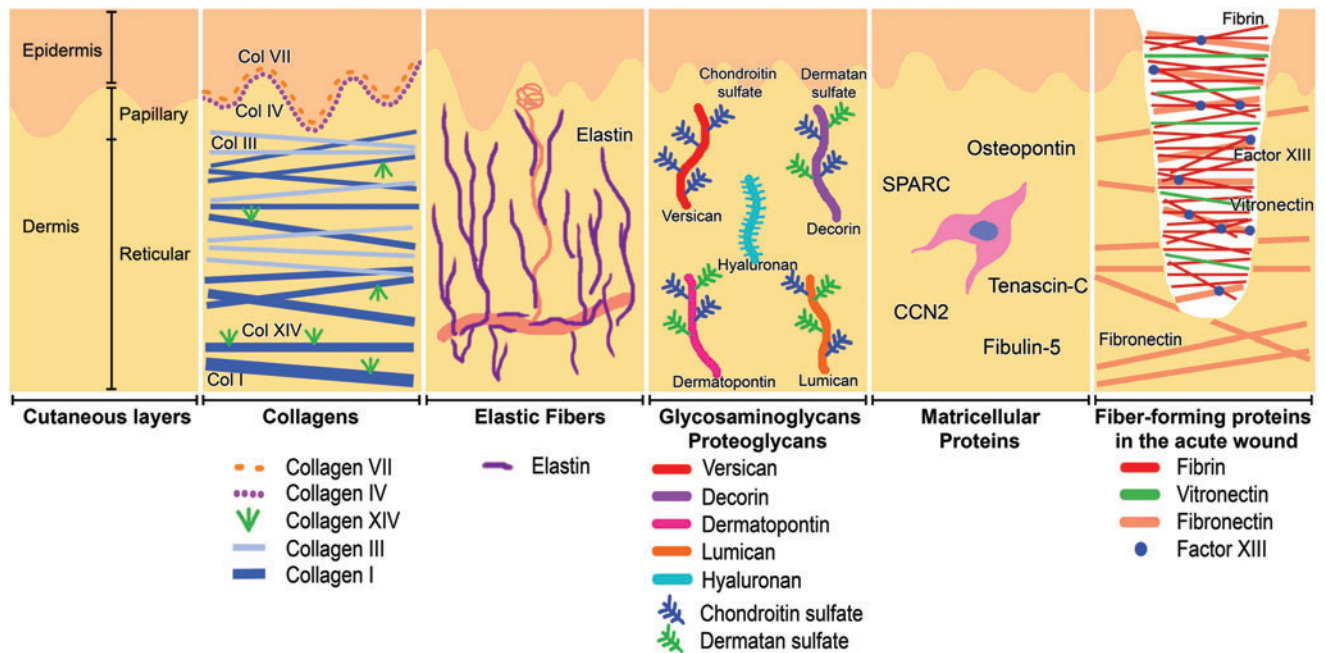
Fibroblasts are also remarkably plastic in nature. Cells that share fibroblasts' mesenchymal origin, including adipocytes and pericytes, are able to de-differentiate into fibroblasts with an appropriate stimulus.<sup>10-14</sup> Even cells of endothelial and epithelial origin are capable of taking on the phenotype into fibroblasts, a fact that has emerged as an important pathologic etiology for fibrotic disease and likely tumor metastasis.<sup>15-18</sup> Likewise, fibro-

blasts themselves are capable of altering their cellular profile, with the most common transition being that into contractile myofibroblasts. The dermal fibroblast also has the unique title of being the first human somatic cell to be induced into a pluripotent stem cell line.<sup>19-21</sup>

The fibroblast is a malleable cell, capable of altering its function and physiology or even transforming into a new cell type, based on its location within the body. The mechanisms driving these alterations are still poorly understood; however, it is thought that both soluble signaling molecules and the resident molecular framework of the fibroblast's extracellular microenvironment can act to modify the activity of the fibroblast. The role of growth factors and cytokines in the fibroblast during wound healing has been discussed in other excellent reviews.<sup>22-25</sup> Since its manipulation and augmentation is emerging in clinical treatment (including use of acellular dermal matrices and topical wound dressings), the influence of the ECM on the fibroblast, both mechanically and through modulation of molecular pathways, warrants further discussion.

### Defining the ECM

The extracellular components of the skin can be divided into fiber-forming structural molecules, nonfiber-forming structural molecules, and "matrix-cellular proteins" that do not function structurally but modify cell-matrix interactions (Fig. 1).<sup>26</sup> Fiber-forming molecules provide a structure to the ECM by creating a complex three-dimensional framework of rigid proteins. The nonfiber-forming molecules, mostly proteoglycans and glycosaminoglycans (GAGs), function to create a charged, dynamic, and osmotically active space. In the skin, the most prevalent fiber-forming protein, by far, is collagen, which comprises 77% of the fat-free dry weight of human skin.<sup>27</sup> Other fiber-forming proteins in the skin include fibrin, fibronectin, vitronectin, elastin, and fibrillin.<sup>28</sup> Glycoproteins such as laminins, secreted molecules that comprise the basement membrane, and integrins, which act as cell membrane-bound receptors, are also structural proteins that facilitate skin cell adhesion and migration. While these fibrous proteins define the rigidity and elasticity of a tissue, it is the nonfiber-forming proteoglycans and GAGs that fill the majority of the tissue's interstitial space.<sup>26</sup> Their negatively charged and hydrophilic nature enable proteoglycans and GAGs to function in hydration, buffering, and force dispersion within tissues. The most abundant proteoglycans in the skin include hyaluronan (HA), decorin, versican, and dermatopontin.<sup>29,30</sup> Also present in the ECM are the matrix-cellular proteins, a recently described group of



**Figure 1.** The extracellular matrix (ECM) of normal skin with selected components depicted. Fiber-forming proteins and collagens define the rigid mechanical structure of skin, while elastic fibers enable stretching. Proteoglycans and glycoproteins create an osmotically active hydrated interstitial space. Matricellular proteins do not contribute to the mechanical structure of the ECM but instead act as paracrine signaling molecules. To see this illustration in color, the reader is referred to the web version of this article at [www.liebertpub.com/wound](http://www.liebertpub.com/wound)

secreted local proteins that interact in autocrine or paracrine cell–matrix signaling while not contributing significantly to ECM mechanical structure. Included among the matricellular proteins are osteopontin, secreted protein, acidic and rich in cysteine (SPARC) (also known as osteonectin), tenascin-C, fibulins, and the CCN family.<sup>31</sup> Unlike most ECM components that are constantly present in normal skin and repopulate wounded skin after predictable intervals, matricellular proteins can be absent in healthy skin and expressed temporarily only after skin wounding (Table 2). In the healing wound, fibroblasts produce the majority of these ECM components while these same molecules simultaneously act to modify the function of the fibroblast.<sup>24</sup> In this sense, the interaction of the fibroblast with the ECM can be thought of as a form of autocrine regulation that is crucial in the process of wound healing. The fibroblast should continually create new matrix proteins to properly heal a cutaneous wound, while concurrently being regulated by proteins of its own creation.

## COLLAGEN

The tensile strength and compressibility of fiber-forming collagen make it a ubiquitous protein in the human body. In fact, collagen is thought to be the single most abundant protein in the animal

kingdom.<sup>32</sup> Although 28 types of collagen have been identified, collagens I and III, respectively compose approximately 90% and 10% of the total collagen in the skin, with collagen V representing about 2%.<sup>33</sup> However, less prevalent collagen types are also integral to normal skin functioning as indicated by the pathologic skin conditions associated with mutations in collagens IV, VII, and XVII (Table 3).

### Collagen-mediated mechanical tension

Interwoven collagen fibrils form the structural scaffold of the healing wound and enable cell adhesion, chemotaxis, and migration. Since collagen fibers align in different orientations, they are able to produce mechanical tension vectors within the skin. Surgeons have long noted this property in the existence of Langer's lines, or skin tension lines, which are believed to correlate histologically with the orientation of collagen fibrils in the dermis.<sup>34–38</sup> Although the tensile strength of collagen is integral to appropriate acute wound healing, too much tautness of collagen bundles in the early wound, among other variables, is thought to contribute to later formation of hypertrophic scars.<sup>39,40</sup> Conversely, the chronically diminished mechanical stimulation imposed by the ECM on dermal fibroblasts has been linked to the reduced production of collagen type I and III and associated dermal laxity characteristic of aging skin.<sup>41</sup> While fibroblasts are

**Table 2.** Chronology of selected extracellular matrix component expression in healing human wounds

	Earliest Appearance	Regular Appearance	References
Fiber-forming collagens			
Collagen I	4 days	7 days	160, 161, 164
Collagen III	2 days	6 days	163
Collagen V	3 days	7 days	161, 185
Nonfiber-forming collagens			
Collagen IV	4 days	7 days	72, 161, 185
Collagen VI	3 days	7 days	161, 165
Collagen VII	4 days	12 days	161, 166, 185
Collagen XIV	Unknown	Unknown	N/A
Other fiber-forming proteins			
Fibrin	Seconds	30 s	167
Fibronectin	10 min	4 h	72, 164
Vitronectin	Unknown	3 days	168
Elastin	3 days	14 days	170
Proteoglycans/glycosaminoglycans			
Hyaluronic acid	1 day	> 1.5 days	171, 172
Chondroitin sulfate	Unknown	3 days	169, 173, 174
Dermatan sulfate	Unknown	3 days	169, 173, 174
Decorin	7 days	12 days	171, 173
Lumican	3 days	7 days	175
Versican	Unknown	14 days	176, 177
Dermatopontin	2 days	14 days	30
Basement membrane			
Complete intact basement membrane	8 days	21 days	161, 178, 185
Matricellular proteins			
Osteopontin	4 days	14 days	121, 179
SPARC	2 days	7 days	180, 181
CCN2	1 day	5 days	161, 182
Tenascin-C	1 day	5 days	161, 182
Fibulin-5	Unknown	14 days	147
Cell types			
Fibrocytes	Unknown	> 4 days	183
Myofibroblasts	Unknown	5 days	72, 161, 162

Earliest demonstrable histologic appearance and regular histologic appearance of ECM components in human wounds is indicated.

responsible for the production and early tensing of the ECM collagen, they are also considerably affected by it. Since the myofibroblast was first described in 1971, molecular mechanical stress (via tension in the ECM) has been implicated in fibroblast differentiation to this cell type.<sup>3</sup> More recent work has shown that fibroblast gene expression, including apoptosis signaling, matrix metalloproteinase (MMP) activity, and differentiation into myofibroblasts, are strongly linked to the rigidity or laxity of the ECM, even independent of transforming growth factor (TGF)- $\beta$  signaling.<sup>42–48</sup> Even a 15% increase in cell stretch is capable of altering a fibroblast's orientation in relation to the ECM, inducing alterations in focal adhesion kinase signaling, or up-regulating expression of alpha smooth muscle actin ( $\alpha$ SMA), consistent with differentiation into the contractile myofibroblast.<sup>49,50</sup> Some clinicians have theorized that reducing the tension on

wound fibroblasts by paralyzing local muscle fibers may reduce myofibroblast proliferation and collagen production, thereby decreasing scarring. Botulinum A toxin is an injectable neurotoxin that causes flaccid paralysis of muscles, and if injected around a wound would thus lessen the mechanical stretch on nearby fibroblasts. Attempts at reducing hypertrophic scar formation through the use of botulinum A have thus far yielded conflicting results.<sup>51,52</sup>

Similarly, fibroblasts grown on type I collagen substrates with greater stiffness show increased expression of  $\alpha_v\beta_3$  integrin, which binds to noncollagen ECM proteins including vitronectin and fibronectin. The same fibroblasts also show simultaneous decreased expression of  $\alpha_2\beta_1$  integrin, which is recognized as the fibroblast-collagen binding integrin and is required for fibroblast migration.<sup>50,53</sup> Conceptually, this correlates with the idea that as an open wound contracts and builds up tension through tightening of collagen fibrils, myofibroblasts transition away from  $\alpha_2\beta_1$  integrin-mediated organization of collagen fibers, and toward  $\alpha_v\beta_3$  integrin-mediated migration along the noncollagen proteins present in acute wounds. This wound contraction and subsequent reorganization of collagen fibers can be seen histologically (Fig. 2). The pathway by which mechanical strain induces fibroblast differentiation or modifies myofibroblast behavior is still unclear, although pathways involving src kinase substrate p130cas, FAK-ERK pathway, PI3K-Akt anti-apoptotic signaling, or specific calcium channel signals are thought to be involved.<sup>54–60</sup>

### Fibrillar collagens

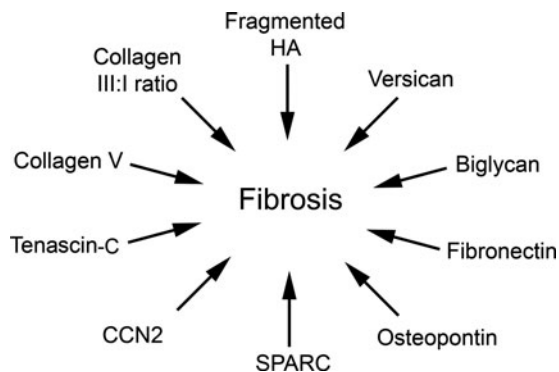
Collagen can broadly be divided into fibrillar and nonfibrillar families. Fibrillar collagens include types I-III, and V. The presence, absence, or ratio of specific collagen types can affect fibroblast function apart from the mechanical stress imposed by collagen fibrils. For example, mice with a genetic deficiency in collagen III exhibit greater myofibroblast differentiation and more pronounced wound contracture.<sup>61</sup> These same mice also demonstrate abnormal collagen I fibrillogenesis in the skin and other organs, indicating that collagen III may play a regulatory role in collagen I synthesis.<sup>62</sup> This correlates with the observation that scarless fetal wound healing is associated with a higher ratio of collagen III to collagen I in multiple small and large mammal models.<sup>63–66</sup> Likewise, collagen type V has been shown to regulate collagen I fiber assembly, with mice deficient in collagen V demonstrating large structurally abnormal collagen fibrils.<sup>67,68</sup>

However its presence has been associated with decreased dermal fibroblast apoptosis, increased

**Table 3.** Genetic disorders involving extracellular matrix proteins, and the characteristic effect these gene mutations have on wound healing

Extracellular Matrix Component	Encoding Gene	Disease Associated with Gene Defect	Characteristic Wound-Healing Pathology
Collagen I	<i>COL1A1</i>	Arthrochalasia EDS Caffey disease Osteogenesis imperfecta	Tissue fragility Atrophic scar Hyperextensible skin Delayed wound healing
Collagen III	<i>COL3A1</i>	Vascular EDS	Tissue fragility Delayed wound healing
Collagen IV	<i>COL4A4</i>	Familial porencephaly Hereditary angiopathy Alport syndrome Leiomyomatosis	Unknown
Collagen V	<i>COL5A2</i>	Classic EDS	Impaired wound healing Atrophic "cigarette paper" scars Hyperextensible skin
Collagen VI	<i>COL6A1</i>	Bethlem myopathy Ulrich muscular dystrophy	Wound dehiscence Atrophic scars
Collagen VII	<i>COL7A1</i>	Dystrophic epidermolysis bullosa	Extreme skin fragility Frequent wounding from minor trauma Delayed wound healing
Collagen XVII	<i>COL17A1</i>	Epidermolysis bullosa junctionalis	Extreme skin fragility Frequent wounding from minor trauma Delayed wound healing
Decorin	<i>DCN</i>	Congenital stromal corneal dystrophy	Unknown
Elastin	<i>ELN</i>	Cutis laxa Williams syndrome	Loose skin Normal wound healing
Fibrillin 1	<i>FBN1</i>	Marfan's syndrome Weill-Marchesani syndrome Congenital scleroderma	Impaired wound healing Atrophic or distended scar Thick skin covering most of body (in congenital scleroderma only)
Fibrillin 2	<i>FBN2</i>	Contractural arachnodactyly	Unknown
Fibulin 4	<i>EFEMP2</i>	Cutis laxa	Loose skin Normal wound healing
Fibulin 5	<i>FBLN5</i>	Cutis laxa	Loose skin Normal wound healing
Laminin-332	<i>LAMA3, LAMB3, LAMC2</i>	Epidermolysis bullosa junctionalis	Extreme skin fragility Frequent wounding from minor trauma Delayed wound healing
Tenascin-X	<i>TNXB</i>	Hypermobility EDS	Hyperextensible skin Normal wound healing

Genetic mutations affecting ECM proteins have varying characteristic effects on skin and wound healing. EDS, Ehlers Danlos syndrome.



**Figure 2.** ECM components implicated in fibrosis or hypertrophic scarring. Overexpression or modification of certain ECM components is associated with myofibroblast differentiation and fibrotic wound healing.

skin thickness (as observed in systemic sclerosis), and, in fibroblasts isolated from patients with Ehlers–Danlos Syndrome, rescue of fibronectin fibrillogenesis and improved  $\alpha_2\beta_1$ -mediated fibroblast migration.<sup>69–71</sup>

Experience in reconstructive surgery and wound care show that fibroblasts are able to successfully migrate and infiltrate acellular dermal matrices that are rich in collagen I; however, the effect of isolated zoonotic collagen I on fibroblasts in human wounds remains uncertain. Topical collagen dressings are effective in reducing wound size of diabetic foot ulcers; however, they showed no effect on healing of facial wounds after excisional Mohs surgery.<sup>72,73</sup> Further studies on stage II and



III pressure ulcers showed good healing after collagen application, but the same effect was also achieved with neutral hydrocolloid control.<sup>74</sup>

### Nonfibrillar collagens

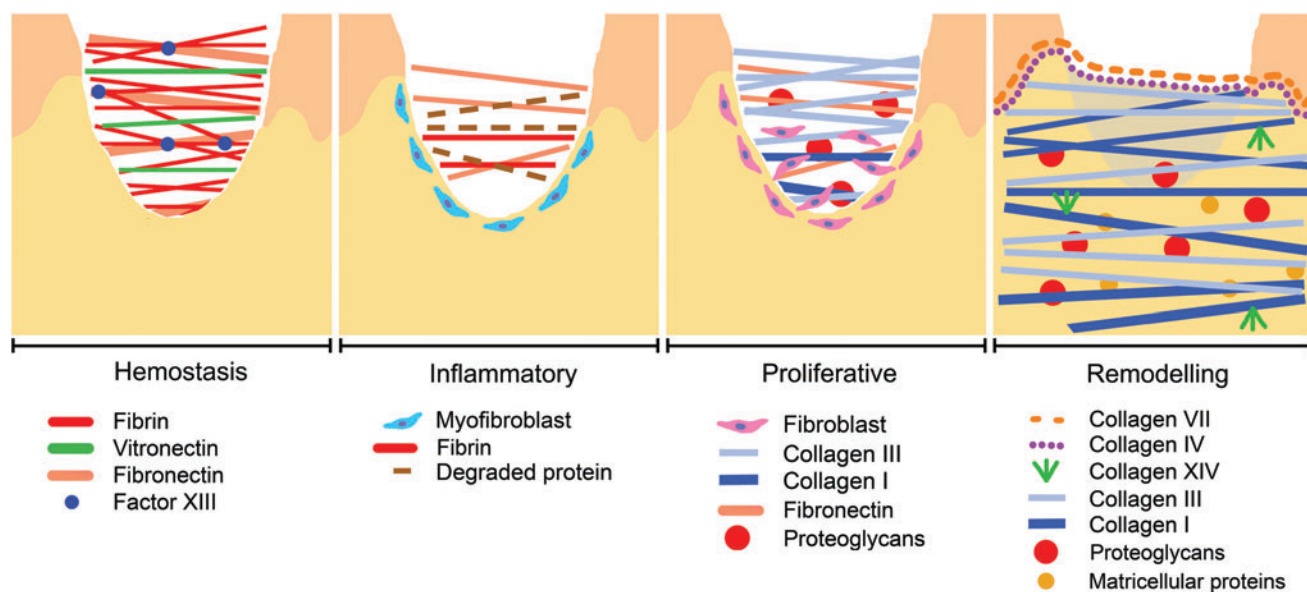
Nonfibrillar collagens, including collagen IV, VI, VIII, and XIV, do not form classic fibrils but instead compose reticular nets, connect cells to the basement membrane, or in the case of FACIT collagens (Fibril-Associated Collagens with Interrupted Triple helices) aid in the organization of other collagens fibers. Despite forming the basal lamina and enabling keratinocyte and fibroblast migration and adhesion to the basement membrane, the role of collagen IV in wound healing has remained relatively unstudied.

In contrast to this, collagen VI has been shown to drive *in vitro* serum-starved fibroblasts through the S phase, and prevent apoptosis via down-regulation of Bax compared with controls. This effect was more pronounced with soluble collagen VI than immobilized collagen VI, and was partially independent from  $\beta_1$  integrin signaling and collagen VI's ability to anchor cells.<sup>75</sup> Collagen VI persists in wound histology from 3 days till many months postwounding; this perhaps suggests that the ability of collagen VI to inhibit fibroblast apoptosis could be relevant during this critical time in wound healing (Fig. 3).<sup>76</sup> The gene encoding collagen VI is located on chromosome 21. Interestingly, dermal fibroblasts isolated from patients with tri-

somy 21 show increased expression of collagen VI, greater adherence to collagen VI, and also increased hyaluronan (HA) synthase correlated to the overexpression of collagen VI.<sup>77,78</sup> These facts, coupled with the progeroid skin aging and sometimes impaired healing of surgical wounds characteristic of trisomy 21, could provide future avenues of study.<sup>79</sup>

Collagen VII has been shown to support dermal fibroblast migration and, in addition, regulates cytokine expression in wound-infiltrating macrophages.<sup>80</sup> Topically applied human recombinant collagen VII was incorporated into skin wounds on mice and decreased expression of fibrogenic TGF- $\beta$ 2 and  $\alpha$ SMA while increasing the expression of anti-fibrogenic TGF- $\beta$ 3. This resulted in less collagen deposition in the healed wound, and may be of interest in controlling fibrosis in normal healing wounds as well as in treating patients with recessive dystrophic epidermolysis bullosa (RDEB), a disease characterized by absence of collagen VII.<sup>81</sup> Fibroblasts from RDEB patients who underwent restorative lentiviral transfection with the gene encoding collagen VII demonstrated homing, incorporation, and production of functional collagen VII in mice after an intravenous injection of these cells.<sup>82</sup>

Collagen XIV is a member of the increasingly studied FACIT collagen family. It has been shown to decrease dermal fibroblast differentiation and reduce fibroblast DNA synthesis by 75% while maintaining normal viability and stable cell numbers.<sup>83</sup>



**Figure 3.** The appearance of ECM proteins correlates with phases of wound healing. The ECM composition of a wound changes as it progresses through the hemostatic, inflammatory, proliferative, and remodeling phases of healing. Fibrin, fibronectin, and other clotting proteins dominate in the hemostatic acute wound, but as fibroblasts migrate into the wound they produce proteoglycans and collagen that are associated with mature healing wounds. To see this illustration in color, the reader is referred to the web version of this article at [www.liebertpub.com/wound](http://www.liebertpub.com/wound)

**Table 4.** Effect of selected extracellular matrix components on dermal fibroblast functions

Extracellular Matrix Component	Fibroblast Proliferation	Fibroblast Migration	Collagen Production/Assembly	Myofibroblast Differentiation/Contraction	References
Collagen I	↓↑	↑	↑	↑	39, 40, 49, 50, 53
Collagen III	—	—	↑	↓	61, 62
Collagen IV	—	—	—	—	NA
Collagen V	↑	↑	↑	—	63, 64, 66,
Collagen VI	↑	↓↑	↑	—	154
Collagen VII	—	↑	↓	↓	75, 77
Collagen XIV	↓	—	↑	↓	78, 79
Fibrin	↑	↑	↑	↑	83, 85
Fibronectin	↑	↑	↑	↑	158
Vitronectin	↓	↑	—	↑	84, 88, 90
Elastin	↑	↑	↑	↓	91, 92, 93
Hyaluronan	↓↑	↑	↓↑	↓↑	158, 186
Dermatan sulfate	↑	↑	—	—	187
Chondroitin sulfate	↑	↑	—	—	188
Decorin	↓	↓	↓	↓	115, 158
Versican	—	↓↑	↑	↑	184
Lumican	↓	—	↑	↑	121
Dermatopontin	—	↑	↑	↓	158
Osteopontin	↑	↑	↑	↑	127
SPARC	—	↓	↑	—	130
CCN2	↑	↑	↑	↓↑	134, 137, 140
Tenascin-C	↓	↓↑	↑	↑	144, 158, 159
Fibulin-5	↓↑	↓↑	↓↑	↑	145, 146

Up-arrows (↑) show an increase or improvement, down-arrows (↓) show a decrease or impairment, bidirectional arrows (↓↑) show no apparent effect, and dashes (—) indicate an unknown or unstudied effect.

(↑) Increased; (↓) Decreased; (↓↑) No effect; (—) Unknown effect.

In mechanical studies, skin isolated from collagen XIV null mice exhibited significantly decreased stretch compared with normal skin.<sup>84</sup> Further research into collagen XIV is needed to assess whether its role in decreasing fibroblast differentiation could perhaps reduce scar formation.

## OTHER FIBER-FORMING PROTEINS

Other fiber-forming proteins include fibrin, fibronectin, vitronectin, and elastin. Elastin helps define the rigidity and elasticity of the normal skin, while fibrin, fibronectin, and vitronectin are key mediators in hemostasis and cell migration in the healing wound.

### Fibrin

In the earliest hemostatic stages of tissue repair, a provisional clot matrix is formed with fibrin, a fibrillar protein derived from soluble plasma fibrinogen.<sup>85</sup> Fibroblasts quickly contract the loose fibrin matrix, and then utilize the contracted matrix as a surface for migration and tissue remodeling. As the wound progresses into the proliferative phase of healing, fibroblasts are then able to synthesize collagen and other ECM proteins to replace the contracted fibrin matrix and progress toward a granulation tissue-like matrix (Fig. 3).<sup>86</sup>

It has been shown that the contraction of the fibrin matrix can influence fibroblast responsiveness

to TGF- $\beta$ , which, in turn, activates collagen synthesis. Fibroblasts placed within noncontracted fibrin matrices *in vitro* had increased collagen and total protein synthesis when stimulated by various concentrations of TGF- $\beta$  compared with fibroblasts embedded in contracted fibrin matrices with identical exposures.<sup>87</sup> In addition, manipulation of the fibrin fiber alignment and overall fibrin matrix geometry has also been implicated in influencing fibroblast matrix remodeling.<sup>88</sup> For example, an *in vitro* study utilizing cruciform fibroblast-populated fibrin gels that varied in arm length and fiber alignment demonstrated that collagen synthesis, and to a lesser extent elastin synthesis, was increased in specific regions of gels of certain geometries. Each of the cruciform fibrin gels were contracted and remodeled by fibroblasts without manipulation; however, the areas with the greatest collagen synthesis per cell correlated to the areas in which the greatest amount of tensile stress was imposed by the fibrin fiber alignment, as was predicted by anisotropic biphasic theory simulations.<sup>88</sup> These data imply that fibroblast remodeling is influenced by both regional mechanical stress and the vector geometry of the fibrin matrix.

### Fibronectin

Fibronectin is a complex component of the ECM, and the target of a growing body of research



analyzing its ability to stimulate fibroblast growth, spreading, migration, and contractility.<sup>89</sup> It is a dimeric glycoprotein that exists in a modified soluble form primarily found in plasma and an insoluble cellular form found in the ECM. Soluble plasma fibronectin assembles into insoluble ECM fibrils in the acute wound through a complex, cell-dependent process involving conformational changes that expose specific matricryptic sites.<sup>85,89</sup> One of these sites is an RGD (Arg-Gly-Asp) sequence known as the “cell-binding domain,” or “growth promoting site” through which fibronectin binds to the  $\alpha_v\beta_3$  integrin expressed by fibroblasts, stimulating their migration into the wound.<sup>85</sup> After tissue injury and deposition of a fibrin clot, fibronectin, in addition, has a unique site to bind and stabilize fibrin. This covalent bond is further stabilized by factor XIII, and is an absolute requirement for migration of fibroblasts into plasma clots, at least *in vitro*.<sup>90</sup> Due to its many binding domains, ECM fibronectin fibrils have the ability to interact with cells, other fibronectin fibrils, and multiple other proteins within the ECM, making it a unique responder in tissue injury.

In wound healing, fibronectin plays a critical role in ECM organization and stability. *In vitro* studies revealed that fibronectin is actually required for deposition of collagen I and other structural proteins into the ECM.<sup>91</sup> Further, maintenance of the fibrillar organization of both collagen and fibronectin *in vitro* requires the active polymerization of fibronectin. Fibrils were lost from the ECM when fibronectin polymerization was inhibited.<sup>91</sup> Fibronectin has also been shown to regulate the proteolytic activation of lysyl oxidase (LOX), the enzyme responsible for covalent cross-linking of collagen fibrils into mature collagen fibers. In the absence of fibronectin, the catalytic activity of LOX is dramatically decreased, resulting in less mature collagen matrices.<sup>92</sup> Furthermore, fibronectin has been shown to play a role in fibroblast-mediated collagen gel contraction *in vivo*. Inhibition of a specific 122-amino-acid sequence within the III<sub>4</sub>-III<sub>5</sub> repeats of fibronectin leads to inhibition of the initial phase of fibroblast-mediated collagen gel contraction.<sup>93</sup>

Fibronectin and fibroblasts share a dynamic, reciprocal relationship. While the intracellular cytoskeleton of fibroblasts can influence the orientation of fibronectin fibrils, the fibronectin matrix can also influence intracellular events. For example, fibroblasts grown on a fibronectin matrix orient their intracellular actin filament bundles in alignment with the matrix.<sup>2</sup> In addition, stimulation of fibroblast proliferation by TGF- $\beta$  has been shown to be dependent on preassembly of a fibronectin matrix. *In vivo* studies in a rat model have shown that

topical application of fibronectin enhances wound-healing parameters, including migration of a greater numbers of fibroblasts expressing TGF- $\beta$  after wounding.<sup>94</sup>

### Vitronectin

Vitronectin is important for early contraction of wounds.<sup>95</sup> In an *in vitro* model of wound healing, fibroblasts were shown to generate tension in the ECM by binding proteins in a chronological manner. In order to generate tension, fibroblasts first attach to fibronectin, followed by attachment to vitronectin, and finally attachment to collagen.<sup>92</sup> These processes were shown to be interdependent, with fibroblasts only generating full force through normal collagen attachment after having previously bound fibronectin and vitronectin.<sup>92</sup> Thus, vitronectin serves as a facilitating step toward maximal wound contraction.

Vitronectin has also been shown to influence fibroblast proliferation as mediated by fibronectin. In an *in vitro* study, fibroblasts bound to vitronectin produced fibronectin fibrils that were significantly less effective at stimulating fibroblast proliferation.<sup>89</sup> Vitronectin is thought to induce this effect by guiding formation of fibronectin fibrils with less exposed “growth promoting sites,” thereby preventing effective binding between their RGD sequences and the  $\alpha_v\beta_3$  integrins expressed by fibroblasts.<sup>89</sup> In this sense, vitronectin acts to modulate or balance the migratory and proliferative fibroblast responses induced by fibronectin. By reducing the “growth promoting sites” exposed on fibronectin, vitronectin when introduced early into a healing wound may temper the pro-fibrotic effect of fibronectin.

### Elastin

Rubber-like elastin fibers contribute to resilience and elasticity in tissues. Long cross-linked elastin fibers are intertwined with the relatively rigid collagen fibers in the ECM.<sup>96</sup> Although elastin is conventionally known for its structural role, elastin-derived peptides have shown beneficial effects on wound repair and regeneration. Within skin wounds, various proteases such as MMPs act on elastin to liberate these fiber-forming proteins. This leads to accelerated fibroblast proliferation, increased collagen type I, and increased tropoelastin.<sup>97</sup> Tropoelastin has been shown to promote attachment, spreading, and proliferation of fibroblasts through binding integrin  $\alpha_v\beta_3$  and elastin binding protein (EBP) in an *in vitro* model. Although the mechanism of tropoelastin's effects are not completely understood, signaling involving EBP has been found to be mediated by a protein kinase A circuit.<sup>98</sup>

## PROTEOGLYCANS AND GAGS

Proteoglycans consist of a core protein that is bound to one or more GAG side chains. In turn, GAGs are linear polysaccharides that can exist independently or bound to a proteoglycan core. Both types of these polymeric molecules have extreme variability in size.

### Hyaluronan

HA, also known as hyaluronic acid, is one of the most common GAGs in the skin and has been a topic of growing interest over the last two decades. In addition to its role in maintaining tissue hydration and osmotic balance, HA plays key roles in both fibrotic and regenerative wound healing. Increased expression of HA and its receptor CD44 have classically been associated with fibrotic disease in a variety of tissues. This concept is recapitulated by the observation that human oral fibroblasts, which are associated with rapid and scarless wound healing, are unable to express HA synthase and have decreased pericellular assembly of HA.<sup>99</sup> Divergently, research in fetal scarless wound healing has demonstrated much greater expression of HA in fetal rabbit wounds, and treatment of *in vitro* human adult wounds with HA-rich amniotic fluid results in improved re-epithelialization.<sup>100,101</sup> Likewise, human keloid scars contain less HA and fibroblasts isolated from keloid scars produce less HA than their nonfibrotic counterparts.<sup>102</sup>

These conflicting actions of HA were recently resolved with the discovery that changing the size of the HA molecule modifies its effect. Native high-molecular-weight HA (>500 kDa) is associated with decreased inflammation, increased expression of collagen III, and increased activity of anti-fibrotic TGF- $\beta$ 3. Conversely, fragmented HA (<400 kDa) has been linked with increased inflammation, greater collagen I expression, increased proliferation of fibroblasts, and increased myofibroblast differentiation.<sup>103,104</sup> Adding interest, if not a little confusion, it has further been shown that specific very small-sized hexameric HA fragments (~1 kDa) improve fibroblast migration and promote wound closure, while decreasing myofibroblast differentiation and fibrosis.<sup>105</sup> These data suggest that hexameric HA might be of interest in the healing of chronic wounds.

Although HA likely exerts its effect on the fibroblast via its CD44 and RHAMM (Receptor for HA Mediated Motility) receptors, the large branching pericellular molecule is also known to trap TGF- $\beta$ 1 molecules close to the fibroblast. This creates a positive feedback autocrine loop that contributes to the fibroblast's differentiation into the myofibroblast.<sup>106,107</sup> Fibroblasts taken from aging patients

demonstrate decreased pericellular HA, and accordingly have reduced TGF- $\beta$ 1-mediated CD44-EGF-R-ERK interaction and, thus, resist differentiation into myofibroblasts.<sup>108</sup> This perhaps contributes, in part, to the impaired healing observed in some elderly patients.

Unlike most other ECM GAGs, HA does not exist naturally in a sulfated form. However, artificially sulfated HA has dramatic and complex effects on the dermal fibroblast that are of interest in the engineering of artificial tissues. A quantitative proteomics study revealed that sulfated HA upregulates 84 proteins, and downregulates various MMPs and collagen I.<sup>109</sup> These effects are likely related, in part, to the ability of sulfated HA to bind even more avidly to TGF- $\beta$ 1 than does native HA.<sup>110</sup> Investigation of artificial tissue scaffolds containing sulfated HA is an ongoing topic of research.

### Dermatan sulfate, chondroitin sulfate

Dermatan sulfate (DS) and chondroitin sulfate (CS) are the GAGs most commonly bound to proteoglycans, along with keratan sulfate and heparan sulfate. When they are not bound to proteoglycans, they can also exist, similar to HA, in a free unbound state. DS and CS are very similar in structure, with DS distinguishing itself only by the presence of iduronic acid, a modified sugar. *In vitro* fibroblasts have been observed to express greater quantities of free DS and CS while in their subconfluent growing phase. The presence of these loosely bound GAGs may be a useful substrate for early fibroblast migration.<sup>111</sup> DS is the most prevalent GAG in the skin, and has been shown to be the most common soluble GAG in acute human wounds.<sup>112</sup> It has been demonstrated that DS is required to modify CD44 and enable fibroblast migration in fibronectin/fibrin gel during the earliest phases of wound healing. In addition, fibroblast growth factor-2 (FGF-2) and FGF-10 are known to bind DS; this affinity is increased even further for DS containing more iduronic acid, indicating that the iduronic acid component of DS is responsible for mediating FGF responsiveness in the fibroblast.<sup>112,113</sup> CS and DS may also prove to be players in scarless fetal wound healing, as proteoglycans isolated from human fetal skin have been shown to contain much longer GAG chains compared with those of the adult human skin.<sup>29</sup>

### Decorin

Decorin is a proteoglycan of the small leucine-rich proteoglycan (SLRP) family that is bound to a variable number of CS or DS GAG chains. Decorin is the most prevalent proteoglycan in adult human skin and demonstrates a binding affinity for TGF- $\beta$ 1 that is even further increased by removing its

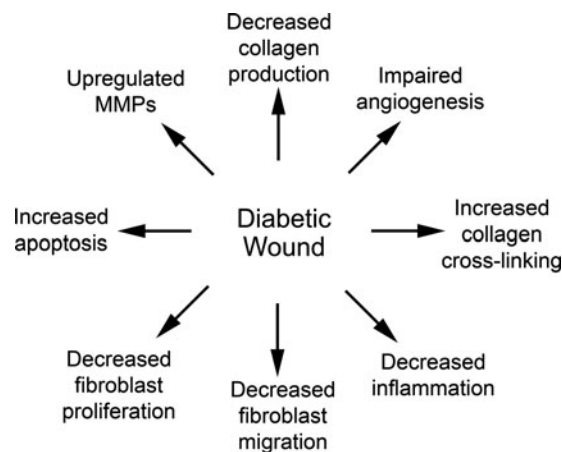
chondroitin and dermatan side chains.<sup>114</sup> In this way, decorin can act as a natural inhibitor of TGF- $\beta$ 1 and is associated with decreased fibrosis and formation of normal scars.<sup>115</sup> In addition to TGF- $\beta$ 1, decorin also effectively binds collagen at  $\beta$ <sub>1</sub>-integrin binding sites and is required for normal collagen fibrillogenesis. The dramatically reduced expression of decorin (25% that of normal) in keloid tissue and hypertrophic burn scars may explain why collagen fibrils within these fibrotic scars are thicker and less organized than in normally healed skin.<sup>116,117</sup> Likewise, delayed expression of decorin has been observed in the healing of hypertrophic burns.<sup>118</sup> Experiments in a knock-out mouse model indicate that the absence of decorin results in increased fibroblast proliferation, increased fibroblast adhesion to collagen or fibronectin, increased fibroblast migration *in vitro*, and paradoxically significantly delayed wound healing and cutis laxa *in vivo*.<sup>119,120</sup> These experiments are consistent with a role of decorin in reduced fibroblast migration and decreased fibrosis in healing wounds. Interestingly, exogenous decorin added to cultured keloid fibroblasts resulted in a reduction of collagen I, MMP1, MMP9, and MMP13.<sup>121</sup>

### Versican

Hypertrophic burn scars in humans show a six-fold higher expression of versican (and biglycan) compared with normal skin.<sup>117</sup> Further evidence that versican is associated with fibrotic healing is the observation that deep dermal fibroblasts, which have a proven role in hypertrophic scars after deep dermal injury, produce significantly more versican than do superficial fibroblasts.<sup>122,123</sup> Fibrotic or hypertrophic scars have multivariate pathologies, with many contributing factors. In addition to genetics and initial wound tension, many extracellular molecules (including versican) have been associated with fibrotic healing (Fig. 4). Interestingly, targeted RNAi against versican resulted in less aggregative behavior in dermal papilla fibroblasts associated with hair follicles.<sup>124</sup> Although these data are of unclear importance for wound healing, they point toward a further investigation into the effect of versican on fibrosis.

### Lumican

Lumican is so named, because it is a translucent proteoglycan that is abundant in the cornea, and also present in the skin. Lumican promotes myofibroblast differentiation and contraction via an  $\alpha$ <sub>2</sub> integrin-mediated pathway.<sup>125</sup> Mice that are deficient in lumican develop abnormally thick collagen fibrils and fragile skin, which are associated with increased proliferation, decreased apoptosis, and downregulation of p53 in fibroblasts *in vitro*.<sup>126</sup>



**Figure 4.** Glycosylated matrix proteins impair and delay healing of diabetic wounds. Many factors, including reduced vasculogenesis, impair the healing of diabetic wounds. The presence of advanced glycosylation end products in diabetic wounds is also associated with decreased collagen production, migration, and proliferation of fibroblasts.

These same fibroblasts exhibited a decreased response to apoptotic Fas-Fas ligand signaling. These data suggest that lumican plays a significant role in pro-apoptotic signaling in fibroblasts, and may be important in fibrotic healing.

### Dermatopontin

Dermatopontin is a recently described SLRP proteoglycan that increases skin elasticity, tensile strength, and collagen fibrillogenesis.<sup>127</sup> Reduced expression of dermatopontin is associated with hypertrophic scar and systemic sclerosis.<sup>128</sup> Dermatopontin has been shown to increase fibroblast adhesion to fibrin matrices, and promote fibronectin fibril formation in a dose-dependent manner.<sup>129</sup> There is some indication that pro-migratory dermatopontin and anti-migratory decorin act to balance and modify each other's activity in the healing wound.<sup>30</sup> Since it was only fully described less than 25 years ago, the full physiology of dermatopontin in wound healing awaits further investigation.

### Biglycan, aggrecan

Biglycan is a proteoglycan commonly present in the skin, while aggrecan is only minimally expressed in the dermis. Although biglycan has been found to be upregulated in fibrotic skin diseases, little else is known about the effect of these proteoglycans on fibroblasts in wound healing.<sup>130</sup>

## MATRICELLULAR PROTEINS

Matricellular proteins are secreted proteins that are incorporated into the ECM while not significantly defining its structure. These proteins are often completely absent or present only in low levels

in uninjured skin. However, they are upregulated after tissue injury. While they do not contribute to the mechanical organization of the ECM, they act as temporally dynamic signaling molecules.

### Osteopontin

Osteopontin is a relatively recently described glycoprotein that was first characterized in the bone. In addition to its role in biomineralization, osteopontin has also been implicated in dermal fibrosis.<sup>131</sup> Osteopontin is required for the differentiation of myofibroblasts in response to TGF- $\beta$  signaling, and increases fibroblast migration and proliferation.<sup>132,133</sup> By decreasing osteopontin concentration via delivery of osteopontin antisense oligodeoxynucleotides to a mouse model wound bed, the wound healed significantly faster with less scarring and granulation tissue formation.<sup>134</sup>

### Secreted protein, acidic and rich in cysteine

SPARC, also known as osteonectin, is another glycoprotein most commonly found in the bone. Similar to osteopontin, increased presence of SPARC is also linked to fibrotic disease in the skin and other tissues. *In vitro* experiments on dermal fibroblasts show SPARC increased gene expression, protein assembly, and ECM incorporation of collagen I.<sup>135,136</sup> Silencing SPARC using siRNA reduced these effects, even in fibroblasts stimulated with TGF- $\beta$  and fibroblasts from patients with systemic sclerosis.<sup>135,137</sup> The *in vivo* data for SPARC are less clear. SPARC-null mice demonstrated accelerated wound closure with decreased collagen production when subjected to 5 mm wounds, but showed significantly delayed wound healing with impaired ECM production in larger 25 mm wounds.<sup>138</sup> The effect of SPARC inhibition in the prevention of fibrosis in human wounds remains unknown.

### CCN2

CCN2, previously known as CTGF, is a matricellular peptide that is not usually expressed in the skin, but is upregulated during tissue injury. CCN2 increases fibroblast expression of collagen I, collagen III, tissue inhibitors of MMP and basic FGF while not affecting expression of proteoglycans.<sup>139</sup> Although CCN2 does not appear to affect local myofibroblast differentiation, it is believed to stimulate mesenchymal stem cell recruitment to the wound site and subsequent differentiation into dermal fibroblasts.<sup>140</sup> Although this implies that CCN2 contributes to the anabolic environment of dermal wound healing, it has also been shown that CCN2 is not a requirement for dermal healing, as fibroblast CCN1/CCN2 knock-out mice are capable of unimpaired wound healing.<sup>141</sup> In fact, CCN2 expression

in skin is associated with hypertrophic scar and fibrosis.<sup>142,143</sup> Conversely, CCN2 seems to be involved in promoting keratinocyte migration during wound healing via integrin  $\alpha_5\beta_1$ .<sup>144</sup> Interestingly, collagen I production and scar were reduced by inhibiting the ERK and JNK signaling downstream of CCN2 in a rabbit model of hypertrophic scarring.<sup>145</sup>

### Tenascin-C

Tenascin-C is a glycoprotein that, while sparsely distributed in the skin, is upregulated in areas of tissue injury, and especially localized to wound edges. Tenascin-C is persistently upregulated in fibrotic disease, including keloids and photo-damaged skin.<sup>146,147</sup> Native full-length tenascin-C arrests fibroblast cell-cycle progression in G1 phase and promotes fibroblast migration along the fibrin-fibronectin matrices characteristic of early wounds.<sup>148</sup> Conversely, fragmented tenascin-C was shown to almost completely inhibit fibroblast migration. This behavior pattern of tenascin-C complements the physiology of wound healing. Initially, tenascin-C is expressed at the wound edge and encourages fibroblast migration. As the wound matures, proteinases are expressed that generate fragmented tenascin-C, which, in turn, inhibits further fibroblast migration and limits hypertrophic scar growth. Perhaps the persistence of full-length tenascin-C in fibrotic disease contributes to this pathologic healing.<sup>149</sup>

### Fibulin-5

Fibulin-5 is a secreted glycoprotein that binds to and mediates development of elastin fibers in the ECM. Overexpression of fibulin-5 by retroviral transfection in rabbits was shown to induce formation of granulation tissue and instigate remodeling of the ECM.<sup>150</sup> The fact that fibulin-5 regulates remodeling of granulation tissue correlates with the fact that under normal conditions fibulin-5 does not appear until 14 days after wounding. Despite this promising result of overexpression, it was found that fibulin-5 null mice had normal wound healing, with the exception of a complete absence of elastin in their skin. Experiments performed on fibroblasts isolated from these mice indicate that fibulin-5 does not affect fibroblast migration or proliferation.<sup>151</sup>

## NONENZYMATICALLY GLYCOSYLATED MATRIX PROTEINS

Nonenzymatic glycosylation is a process in which reducing sugars irreversibly bind to the amino groups of proteins, producing Advanced Glycosylation Endproducts (AGEs). It is commonly observed in ECM proteins of patients with diabetes mellitus (DM) and increased age.<sup>152</sup> AGEs contrib-

ute to a number of the pathologic processes caused by hyperglycemia, affecting everything from entire organ systems (as in diabetic renal nephropathy) to the individual fibroblast in a healing wound. Some of these pathologic processes are thought to be related to the inflammatory function of an immunoglobulin receptor that targets AGEs, Receptor for Advanced Glycation Endproducts (RAGE).

### Advanced glycation endproducts

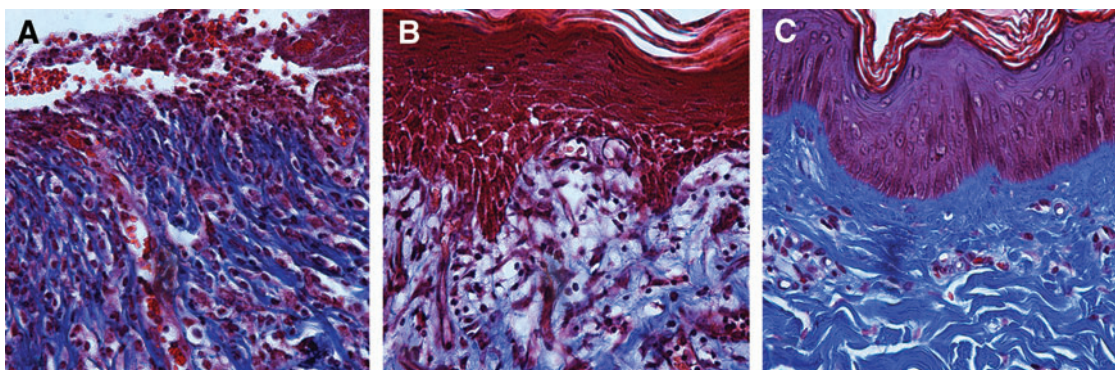
Since dermal macromolecular ECM has a slow turnover rate, the presence of glycosylated ECM can be especially problematic in the skin, with approximately 30% of diabetic patients experiencing some cutaneous involvement during their lifetime (Fig. 5).<sup>153</sup> Accumulation of AGEs on collagen in the ECM of skin has been widely studied.<sup>152,154,155</sup> Glycosylation of collagen leads to increased cross-linking between collagen fibers, increased stiffness, decreased solubility, and decreased susceptibility to proteolysis.<sup>152,155,156</sup> Some authors highlight differences between the collagen cross-linking due to DM compared with aging, stressing that diabetic cross-linking is more extensive and causes a further decrease in solubility. AGEs have also been found to accumulate on elastin fibers within skin that has undergone solar elastosis.<sup>154</sup> AGEs generate active oxygen-free radical species during UVA irradiation, thereby increasing damage to and photoaging of the skin.<sup>154</sup>

With regard to wound healing, nonenzymatic glycosylation of collagen leads to impaired fibroblast-induced contraction.<sup>152</sup> During ECM remodeling in the normal healing process, contraction of collagen matrices is associated with downregulation of type I collagen and upregulation of MMPs by fibroblasts. *In vitro* analysis revealed that collagen glycosylation

inhibited production and activation of MMP-1 and MMP-2 by fibroblasts, suggesting a mechanism for the decreased lattice contraction.<sup>152</sup> Other studies which support the finding of decreased MMP activity found that fibroblast synthesis of HA was also decreased by AGE accumulation within skin ECM. These changes elicited by AGEs are thought to be due to interactions with fibroblast cell membranes, as both HA and MMPs are located within fibroblast cell membranes.<sup>154</sup>

AGE accumulation-induced cross-linking of collagen within the ECM has been implicated in impaired neovascularization of chronic diabetic wounds.<sup>156</sup> In addition to release of angiogenic factors, ECM proteins are necessary for mechanical support of capillary buds during normal angiogenesis. AGEs affect the ability of the ECM to support this regulated vasculogenesis. Endothelial cells studied in glycated collagen lattices were shown to be significantly impaired, with inhibited invasion, decreased proliferation, and significantly reduced capillary formation.<sup>156</sup> While outside the scope of this review, AGEs can greatly impair vasculogenesis in diabetic wounds, thereby significantly impairing healing in addition to their effect on dermal fibroblasts.

A study of human diabetic skin specimens revealed more apoptotic cells and increased expression of AGE and RAGE compared with nondiabetic skin.<sup>153</sup> It was also shown that fibroblasts within the glycosylated ECM of these skin samples exhibited cell-cycle arrest between G2/M phases, leading to apoptosis. This arrest was reversed by application of RAGE-blocking antibodies, demonstrating causality.<sup>153</sup> In addition, it was shown that the fibroblasts within these glycosylated ECMs had modified actin cytoskeletons and increased release of cytoplasmic lactate dehydrogenase, indicating damage to their cell membranes.<sup>152,154</sup>



**Figure 5.** Histological evaluation of wound contraction and collagen fiber orientation in a healing full-thickness excisional porcine wound stained with Masson Trichrome (collagen appears blue). (A) After 7 days of healing, the wound is contracted and loosely organized collagen is present in granulation tissue. (B) 14 days after wounding, the wound is re-epithelialized with reduced contraction and gradual re-modeling of collagen fibers. (C) Intact porcine skin demonstrates highly organized collagen bundles and fibers in dermis. All images are taken with a 20× objective. To see this illustration in color, the reader is referred to the web version of this article at [www.liebertpub.com/wound](http://www.liebertpub.com/wound)

*In vivo* studies involving genetically diabetic mice demonstrated that delayed healing of diabetic wounds is accompanied by a delayed localization of inflammatory cells to the wound, diminished generation of growth factors by inflammatory cells, exaggerated generation of pro-inflammatory factors (e.g., cytokines and MMPs), and delayed egress of inflammatory cells.<sup>157</sup> By inducing a RAGE blockade in the diabetic mice, beneficial inflammatory responses were restored, including re-established migration of inflammatory cells both into and out of wounds and their appropriately limited activation. RAGE-bearing cells responding to AGE accumulation within the ECM of skin have thus been implicated as having a global effect on diabetic wound healing in this *in vivo* study.<sup>157</sup>

The ability of AGEs in the ECM to negatively impact almost every component of wound healing, including migration and proliferation of the fibroblast, matrix remodeling, angiogenesis, and inflammatory signaling, is profound. Methods of preventing AGE formation or modulating their effect through wound interventions are an important topic of current research.

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#### TAKE-HOME MESSAGES

- The ECM plays important signaling roles during dermal wound healing, independent from its ability to sequester growth factors.
- Individual ECM components have varied effects on fibroblast proliferation, migration, collagen production, and differentiation into myofibroblasts (Table 4).
- Different fragment sizes of ECM molecules, such as HA and tenascin-C, can induce opposing effects on dermal fibroblasts.
- Manipulation of certain ECM components may reduce fibrosis in healing wounds.
- Glycosylated matrix proteins are major factors in compromised healing of diabetic wounds, due to both weakened vasculogenesis and impaired fibroblast function caused by AGEs.

authors listed. No ghostwriters were used to write this article.

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### Abbreviations and Acronyms

- $\alpha$ SMA = alpha smooth muscle actin  
 AGE = advanced glycosylation end product  
 CS = chondroitin sulfate  
 CCN2 = Cyr61-Ctgf-Nov-2  
 DM = diabetes mellitus  
 DS = dermatan sulfate  
 EBP = elastin binding protein  
 ECM = extracellular matrix  
 FACIT  
 collagens = fibril-associated collagens with interrupted triple helices  
 FGF-2 = fibroblast growth factor-2  
 GAG = glycosaminoglycan  
 HA = hyaluronan  
 LOX = lysyl oxidase  
 MMP = matrix metalloproteinase  
 RAGE = receptor for advanced glycation endproducts  
 RDEB = recessive dystrophic epidermolysis bullosa  
 RHAMM = receptor for hyaluronan-mediated motility  
 SPARC = secreted protein, acidic and rich in cysteine  
 SLRP = small leucine-rich proteoglycan  
 TGF- $\beta$  = transforming growth factor beta