A Primer of Collagen Biology: Synthesis, Degradation, Subtypes, and Role in Dupuytren's Disease

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Contents

17.1 Introduction

Historically, investigations into the pathogenesis of Dupuytren's disease have focused on the roles of genetic and epigenetic factors (such as growth factors, trauma, concurrent medications and/or disease states) and their influence on the cellular components of the subcutaneous tissues (fibroblasts and myofibroblasts) (reviewed in Cordova et al. 2005; Al-Qattan 2006; Rayan 2007) The collagen matrix (the primary constituent of the cords found in late stage Dupuytren's contracture) has been considered merely a biomarker of the cellular processes, with little importance as a therapeutic target (except as an inert structure whose disruption or removal will result in symptomatic relief to the patient).

However, more recent investigations into the genomics and proteomics of cells and tissues isolated from surgically excised Dupuytren's tissue have revealed significant overlap with the patterns of gene and protein expression in normal wound healing. These findings suggest that the abnormalities in Dupuytren's disease may represent an aberrant or exaggerated wound healing response in affected patients, a process which is largely mediated through the interaction of the fibroblast with the extracellular matrix, specifically via attachments to collagen fibers. The genomic and proteomic changes detected at the cellular level, coupled with structural differences in collagen components of Dupuytren's tissue compared to those in normal palmar fascia, indicate an additional contribution to the pathogenesis of this disease from an upset in the normal balance of extracellular matrix remodeling.

Thus, the collagen matrix continues to represent a key therapeutic target in Dupuytren's disease, both as

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Table 17.1 Functional/structural classification and tissue distribution of collagen subtypes

Classification	Types	Tissue distribution	General functions
Fibril forming	I, II, III, V, XI, XXIV, XXVII	Skin, bone, tendon, blood vessels, cornea (I, III)	Primary structural components of extracellular matrix (ECM)
		Reticular fibers (III)	
		Associated with Type I (V) or Type II (XI)	
		Cartilage, intervertebral disk, vitreous (II)	
		Developing/embryonic tissues (XXIV, XXVII)	
Network	IV, VIII, X	Widespread, all basement membranes (IV)	Adhesion
		Descement's membrane, endothelial cells (VIII)	Occlusion
		Hypertrophic cartilage (X)	Source of matricryptins (IV, VIII)[b]
Anchoring fibrils	VII	Dermal–epidermal junctions (skin, oral mucosa, genitourinary tract)	Anchoring
FACIT	IX, XII, XIV, XIX, XX, XXI, XXII	Widespread, esp. corneal epithelium (XX)	Integration, stabilization, and structural regulation of ECM
		Associated w. Type I (XII, XIV) or Type II (IX)	Signal transduction
		Skeletal muscle (XIX)	
		Cornea and vitreous (IX)	
		Blood vessel wall (XXI)	
Transmembrane (MACIT)	XIII, XVII, XXIII, XXV	Hemidesmosomes (XVII)	Cell–cell/cell–matrix adhesion
		Neuromuscular junction (XIII)	Regulation of embryonic development
Beaded filament (microfibrillar)	VI , XXVIII	Widespread in connective tissues, especially adipose and synovial membranes (VI)	Integration
		Peripheral nerves (XXVIII)	Myelination (XXVIII)
		Associated with Type VI in blood vessels (VI)	
Endostatin producing (multiplexins[a])	XV, XVI, XVIII	Associated with basement membranes (widespread), but especially blood vessels, skeletal muscle (XV), and eye (XVIII)	Antiangiogenic
			Source of matricryptins[b]
Unknown	XXVI	Testis and ovary (during development), elastic fibers	Unknown

Information from the following sources: van der rest and Garrone (1991); Prockop and Kivirikko (1995); Kuo et al. (1997); Zeichen et al. (1999); Ortega and Werb (2002); Gelse et al. (2003); Ricard-Blum and Ruggerio (2005); Khoshnoodi et al. (2006); Veit et al. (2006); Shoulders and Raines (2009)

FACIT *F*ibril *A*ssociated *C*ollagens with *I*nterrupted *T*riple Helices, MACIT *M*embrane *A*ssociated *C*ollagens with *I*nterrupted *T*riple Helices

[a]Multiplexin indicates collagen with multiple triple-helix domains and interruptions

[b]Matricryptin indicates C-terminal fragment with growth factor or other pharmacologic effect derived by proteolytic cleavage from the parent collagen molecule

a structural and as a functional component. An understanding of its structure, biology, regulation, and potential roles in Dupuytren's disease are important to development of novel approaches in modulating the disease via this target.

17.2 Collagens: Structurally and Functionally Diverse Proteins with Common Features

Collagens are an extremely structurally and functionally diverse group of proteins that are defined as such based on the following common features:

- Somewhere within the molecule, one or more triple-helical motifs consisting of three polypeptide chains (McAlinden et al. 2003; Khoshnoodi et al. 2006)
- The presence of multiple Gly-X-Y repeats (with X and Y usually being proline or hydroxyproline), which permit the formation of the helical structures (van der Rest and Garrone 1991; Ricard-Blum and Ruggerio 2005)
- A high content (up to 50% of the proline residues) of hydroxyproline, required for stabilization of the triple helix (Gelse et al. 2003; Shoulders and Raines 2009)

To date, 28 different types of collagen (the products of 34 distinct genes) have been identified; these have been

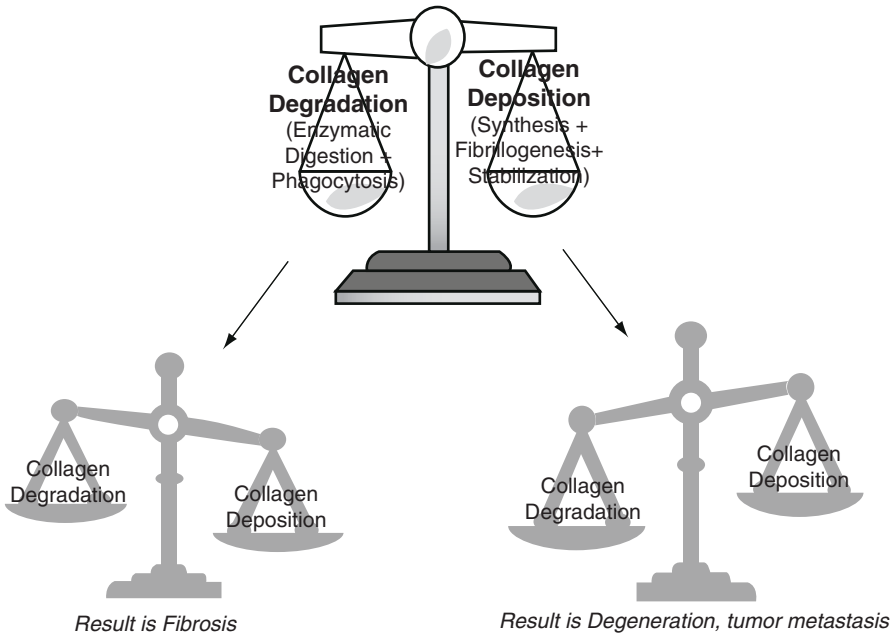

Fig. 17.1 Remodeling of the extracellular matrix. Remodeling of the extracellular matrix requires both removal and deposition of collagen. The processes are normally balanced so that there is no net increase or decrease in the amount of matrix present. The balance is altered in pathologic conditions. Increased deposition or decreased degradation results in fibrosis; the opposite scenario results in tissue degeneration and facilitation of tumor metastasis (Mansell and Bailey 2004)

classified, based on their structural and/or functional similarity, into eight different classes (reviewed in van der Rest and Garrone 1991; Prockop and Kivirikko 1995; Gelse et al. 2003; Ricard-Blum and Ruggerio 2005; Khoshnoodi et al. 2006). An overview of collagen classification and tissue distribution is presented in Table 17.1.

The fibrillar collagens are the most abundant class of collagens and are the best characterized; all of them consist almost exclusively of triple-helical collagen composed of Gly-X-Y repeats which is deposited in the extracellular space in the form of overlapping fibrils. Type I is the primary structural component of the extracellular matrix in most tissues. In fibrils, it is usually found as a complex protein in combination with Type V collagen, which serves as a nucleus for fibril formation. Type III collagen fibrils are also referred to as reticulin; this collagen type is found in healing wounds, embryonic connective tissues, and as a primary structural component in tissues such as skin, lung, and blood vessels where greater elasticity is needed (reviewed in Prockop and Kivirikko 1995; Ricard-Blum and Ruggerio 2005; Kadler et al. 2008; Shoulders and Raines 2009). An increase in the content of Type III relative to Type I collagen compared to the normal palmar fascia is considered a hallmark feature of Dupuytren's disease tissue, regardless of the stage of the disease (Hanyu et al. 1984; Melling et al. 1999, 2000).

Other collagens can be found within Dupuytren's tissue or associated with adjacent structures, although they have not been implicated in the pathogenesis of this condition. These include Type IV (a primary component of epidermal and vascular basement membranes as well as perineural structures), Type VI (an important component of synovial membranes and blood vessel basement membranes), and Type VII (plays a role in anchoring the epidermis to the dermis).

17.3 Collagen Remodeling: Process and Controls

The components of the extracellular matrix are in a constant state of flux, with the changes in the composition and quantity of the matrix collagen dictated by environmental stimuli and stage of development. In order for new collagen to be deposited, the existing collagen must be removed; these two processes occur simultaneously in a balanced fashion in normal tissues so that there is no net change in either the quantity or composition of the extracellular matrix present (Fig. 17.1). Disruption of the dynamic balance between these two processes can occur in response to physiologic need but also occurs in a number of pathologic conditions, including Dupuytren's disease (Mansell and Bailey 2004; Stetler-Stevenson 1996).

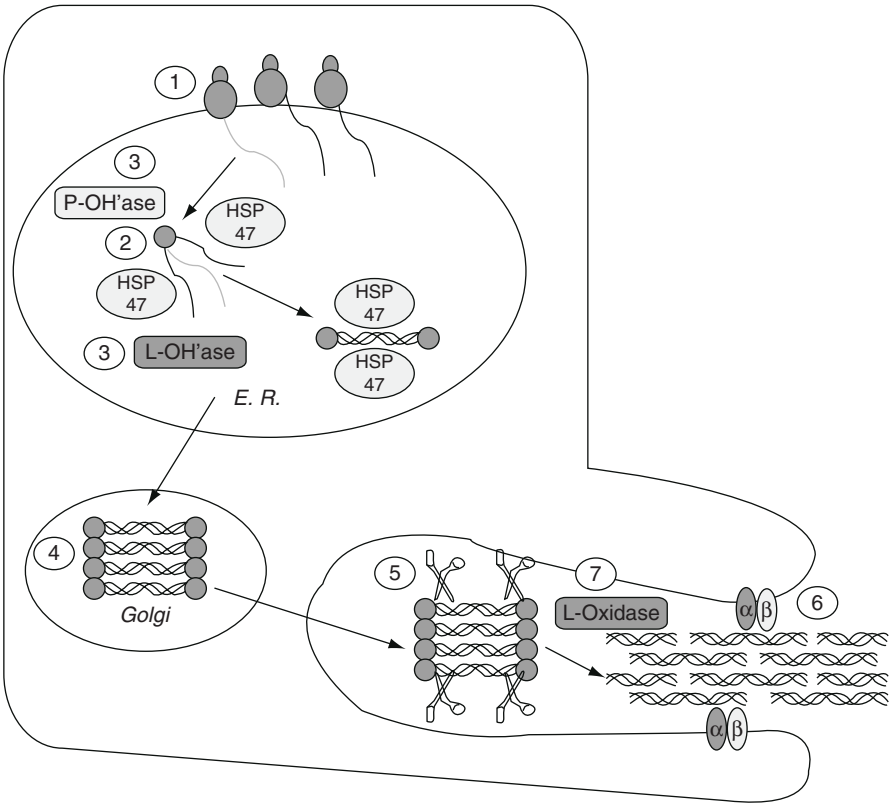

Fig. 17.2 Key control points in collagen synthesis. The rate of collagen deposition is determined by the sum of the following processes: (*1*) the rate of COL gene expression and translocation of collagen monomers into the endoplasmic reticulum (ER), (*2*) aggregation of monomers into trimers via their C propeptides, which initiates triple-helix formation. Heat shock protein 47 (Hsp47) is required to prevent "tangling" of the monomers and to slow helix formation to allow for (*3*) hydroxylation of proline and lysine residues by prolyl-hydroxylase (P-OH'ase) and lysyl hydroxylase (L-OH'ase) and formation of intramolecular cross-links by these hydroxy amino acids, stabilizing the triple helix, (*4*) translocation into the Golgi apparatus and aggregation into fibril "stacks" (speed of translocation depends on speed and effectiveness of triple-helix formation), (*5*) deposition of stacks into the extracellular space via the formation of "fibripositors" and cleavage of the N- and C-terminal propeptides by N- and C-terminal propeptidases (represented by scissors), (*6*) overlapping aggregation of collagen tripeptides into fibrils, enhanced by the presence of β_1 integrins ($\alpha\beta$) on the cell surface, and (*7*) stabilization of the collagen fibrils by intermolecular cross-links through the action of lysyl oxidase (L-oxidase) (Figure adapted from material in Kadler et al. 1996; Persikov and Brodsky 2002; Canty and Kadler 2005; Khoshnoodi et al. 2006; Kadler et al. 2008)

Of the two processes involved in collagen remodeling, the process of collagen deposition is more complex and thus can be regulated at multiple levels. In contrast, collagen degradation is primarily regulated by controlling the quantity and activity of collagen digesting enzymes.

17.3.1 Collagen Deposition

The transformation of labile newly synthesized collagen polypeptides into the stable collagen fibrils present within the extracellular matrix is a slow and complex process. The rate and effectiveness of collagen deposition (the net result of synthesis and fibrillogenesis) is thus governed at many levels (as depicted in Fig. 17.2). These are described specifically as they relate to the fibrillar collagens in the following sections.

As with all proteins, the rate of collagen deposition can be regulated by up- or downregulation of the *COL* genes responsible for the synthesis of the collagen α chains. These are synthesized directly into the endoplasmic reticulum to undergo the posttranslational modifications needed for both the formation and stabilization of triple-helical procollagen. Assembly of collagen trimers from the monomeric α chains occurs due to the interaction of the C-NC domains located near the C termini of these molecules (Khoshnoodi et al. 2006).

Once trimerization is complete, triple-helix formation commences in a C to N direction; formation and stabilization of the helix requires conversion of proline residues located in the –Y position of the Gly-X-Y collagen motif to hydroxyproline through the action of prolyl-4-hydroxylase and its essential cofactor, ascorbic acid (vitamin C). Further stabilization of the triple helix results from the action of lysyl hydroxylase which generates hydroxylysine from lysine residues within the collagen molecule; the hydroxylysine residues provide sites for the addition of glucose and galactose molecules to the procollagen molecules and are also critical to extracellular cross-linking of collagen fibrils. These modifications serve to increase the thermal stability of the collagen molecules (Prockop and Kivirikko 1995; Persikov and Brodsky 2002; Canty and Kadler 2005).

Additional stabilization of the forming procollagen triple helices results from their association with the molecular chaperone heat shock protein 47 (Hsp47). This protein becomes associated with the newly synthesized collagen α chains, facilitating trimerization; it also is critical to the correct formation of the procollagen triple helix by preventing premature and incorrect "folding" of the procollagen monomers as well as preventing their thermal denaturation until the necessary posttranslational modifications of proline and lysine are complete and triple-helix formation can take place. Additionally, Hsp47 assists with the translocation of the completed procollagen trimers into the Golgi apparatus for transportation to the extracellular space (Prockop and Kivirikko 1995; Canty and Kadler 2005).

Within the Golgi apparatus, the procollagen trimers are aligned into stacks and are transported to the cell membrane of the fibroblasts in specialized elongated vacuoles; these fuse with the plasma membrane surface to form specialized structures known as "fibripositors" in which the lumen of the vacuole is contiguous with the extracellular space but is still somewhat protected from the extracellular environment (Canty et al. 2004). The process known as "fibrillogenesis" is initiated; this requires cleavage of the C- and N-terminal propeptides by the appropriate peptidases, assembly of the cleaved collagen triple helices into fibrils, and stabilization of the forming fibrils by cross-linking the individual triple-helical collagen monomers through their hydroxylysine residues by the activity of extracellular lysyl oxidase. The confinement of these processes within the fibripositor not only protects the labile collagen monomers from degradation until stable fibrils can be formed but also directs the placement of the forming collagen fibrils where they are needed, along the lines of force (Gelse et al. 2003; Canty and Kadler 2005; Canty et al. 2006).

Once the propeptides are cleaved, collagen monomers can self-assemble into fibrils at neutral pH, but the speed and effectiveness of fibrillogenesis as well as the thermal and enzymatic stability of the resulting fibrils are enhanced when this process is assisted (Prockop and Kivirikko 1995). Cell surface β_1 integrins (primarily α_2 and α_5) can bind to collagen fibrils and assist in fibrillogenesis by keeping the monomers in close proximity with each other (facilitating cross-linking) as well as with Type V collagen fibrils (which serve as a nucleus for the formation of mature Type I collagen fibrils) and cell surface bound fibronectin (which assists in fibril assembly and protects newly formed fibrils from degradation) (Wenstrup et al. 2004; Kadler et al. 2008). Fibrillogenesis is also potentiated by the action of the matricellular protein periostin, which also plays a role in determining the ultimate diameter and orientation of newly laid collagen fibrils (Norris et al. 2007).

Additional stabilization of the collagen fibrils occurs once fibrillogenesis is complete. Covalent modification of the maturing fibrils (lysyl oxidase mediated cross-linking, along with enzymatic and nonenzymatic glycation), association with matrix proteins and small leucine-rich proteoglycans (such as periostin, decorin, and the FACIT collagens), increased fibril length and/or diameter, and increased mechanical tension all serve to increase the resistance of the collagen fibrils to enzymatic and thermal degradation (Canty and Kadler 2005; Minond et al. 2006; Norris et al. 2007; Hamilton 2008; Perumal et al. 2008; Flynn et al. 2010; Kalamajski and Oldberg 2010).

17.3.2 Collagen Degradation

17.3.2.1 Collagenases

The collagen triple helix, once stabilized by intra- and intermolecular cross-linking, is remarkably resistant to degradation, as the labile peptide bonds are buried within the interior of the collagen molecule. For an enzyme to effectively initiate degradation of an intact triple-helical collagen molecule, it must possess the ability to identify and bind to "weaker" sites on the molecule, locally unwind the triple helix, and successfully cleave one or more strands of the triple helix. A collagenase is defined as an enzyme which can effectively perform all three of these activities (Lauer-Fields et al. 2002).

Table 17.2 Mammalian collagenases and their substrates

Enzyme designation	Common name(s)	Substrate(s)	Function(s)
MMP-1	Collagenase 1	Fibrillar collagens (Type III preferred)	Extracellular cleavage (physiologic and pathologic)
	Interstitial collagenase	Collagens VI, VII, X, IX	Wound healing
	Fibroblast collagenase	Gelatin	
	Synovial collagenase		
MMP-2	Gelatinase A	Gelatin (preferred)	Pericellular collagenolysis (phagocytosis)
		Fibrillar collagens	Inflammation
		Collagens IV, V, VII, X, XI	Activates proMMPs (8, 9, 13)
MMP-3	Stromelysin 1	Gelatin (preferred)	Activates most proMMPs
		Collagens III, IV, V, VII, IX, X, XI	
MMP-7	Matrilysin 1	Gelatin (preferred)	Activates many proMMPs
		Collagens I and V	
MMP-8	Collagenase 2	Fibrillar collagens (Type I preferred)	Inflammation
	Neutrophil collagenase		Wound healing
MMP-9	Gelatinase B	Gelatin	Inflammation
		Collagen I(?)	Pathologic remodeling
		Collagens IV, V, VII, X, XI	
MMP-10	Stromelysin 2	Gelatin (preferred)	Activates many proMMPs
		Collagens I, III, IV, V, IX, X	Wound healing
MMP-12	Metalloelastase	Gelatin	Basement membrane remodeling
		Collagens I, IV	
MMP-13	Collagenase 3	Gelatin	Cartilage and bone remodeling
		Fibrillar collagens (Type II preferred)	Wound healing
		Collagens VI, IX, X, XIV	
MMP-14	MT1-MMP[a]	Fibrillar collagens	Activates proMMPs (2, 8, 13)
		Gelatin	Pericellular collagenolysis (phagocytosis)
			Fibroblast migration
MMP-15	MT2-MMP[a]	Gelatins	Activates proMMP-2
		Collagen III	
MMP-16	MT3-MMP[a]	Fibrillar collagens	Activates proMMP-2

Information from the following sources: Everts et al. (1996); Stetler-Stevenson (1996); Sternlicht and Werb (2001); Lauer-Fields et al. (2002); Visse and Nagase (2003); Gutiérrez-Fernández et al. (2007); Barbolina and Stack (2008); Sabeh et al. (2009); Minond et al. (2006); Nagase et al. (2006); Pasternak and Aspenberg (2009)

[a]MT-MMP indicates membrane-associated (transmembrane) MMP. These are not secreted but remain associated with the cell membrane and are activated intracellularly by furin-mediated cleavage

Mammalian collagenases fall into two enzyme classes: the zinc-dependent metalloproteinases (represented by a subset of the 23 known mammalian matrix metalloproteinases, or MMPs) and the cysteine proteases (represented by cathepsins K and L). Of the two classes, the MMPs are the primary enzymes responsible for both physiologic and pathologic remodeling of extracellular matrix collagen (Lauer-Fields et al. 2002; Pasternak and Aspenberg 2009); cathepsins primarily function as lysosomal proteases but do play a role in extracellular collagenolysis in bone and cartilage remodeling (Turk et al. 2001; Daley et al. 2007). The MMPs that can act as collagenases and/or have been shown to have a role in collagen remodeling are listed in Table 17.2.

Cleavage of fibrillar collagens by MMPs occurs in the same fashion, regardless of the enzyme involved, as all of them recognize a single site on the collagen molecule (Gly_{775}-Ile/Leu_{776} on the alpha chain). Cleavage at this site results in the generation of two fragments of approximately one-fourth and three-fourths the length of the original fibril (Lauer-Fields et al. 2002; Nagase et al. 2006). The resultant cleavage fragments are known as gelatin and are rapidly cleared from the site of their formation, either by additional proteolysis (in addition to the gelatinolytic MMPs, a number of nonspecific

proteases can digest gelatin), by denaturation (gelatin is thermically unstable at physiologic temperatures), and by phagocytosis, a process that is mediated in part by the β_1 integrin-mediated interaction of collagen fibrils with the fibroblast cell surface (Evanson et al. 1968; Fesus et al. 1981; Everts et al. 1996; Arora et al. 2000; Leikina et al. 2002; Stultz 2002).

17.3.2.2 Control of Collagen Degradation

Control of collagen degradation depends on tight regulation of the activity of the MMPs as well as temporal and spatial limitation of these enzymes. There are four levels at which these enzymes are regulated:

Transcription

Only a few of the MMPs (notably MMP-2 and MMP-14) are constitutively expressed, and then only at low levels and in a few tissues. As a general rule, up- and downregulation of the genes responsible for MMP synthesis is required for these enzymes; additional regulation occurs via stabilization of the transcripts (Sternlicht and Werb 2001; Ra and Parks 2007).

Activation of Inactive Enzyme

With the exception of the matrix metalloproteinases (MT-MMPs) which are activated intracellularly, most MMPs are synthesized and secreted as inactive zymogens as the result of a direct internal interaction between a cysteine on the polypeptide and the zinc bound to the catalytic site of the molecule. Disruption of this interaction (resulting in the generation of catalytically active enzyme) requires proteolytic cleavage of the proMMP. While this most commonly results from interaction with another MMP, nonspecific proteases (such as plasmin) can activate the membrane-bound MMPs; additionally, chemical disruption of the cysteine-Zn^{+2} interaction by strong oxidizing conditions, metal ions, or disulfides can also lead to MMP activation and autocatalysis of the prodomain (Somerville et al. 2003; Visse and Nagase 2003; Ra and Parks 2007).

Compartmentalization

Inactive forms of MMPs 8, 9, and 12 are retained within the secretory granules of inflammatory cells such as neutrophils and macrophages until released upon stimulation of the cell (Sternlicht and Werb 2001). MMPs that are freely secreted into the extracellular space are generally held close to the surface of the secreting fibroblast via interactions with cell surface proteins such as integrins or membrane-bound MMPs; these interactions are critical to the activity and substrate specificity of each MMP (Ra and Parks 2007).

Inactivation of Active Enzymes

Temporal and spatial regulation of MMP activity results primarily from their inhibition due to interaction with one of the four small glycoproteins known as *t*issue *i*nhibitors of *m*etallo*p*roteinases (TIMPs). Like MMPs, these are not constitutive proteins but are regulated in response to physiologic need (the exception is TIMP-3, which is present in an inactive complex with the carbohydrate moieties of the interstitial matrix and is released in active form during matrix degradation), with the relative balance between the levels of MMP and TIMP determining the net activity of matrix degradation. All MMPs are subject to inactivation by all of the TIMPs, with a few exceptions; TIMP-1 is not effective at inactivating the MT-MMPs, and TIMP-2 actually enhances the activation of proMMP-2 by MMP-14 (MT1-MMP) by facilitating the interaction between these two proteins (Baker et al. 2002; Visse and Nagase 2003). Any MMP that escapes local inactivation by TIMPS and/or enters the systemic circulation is rapidly inactivated by interaction with the nonspecific protease inhibitor α_2-macroglobulin which is ubiquitously present in the serum and interstitial fluid, and to a lesser extent by the cell-surface-associated protein *re*versin-inducing *c*ysteine-rich protein (RECK), which is widely distributed in many tissues (Cawston and Mercer 1986; Baker et al. 2002).

17.4 Roles of Collagen in Dupuytren's Disease

17.4.1 Structural Role of Collagen (Imbalance in Collagen Remodeling)

The hallmark feature of Dupuytren's contracture is the localized deposition of excess and abnormal collagen within the palmar fascia, which suggests that the normal balance of collagen remodeling has been locally perturbed in these patients. A number of studies evaluating genomic and proteomic differences in the diseased palmar fascia (cords, nodules, and/or fibroblasts) from Dupuytren's patients compared to either unaffected fascial components or palmar fascia from unaffected

individuals clearly indicate the presence of a shift in this balance in favor of net collagen deposition. These studies are summarized in Table 17.3.

In addition, a number of structural and biochemical abnormalities have been identified in the collagen comprising the Dupuytren's cords that indicate an increase in the stability of the matrix to enzymatic and/or thermal degradation, which would further tip the balance by decreasing collagen degradation even when collagen degrading enzymes are present in abundance. Increased thermal and enzymatic stability of collagen fibrils resulting from increased cross-linking of collagen due to increased hydroxylysine and/or hydroxyproline and the presence of abnormal collagen cross-links (Brickley-Parsons et al. 1981; Notbohm et al. 1995) or resulting from increased or altered collagen glycation (Brickley-Parsons et al. 1981; Hanyu et al. 1984; Melling et al. 1999) has been described in Dupuytren's disease tissue. The collagen fibrils are also larger in diameter in Dupuytren's disease tissue and are more closely packed in larger fascicles with thicker fascicular sheaths (Mansell and Bailey 2004), which prevents ready access to the labile portions of the individual collagen monomers by collagenases (Perumal et al. 2008). These structural changes in Dupuytren's collagen also result in increased stiffness (decreased elasticity) in the fibrils relative to normal tissue, which renders them more effective in their role as signal transducers to fibroblasts (detailed in the following section) (Melling et al. 2000; Shoulders and Raines 2009).

17.4.2 Functional Role of Collagen (Mechanotransduction and Fibroblast Responses to Force)

More recent studies investigating the pathophysiology of Dupuytren's disease have revealed the significant degree of overlap in its morphology and biology with several phases of wound healing. Features common to the two processes include the transition of fibroblasts to myofibroblasts, generation of tension by myofibroblasts, increased deposition of Type III collagen and fibronectin, the key role of TGF-β, and upregulation of collagen remodeling resulting in shortening of the existing collagen fibrils, which causes contraction (Brickley-Parsons et al. 1981; Tomasek et al. 2002; Rayan 2007). The key difference between these two processes is that the tissue responses in Dupuytren's disease are more exaggerated and/or prolonged than those seen in normal healing wounds. Thus, Dupuytren's disease may be regarded as a manifestation of aberrant wound healing responses.

Fibroblasts are in constant contact with their external environment and monitor the degree of static and dynamic force present through cell surface β_1 integrins (α_2, α_5 and/or α_{11}) and the Type I collagen fibrils present in the extracellular matrix. Any increase in the degree of tension present in the matrix is transmitted to the intracellular cytoskeleton; this transmission of force results in alteration of gene expression which occurs either indirectly via the release and nuclear translocation of preformed transcription factors, or directly, resulting from mechanical deformation of the nuclear DNA due to its physical association with the cytoskeletal elements that cross the nuclear membrane (Fig. 17.3) (Chiquet et al. 2003, 2007; Wang et al. 2007, 2009). The alteration of mechanical force on resting fibroblasts is the primary stimulus for the initiation of wound healing and also for physiologic remodeling to ensure that the quantity and quality of the extracellular matrix is adequate to the load.

It has been shown that the morphologic changes common to both wound healing and the early stages of Dupuytren's disease can be induced in cultured fibroblasts (normal or derived from Dupuytren's disease tissue) or granulation tissue explants by subjecting them to mechanical tension; these include proliferation, transition to myofibroblasts, secretion of TGF-β and other cytokines, generation of contractile force in myofibroblasts, increased fibroblast motility, and increased thickness and stiffness of collagen fibrils (Alman et al. 1996, Hinz et al. 2001; Tomasek et al. 2002; Balestrini and Billiar 2009; Roeder et al. 2009). In addition, many of the same genomic and proteomic differences in the diseased palmar fascia (cords, nodules, and/or fibroblasts) from Dupuytren's patients compared to either unaffected fascial components or palmar fascia from unaffected individuals can be induced in cultured fibroblasts by subjecting them to mechanical force or have been identified as being differentially expressed in tissues under stress (Table 17.3). Conversely, decreased mechanical tension on myofibroblasts results in their disappearance via apoptosis, seen in the resolution phase of wound healing and also in the late stages of Dupuytren's disease (Fluck et al. 1998; Grinnell et al. 1999; Gabbiani 2003).

Table 17.3 Gene and/or protein alterations in Dupuytrens's fibroblasts related to collagen remodeling or fibroblast responses to tension

Gene or protein	Function	Effect on collagen remodeling	Direction of change in:	
			Dupuytren's disease tissue	Fibroblasts under tension
ADAMTS-2	N-terminal propeptidase	↑ Collagen deposition	↑↑	↑
ADAMTS-3	N-terminal propeptidase	↑ Collagen deposition	↑↑	↑
ADAMTS-14	N-terminal propeptidase	↑ Collagen deposition	↑↑	↑
COL1A1	Collagen I alpha1 chain	↑ Collagen deposition	↑↑	↑↑
COL1A2	Collagen I alpha2 chain	↑ Collagen deposition	↑↑	↑↑
COL3A1	Collagen III alpha1 chain	↑ Collagen deposition	↑	↑
COL5A1	Collagen V alpha1 chain	↑ Collagen deposition	↑	
COL5A2	Collagen V alpha2 chain	↑ Collagen deposition	↑	
Integrin β_1	Signal transduction from collagen; facilitates fibrillogenesis	↑ Collagen deposition	↑	↑
Fibronectin	Facilitates fibrillogenesis	↑ Collagen deposition	↑	↑
Hsp47	Intracellular collagen stabilization and triple-helix formation	↑ Collagen deposition	↑	↑
Lysyl oxidase-2	Extracellular collagen cross-linking (enhances fibrillogenesis and fibril stability/stiffness)	↑ Collagen deposition	↑↑	↑
		↓ Collagen degradation		
MMP-1	Collagenase	↑ Collagen degradation	↑	↓↓
MMP-2	Gelatinase; pericellular collagenase	↑ Collagen degradation ("local" remodeling)	↑↑	↑
MMP-3	Activates proMMPs	↑ Collagen degradation	↓↓	↑
MMP-7	Activates proMMPs	↑ Collagen degradation	↑	
MMP-8	Collagenase	↑ Collagen degradation	↓	
MMP-13	Collagenase	↑ Collagen degradation	↑	↑
MMP-14	Collagenase (membrane-associated); activates proMMPs	↑ Collagen degradation ("local" remodeling)	↑ (nodule)	
			↓ (cord)	
Periostin	Enhances fibrillogenesis; stabilizes extracellular collagen fibrils	↑ Collagen deposition	↑↑	↑
		↑ collagen degradation		
TIMP-1	Inhibits MMPs (except 14)	↑ Collagen degradation	↑↑↑	
TIMP-2	Allows activation of MMP-2 by MMP-14; inactivates MMPs	↑ Collagen degradation (via MMP-2); ↓ collagen degradation	↓	↑,↓, or no change
TIMP-3	Inhibits all MMPs	↓ Collagen degradation	↓	
TIMP-4	Inhibits all MMPs	↓ Collagen degradation	↑↑↑	

Information from the following sources: Lambert et al. (1992, 2001); Wilde et al. (2003); Qian et al. (2004); Lee et al. (2006); Webb et al. (2006); Johnston et al. (2007); Merryman et al. (2007); Hamilton (2008); Rehman et al. (2008); Kaneko et al. (2009); Vi et al. (2009); Ziegler et al. (2010)
Unless specified, gene or protein changes are in the same direction in both nodules and cords from Dupuytren's disease patients

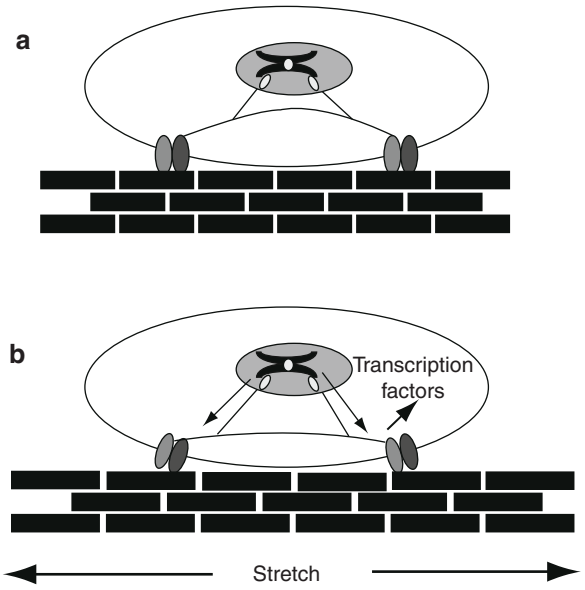

Fig. 17.3 Fibroblast and matrix responses to mechanical forces. Fibroblast responses to mechanical forces are mediated through β_1 integrin interaction with collagen fibrils in the extracellular matrix and the actin cytoskeleton. (**a**) No signaling occurs in the absence of tension on the collagen matrix or the actin cytoskeleton; note normal orientation of the integrin molecules (depicted as perpendicular to the collagen fibrils) that link the collagen matrix to the actin cytoskeleton. (**b**) Increased tension on the collagen matrix is transmitted to the actin cytoskeleton due to altered orientation of the cell surface integrins, resulting in the release of transcription factors and rapid changes in gene/protein expression. Effects may also be mediated due to direct cytoskeletal coupling of the cell membrane to the chromosomal DNA through the nuclear membrane; deformation of DNA by transferred tension results in direct alteration of gene transcription. NOTE: a decrease in the elasticity of either the collagen matrix or the actin cytoskeleton increases the intensity of the signal (Figure adapted from material in Chiquet et al. 2003, 2007; Wang et al. 2007, 2009)

17.5 Conclusions

Collagen has been described as "an unstable molecule that can form stable tissues." This property results from the careful shepherding of the unstable precursor through a highly complex process as well as close regulation over the processes that initiate its degradation, so that the quantity and quality of this essential structural component is balanced to the physical requirements of each tissue. Either as the primary structural component of the Dupuytren's cord or as the primary functional mediator of the pathophysiologic processes that underlie this disease, it remains an important target for therapeutic intervention, and a basic understanding of its biology is critical to therapeutic success.

References

Alman BA, Greel DA, Ruby LK, Goldberg MJ, Wolfe HJ (1996) Regulation of proliferation and platelet-derived growth factor expression in palmar fibromatosis (Dupuytren contracture) by mechanical strain. J Orthop Res 14:722–728

Al-Qattan MM (2006) Factors in the pathogenesis of Dupuytren's contracture. J Hand Surg 31A:1527–1534

Arora PD, Manolson MF, Downey GP, Sodek J, McCulloch CAG (2000) A novel model system for characterization of phagosomal maturation, acidification, and intracellular collagen degradation in fibroblasts. J Biol Chem 275:35432–35441

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

Balestrini JL, Billiar KL (2009) Magnitude and duration of stretch modulate fibroblast remodeling. J Biomech Eng 131:051005-1–051005-8

Barbolina MV, Stack MS (2008) Membrane type 1-matrix metalloproteinase: substrate diversity in pericellular proteolysis. Semin Cell Dev Biol 19:24–33

Brickley-Parsons D, Glimcher MJ, Smith RJ, Albin R, Adams JP (1981) Biochemical changes in the collagen of the palmar fascia in patients with Dupuytren's disease. J Bone Joint Surg Am 63:787–797

Canty EG, Kadler KE (2005) Procollagen trafficking, processing and fibrillogenesis. J Cell Sci 118:1341–1353

Canty EG, Lu Y, Meadows RS, Shaw MK, Holmes DF, Kadler KE (2004) Coalignment of plasma membrane channels and protrusions (fibripositors) specifies the parallelism of tendon. J Cell Biol 165:553–563

Canty EG, Starborg T, Lu Y, Humphries SM, Holmes DF, Meadows RS, Huffman A, O'Toole ET, Kadler KE (2006) Actin filaments are required for fibripositor-mediated collagen fibril alignment in tendon. J Biol Chem 281:38592–38598

Cawston TE, Mercer E (1986) Preferential binding of collagenase to α2-macroglobulin in the presence of the tissue inhibitor of metalloproteinases. FEBS Lett 209:9–12

Chiquet M, Renedo AS, Huber F, Flück M (2003) How do fibroblasts translate mechanical signals into changes in extracellular matrix production? Matrix Biol 22:73–80

Chiquet M, Tunç-Civelek V, Sarasa-Renedo A (2007) Gene regulation by mechanotransduction in fibroblasts. Appl Physiol Nutr Metab 32:967–973

Cordova A, Tripoli M, Corradino B, Napoli P, Moschella F (2005) Dupuytren's contracture: an update of biomolecular aspects and therapeutic perspectives. J Hand Surg 30B:557–562

Daley WP, Peters SB, Larsen M (2007) Extracellular matrix dynamics in development and regenerative medicine. J Cell Sci 121:255–264

Evanson J, Jeffrey JJ, Krane SM (1968) Studies on collagenase from rheumatoid synovium in tissue culture. J Clin Invest 47:2639–2651

Everts V, van der Zee E, Creemers L, Beertsen W (1996) Phagocytosis and intracellular digestion of collagen, its role in turnover and remodeling. Histochem J 28:229–245

Fesus L, Jeleliska MM, Kope M (1981) Degradation by thrombin of denatured collagen and of collagenase digestion products. Thromb Res 22:367–373

Fluck J, Querfeld C, Cremer A, Niland S, Krieg T, Sollberg S (1998) Normal human primary fibroblasts undergo apoptosis in three-dimensional contractile collagen gels. J Invest Dermatol 110:153–157

Flynn BP, Bhole AP, Saeidi N, Liles M, DiMarzio CA et al (2010) Mechanical strain stabilizes reconstituted collagen fibrils against enzymatic degradation by mammalian collagenase matrix metalloproteinase 8 (MMP-8). PLoS One 5:e12337. doi:10.1371/journal.pone.0012337

Gabbiani G (2003) The myofibroblast in wound healing and fibrocontractive diseases. J Pathol 200:500–503

Gelse K, Pöschl E, Aigner T (2003) Collagens – structure, function, and biosynthesis. Adv Drug Deliv Rev 55:1531–1546

Grinnell F, Zhu M, Carlson MA, Abrams JM (1999) Release of mechanical tension triggers apoptosis of human fibroblasts in a model of regressing granulation tissue. Exp Cell Res 248:608–619

Gutiérrez-Fernández A, Inada M, Balbín M, Fueyo A, Pitiot AS, Astudillo A, Hirose K, Hirata M, Shapiro SD, Noël A, Werb Z, Krane SM, López-Otín C, Puente XS (2007) Increased inflammation delays wound healing in mice deficient in collagenase-2 (MMP-8). FASEB J 21:2580–2591

Hamilton DW (2008) Functional role of periostin in development and wound repair: implications for connective tissue disease. J Cell Commun Signal 2:9–17

Hanyu T, Tajima T, Tagaki T, Sasaki S, Fujimoto D, Isemora M, Yosizawa Z (1984) Biochemical studies on the collagen of the palmar aponeurosis affected with Dupuytren's disease. Tohoku J Exp Med 142:437–443

Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G (2001) Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am J Pathol 159:1009–1020

Johnston P, Chojnowski AJ, Davidson RK, Riley GP, Donell ST, Clark IM (2007) A complete expression profile of matrix-degrading metalloproteinases in Dupuytren's disease. J Hand Surg 32A:343–351

Kadler KE, Holmes DF, Trotter JA, Chapman JA (1996). Collagen fibril formation. Biochem J. 316(Pt 1):1–11

Kadler KE, Hill A, Canty-Laird EG (2008). Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. Curr Opin Cell Biol 20(5):495–501

Kalamajski S, Oldberg Å (2010) The role of small leucine-rich proteoglycans in collagen fibrillogenesis. Matrix Biol 29: 248–253

Kaneko D, Sasazaki Y, Kikuchi T, Ono T, Nemoto K, Matsumoto H, Toyama Y (2009) Temporal effects of cyclic stretching on distribution and gene expression of integrin and cytoskeleton by ligament fibroblasts in vitro. Connect Tissue Res 50: 263–269

Khoshnoodi J, Cartailler J-P, Alvares K, Veis A, Hudson BG (2006) Molecular recognition in the assembly of collagens: terminal noncollagenous domains are key recognition modules in the formation of triple helical protomers. J Biol Chem 281:38117–38121

Kuo H-J, Maslen CL, Keene DR, Glanville RW (1997) Type VI collagen anchors endothelial basement membranes by interacting with Type IV collagen. J Biol Chem 272:26522–26529

Lambert CA, Soudant EP, Nusgens BV, Lapière CM (1992) Pretranslational regulation of extracellular matrix macromolecules and collagenase expression in fibroblasts by mechanical forces. Lab Invest 66:444–451

Lambert CA, Colige AC, Lapière CM, Nusgens BV (2001) Coordinated regulation of procollagens I and III and their post-translational enzymes by dissipation of mechanical tension in human dermal fibroblasts. Eur J Cell Biol 80:479–485

Lauer-Fields JL, Juska D, Fields GB (2002) Matrix metalloproteinases and collagen catabolism. Biopolymers (Pept Sci) 66:19–32

Lee LC, Zhang AY, Chong AK, Pham H, Longaker MT, Chang J (2006) Expression of a novel gene, MafB, in Dupuytren's disease. J Hand Surg 31A:211–218

Leikina E, Mertts MV, Kuznetsova N, Leikin S (2002) Type I collagen is thermally unstable at body temperature. Proc Natl Acad Sci 99:1314–1318

Mansell JP, Bailey AJ (2004) Collagen metabolism disorders. Encyclopedia Endo Dis 1:530–537

McAlinden A, Smith TA, Sandell LJ, Ficheux D, Parry DAD, Hulmes DJS (2003) Alpha-helical coiled-coil oligomerization domains are almost ubiquitous in the collagen superfamily. J Biol Chem 278:42200–42207

Melling M, Reihsner R, Pfeiler W, Schnallinger M, Karimian-Teherani D, Behnam M, Mostler S, Menzel EJ (1999) Comparison of palmar aponeuroses from individuals with diabetes mellitus and Dupuytren's contracture. Anat Rec 255:401–406

Melling M, Karimian-Teherani D, Mostler S, Behnam M, Sobal G, Menzel EJ (2000) Changes of biochemical and biomechanical properties in Dupuytren disease. Arch Pathol Lab Med 124:1275–1281

Merryman WD, Lukoff HD, Long RA, Engelmayr GC Jr, Hopkins RA, Sacks MS (2007) Synergistic effects of cyclic tension and transforming growth factor-beta1 on the aortic valve myofibroblast. Cardiovasc Pathol 16:268–276

Minond D, Lauer-Fields JL, Cudic M, Overall CM, Pei D, Brew K, Visse R, Nagase H, Fields GB (2006) The roles of substrate thermal stability and P_2 and P_1' subsite identity on matrix metalloproteinase triple-helical peptidase activity and collagen specificity. J Biol Chem 281:38302–38313

Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 69:562–573

Norris RA, Damon B, Mironov V, Kasyanov V, Ramamurthi A, Moreno-Rodriguez R, Trusk T, Potts JD, Goodwin RL, Davis J, Hoffman S, Wen X, Sugi Y, Kern CB, Mjaatvedt CH, Turner DK, Oka T, Conway ST, Molkentin JD, Forgacs G, Markwald RR (2007) Periostin regulates collagen fibrillogenesis and the biomechanical properties of connective tissues. J Cell Biochem 101:695–711

Notbohm H, Bigi A, Roveri N, Hoch J, Acil Y, Koch HJ (1995) Ultrastructural and biochemical modifications of collagen from tissue of morbus Dupuytren patients. J Biochem 118: 405–410

Ortega N, Werb Z (2002) New functional roles for non-collagenous domains of basement membrane collagens. J Cell Sci 115:4201–4214

Pasternak B, Aspenberg P (2009) Metalloproteinases and their inhibitors – diagnostic and therapeutic opportunities in orthopedics. Acta Orthop 80:693–703

Persikov AV, Brodsky B (2002) Unstable molecules form stable tissues. Proc Natl Acad Sci 99:1101–1103
Perumal S, Antipova O, Orgel JPRO (2008) Collagen fibril architecture, domain organization, and triple-helical conformation govern its proteolysis. Proc Natl Acad Sci 105:2824–2829
Prockop DJ, Kivirikko KI (1995) Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem 64:403–434
Qian A, Meals RA, Rajfer J, Gonzalez-Cavidad NF (2004) Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. Urology 64:399–404
Ra H-J, Parks WC (2007) Control of matrix metalloproteinase catalytic activity. Matrix Biol 26:587–596
Rayan GM (2007) Dupuytren disease: anatomy, pathology, presentation, and treatment. J Bone Joint Surg Am 89:189–198
Rehman S, Salway F, Stanley JK, Ollier WER, Day P, Bayat A (2008) Molecular phenotypic descriptors of Dupuytren's disease defined using informatics analysis of the transcriptome. J Hand Surg 33A:359–372
Ricard-Blum S, Ruggerio F (2005) The collagen superfamily: from the extracellular matrix to the cell membrane. Pathol Biol 53:430–442
Roeder BA, Kokini K, Voytik-Harbin SL (2009) Fibril microstructure affects strain transmission within collagen extracellular matrices. J Biomech Eng 131:031004-1–031004-11
Sabeh F, Li X-Y, Saunders TL, Rowe RG, Weiss SJ (2009) Secreted versus membrane-anchored collagenases: relative roles in fibroblast-dependent collageanolysis and invasion. J Biol Chem 284:23001–23011
Shoulders MD, Raines RT (2009) Collagen structure and stability. Annu Rev Biochem 78:929–958
Somerville RPT, Oblander SA, Apte SS (2003) Matrix metalloproteinases: old dogs with new tricks. Genome Biol 4:216
Sternlicht MD, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 17:463–516
Stetler-Stevenson WG (1996) Dynamics of matrix turnover during pathologic remodeling of the extracellular matrix. Am J Pathol 148:1345–1350
Stultz CM (2002) Localized unfolding of collagen explains collagenase cleavage near imino-poor sites. J Mol Biol 319:997–1003
Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA (2002) Myofibroblasts and mechanoregulation of connective tissue remodeling. Nat Rev Mol Cell Biol 3:349–363
Turk V, Turk B, Turk D (2001) Lysosomal cysteine proteases: facts and opportunities. EMBO J 20:4629–4633
Van der Rest M, Garrone R (1991) Collagen family of proteins. FASEB J 5:2814–2823
Veit G, Kobbe B, Keene DR, Paulsson M, Koch M, Wagener R (2006) Collagen XXVIII, a novel von Willebrand factor A domain containing protein with many imperfections in the collagenous domain. J Biol Chem 281:3494–3504
Vi L, Fenga L, Zhu RB, Wu Y, Satish L, Gan BS, O'Gorman DB (2009) Periostin differentially induces proliferation, contraction and apoptosis of primary Dupuytren's disease and adjacent palmar fascia cells. Exp Cell Res 315:3574–3586
Visse R and Nagase H (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ. Res 92(8): 827–839.
Wang JH, Thampatty BP, Lin JS, Im HJ (2007) Mechanoregulation of gene expression in fibroblasts. Gene 391:1–15
Wang N, Tytell JD, Ingber DE (2009) Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. Nat Rev Mol Cell Biol 10:75–82
Webb K, Hitchcock RW, Smeal RM, Li W, Gray SD, Tresco PA (2006) Cyclic strain increases fibroblast proliferation, matrix accumulation, and elastic modulus of fibroblast-seeded polyurethane constructs. J Biomech 39:1136–1144
Wenstrup RJ, Florer JB, Brunskill EW, Bell SM, Chervoneva I, Birk DE (2004) Type V collagen controls the initiation of collagen fibril assembly. J Biol Chem 279:53331–53337
Wilde J, Yokozeki M, Terai K, Kudo A, Moriyama K (2003) The divergent expression of periostin mRNA in the periodontal ligament during experimental tooth movement. Cell Tissue Res 312:345–351
Zeichen J, van Griensven M, Albers I, Lobenhoffer P, Bosch U (1999) Immunohistochemical localization of collagen VI in arthrofibrosis. Arch Orthop Trauma Surg 119:315–318
Ziegler N, Alonso A, Steinberg T, Woodnutt D, Kohl A, Müssig E, Schulz S, Tomakidi P (2010) Mechano-transduction in periodontal ligament cells identifies activated states of MAP-kinases p42/44 and p38-stress kinase as a mechanism for MMP-13 expression. BMC Cell Biol 11:10