

CELLULAR STRUCTURE AND BIOLOGY OF DUPUYTREN'S DISEASE

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Dupuytren's disease is characterized by shortening of the palmar fascia, leading to progressive digital flexion deformity.³⁹ Since the time Guillaume Dupuytren described the disease named for him, physicians have been interested in the underlying cause of the shortening of the palmar fascia. Early proposals suggested that the force motivating this contraction came from the newly formed collagen within the wound.² Experiments conducted in the 1950s suggested that cells were central to the contraction responsible for tissue contracture.^{1, 27} It has since become clear that the shortening of the palmar fascia responsible for the flexion deformity central to Dupuytren's disease can be attributed to the cells present in the diseased tissue.

The cell believed to generate the contractile forces responsible for tissue contraction is the myofibroblast.⁵⁰ This cell was discovered in electron micrographs from contracting (healing) experimental granulation tissue.²⁰ The myofibroblast appears to have morphologic characteristics of both smooth muscle cells and fibroblasts,⁵⁰ hence its name. The myofibroblast subsequently was identified within

the nodules of Dupuytren's disease,¹⁸ as well as other contracting tissues.⁴⁸ The contractile capability of the myofibroblast has been demonstrated in granulating wounds.³⁷ More recently, Dupuytren's myofibroblasts have been demonstrated to generate contractile force.^{28, 44, 45, 56}

This article reviews what is known about the myofibroblast and its potential role in Dupuytren's disease. The sections that follow examine in more detail these topics: What is the myofibroblast? Does the myofibroblast generate contractile force? What regulates myofibroblast differentiation in Dupuytren's disease?

WHAT IS THE MYOFIBROBLAST?

Morphologic Characteristics of the Myofibroblast

The morphologic characteristics of this specialized cell were first described in studies examining the ultrastructure of granulation tissue and nodules of Dupuytren's disease.^{18, 20} It was termed *the myofibroblast* because it shares morphologic features with fibroblasts and smooth muscle cells (Fig. 1).¹⁹

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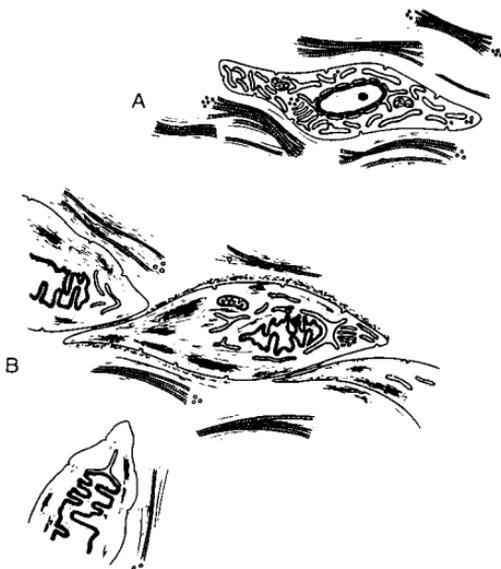


Figure 1. Comparison of the characteristics of fibroblasts and myofibroblasts. *A*, A typical fibroblast with a smooth contoured nucleus, scattered mitochondria, and well-developed secretory organelles including a large Golgi apparatus and dilated rough endoplasmic reticulum. *B*, The myofibroblast is characterized by the presence of large bundles of actin microfilaments. In addition, myofibroblasts have an indented nucleus and fibrillar material at their surface composed, at least in part, of fibronectin. (From Gabbiani G, Majno G, Ryan GB: The fibroblast as a contractile cell: The myofibroblast. In Kulonen E, Pikkarainen J (eds): *Biology of Fibroblast*. New York, Academic Press, 1973, p 139; by permission of the publisher Academic Press Limited, London.)

Fibroblasts in adult connective tissues are elongate cells with a slender, fusiform, and smooth, contoured nucleus. These cells also contain scattered mitochondria and well-developed secretory organelles, including a large Golgi apparatus and numerous, often dilated, rough endoplasmic reticulum. The actin microfilaments are concentrated beneath the plasma membrane, forming a cortical meshwork; few if any discrete bundles of microfilaments are observed. No basal lamina, fibronectin fibrils, or specialized attachment sites, such as plasmalemmal attachment plaques or fibronexus, are present at the fibroblast surface (see Fig. 1).

Smooth muscle cells, in contrast to fibro-

blasts, are surrounded by a basal lamina with numerous plasmalemmal attachment plaques. In addition, the actin in smooth muscle cells is organized into bundles that fill most of the cytosol of the cell and are oriented parallel to the long axis of the cell. Also, in contrast to fibroblasts, the elongated nucleus is deformed by shallow invaginations, and the Golgi apparatus and rough endoplasmic reticulum are poorly developed.²⁷

Myofibroblasts can be distinguished from fibroblasts and smooth muscle cells by a number of morphologic features (see Fig. 1). The predominant characteristic that distinguishes myofibroblasts from fibroblasts is the presence of bundles of actin microfilaments

(termed *stress fibers*), usually arranged parallel to the long axis of the cell (Fig. 2). These bundles of actin microfilaments in myofibroblasts are fewer in number and smaller in diameter than those observed in smooth muscle cells. Myofibroblasts also can be distinguished from smooth muscle cells by the presence of a well-developed Golgi apparatus and dilated rough endoplasmic reticulum, as well as the absence of an enveloping basal lamina. Similar to smooth muscle cells, the elongate nucleus of the myofibroblast frequently is deformed by shallow indentations indicative of cellular contraction.⁵⁰ Myofibroblasts do have specialized attachment sites, but these appear different from the plasmalemmal attachment plaques observed in smooth muscle. The bundles of intracellular actin microfilaments appear to become continuous, with extracellular fibrils composed of fibronectin at the plasmalemma (see Fig. 2). These specialized attachment sites in myofibroblasts are termed the *fibronexus* and are present at the surfaces of myofibroblasts in Dupuytren's diseased tissue.^{54, 58} The fibronexus is proposed to be the mechanism by which contractile force generated by the intracellular bundles of actin microfilaments is transmitted to the surrounding extracellular matrix and tissues.⁴⁹

Myofibroblasts, in paraffin or cryostat sec-

tions, also can be recognized with the light microscope. They are usually large, spindle-shaped, often stellate, cells with indented nuclei. They also can be recognized by their ability to stain intensely with either anti-actin antibody or fluorescent probes that bind filamentous actin, such as rhodamine phalloidin (Fig. 3).⁵⁷ In contrast, fibroblasts do not stain with these probes because of the dispersed nature of their filamentous actin.

Distribution of Myofibroblasts in Dupuytren's Diseased Tissue

Dupuytren's disease was described by Luck³⁵ as progressing through three histologically distinct phases—proliferative, involutinal, and residual. During the proliferative phase, a local fibroplasia in the fascia results in the formation of a nodular lesion. During the involutinal phase, the cells present in the nodule align themselves with the lines of stress in the tissue. The residual phase is characterized by the disappearance of the nodule, leaving a relatively acellular, scar-like tissue.

Dupuytren's diseased tissues at different stages of the disease process have been examined for the presence of myofibroblasts by electron microscopy^{10, 17, 31, 51} as well as by

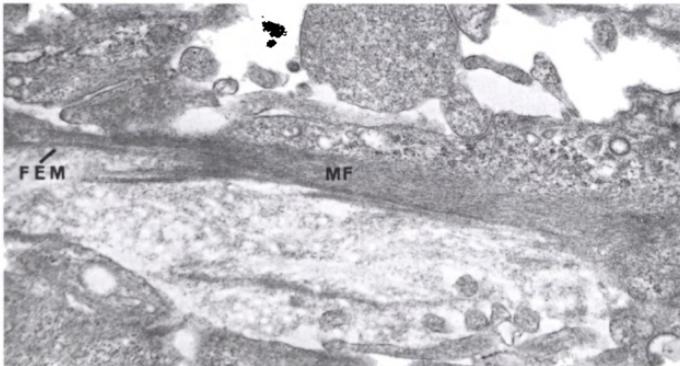


Figure 2. Transmission electron micrograph of a myofibroblast in Dupuytren's diseased tissue. An intracellular bundle of actin microfilaments (MF) appears to be continuous through the cell membrane with extracellular fibronectin fibrils (FEM). A specialized adhesion complex termed the *fibronexus* is present at the cell membrane interconnecting the bundle of actin microfilaments and the fibronectin fibrils (original magnification $\times 12,000$). (From Tomasek JJ, Schultz RJ, Haaksma CJ: Extracellular matrix-cytoskeletal connections at the surface of the specialized contractile fibroblast (myofibroblast) in Dupuytren disease. *J Bone Joint Surg [Am]* 69:1400, 1987, with permission.)

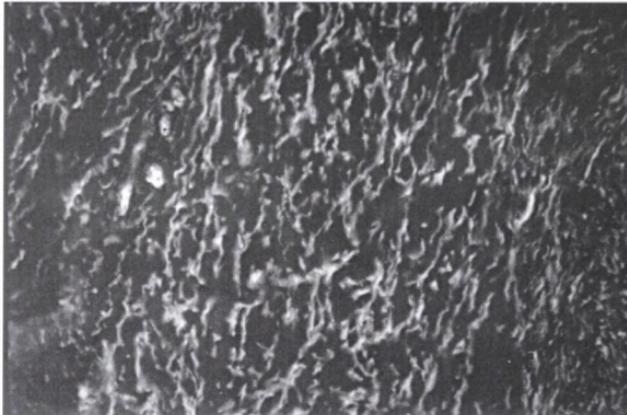


Figure 3. Fluorescence photomicrograph of myofibroblasts in Dupuytren's diseased tissue. Myofibroblasts in Dupuytren's diseased tissue stain intensely with rhodamine phalloidin, a fluorescent probe that binds F-actin (original magnification $\times 600$).

fluorescent staining with probes for filamentous actin.^{51, 57} These studies have demonstrated that myofibroblasts appear during the proliferative phase of the disease and come to comprise almost all of the cells present in the highly cellular nodule. The involucional-phase nodules are also highly cellular, but individual myofibroblasts are smaller and tend to be aligned in the same direction, along lines of stress. In contrast, residual-phase nodules are hypocellular and the cells are slender and aligned with thick bands of collagen, giving them a tendon-like appearance. Myofibroblasts are not observed in residual-phase nodules; rather these nodules are composed of mature fibroblasts.

The appearance and disappearance of myofibroblasts during Dupuytren's disease is similar to that observed in other tissues in which myofibroblasts are present, such as granulation tissue in wounds.¹¹ What regulates the appearance and disappearance of the myofibroblast is still being investigated. Recent studies suggest that mechanical forces present in the tissue, together with growth factors, such as transforming growth factor- $\beta 1$ (TGF- $\beta 1$), may play an important role in promoting the differentiation of the myofibroblast (see the section on TGF- $\beta 1$). The regulation of the myofibroblast's disappearance may be just as important as its appearance. The persistence of myofibroblasts could lead to excessive tis-

sue contraction and matrix deposition. Myofibroblasts in granulation tissue disappear during scar formation by the process of programmed cell death or apoptosis.¹³ Whether apoptosis causes the loss of myofibroblasts during the residual stage of Dupuytren's disease needs to be determined.

Fibronectin in Dupuytren's Diseased Tissue

It has been proposed that extracellular glycoprotein fibronectin plays an important role in cellular processes such as migration, differentiation, and adhesion,³⁰ all of which are important during Dupuytren's disease. This glycoprotein is present in morphogenetically active embryonic tissues and in granulation tissue in adults. Fibronectin is extensively present in nodules in Dupuytren's diseased tissue, where it is organized into fibrils at the surfaces of myofibroblasts (see Fig. 2).³⁸

Fibronectin fibrils at the surfaces of myofibroblasts in Dupuytren's diseased tissue participate in the formation of a specialized attachment site, termed *the fibronexus*, that links the intracellular bundles of actin microfilaments with extracellular fibronectin fibrils. Recent studies have demonstrated the presence of the integrin transmembrane glycoprotein $\alpha 5 \beta 1$ at the surfaces of myofibroblasts in

Dupuytren's diseased tissue.³⁶ It is believed that the $\alpha_5\beta_1$ integrin receptor spans the membrane, linking the intracellular actin and actin-associated proteins with the extracellular fibronectin.³⁷ The fibronexus is proposed to be the structural element by which contractile force generated by the intracellular bundles of actin microfilaments is transmitted to the surrounding extracellular matrix and tissues.¹⁴ In addition to transmitting contractile force, these fibronexus attachment sites recently have been suggested to be sites of signal transduction.⁸ Present in the fibronexus are tyrosine kinases, such as focal adhesion kinase, which have been implicated in regulating cell proliferation, differentiation, and extracellular matrix production, all of which are important in the appearance and function of myofibroblasts in Dupuytren's disease.

The fibronectins are a family of glycoproteins with numerous isoforms that arise by differential splicing of a single gene transcript.²⁹ Sequence variations occur in three areas of the fibronectin monomer. Extra domains A and B (ED-A and ED-B fibronectin) of human fibronectin may be either included or deleted, whereas the IIICS region can vary in length, when present. In the C-terminal region of fibronectin, posttranslational modification with a specific O-linked glycosylation of a single threonine residue results in the formation of what has been called *oncofetal fibronectin* because of its presence in embryonic and tumor tissues.³⁸ The expression of these fibronectin isoforms is regulated in a cell- and tissue-specific manner during development and aging, and they are highly restricted in their distribution in adult tissues. In Dupuytren's disease, the ED-A fibronectin isoform is expressed during the proliferative phase of the disease and less during the involutional phase, whereas the ED-B fibronectin isoform and de novo glycosylated fibronectin are expressed mainly during the proliferative phase of the disease (Fig. 4).^{7, 24} The function of these fibronectin isoforms is unclear, but their presence in Dupuytren's diseased tissue is consistent with their presence in other tissues that are proliferating and undergoing extensive remodeling.

A number of recent studies have demonstrated the presence of TGF- β 1 in Dupuytren's diseased tissue.^{3, 7} The presence of TGF- β 1 in Dupuytren's diseased tissue is consistent with the appearance of fibronectin and the oncofetal isoforms of fibronectin. In vitro studies have demonstrated that TGF- β 1 is a

potent inducer of fibronectin expression and expression of the oncofetal isoforms of fibronectin.^{36, 33} These studies suggest that TGF- β 1 may play an important role in Dupuytren's disease by regulating fibronectin expression and alternative splicing.

Cytoskeletal Proteins in Myofibroblasts in Dupuytren's Diseased Tissue

The distinguishing characteristic of the myofibroblast is the presence of intracellular bundles of microfilaments. These are composed of actin filaments, myosin, and associated proteins. Actin is encoded by a multigene family that produces six different isoforms.⁶¹ These isoforms have been classified according to their pattern of expression—two muscle actins in striated muscle (α -skeletal and α -cardiac), two muscle actins in smooth muscle (α -smooth muscle actin and γ -smooth muscle actin), and two nonmuscle actins present in every cell (β -actin and γ -actin). Fibroblasts express only the β - and γ -actin isoforms. Myofibroblasts, in addition to expressing the β - and γ -actin isoforms, also express α -smooth muscle actin.^{50, 53}

The expression of α -smooth muscle by myofibroblasts appears to be transient. In granulation tissue, the expression of α -smooth muscle actin increases during wound closure and gradually disappears during scar formation.¹¹ In Dupuytren's disease, α -smooth muscle actin expression also appears to be transient. Myofibroblasts expressing α -smooth muscle actin appear during the proliferative phase of the disease, are present during the involutional phase, and disappear by the residual phase.^{51, 53} The functional significance of α -smooth muscle actin expression is currently unclear, but recent studies have suggested that the expression of α -smooth muscle actin in myofibroblasts correlates with increased generation of contractile force (see section on regulation of myofibroblast contraction).⁵⁶

The interaction of myosin with actin is responsible for the generation of contractile force. Myosin is co-localized with actin in stress fibers in myofibroblasts.^{5, 15} Myofibroblasts have been examined to determine which isoform of myosin they express. Myofibroblasts in granulation tissue are never observed to express the smooth muscle myosin isoform.^{15, 90} In Dupuytren's disease, in contrast, smooth muscle myosin has been re-

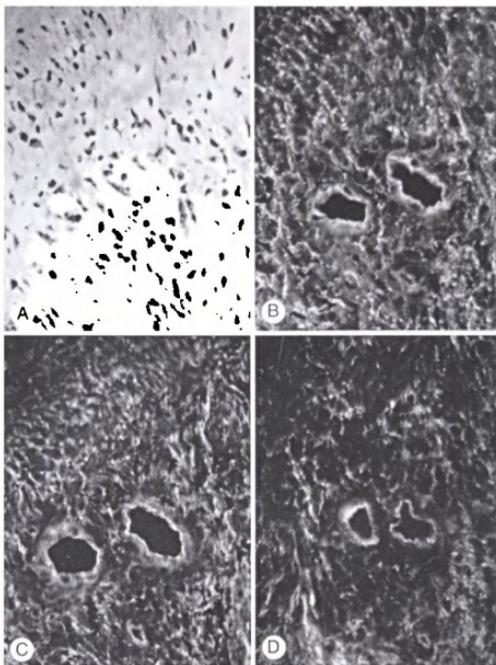


Figure 4. Serial sections showing indirect immunofluorescence staining of proliferative-stage Dupuytren's diseased tissue. A, Hematoxylin- and eosin-stained tissue section demonstrating the large numbers of cells in proliferative-stage Dupuytren's diseased tissue. Immunofluorescence staining demonstrating the distribution of total fibronectin (B), ED-A fibronectin (C), and ED-B fibronectin (D) in Dupuytren's diseased tissue (original magnification $\times 250$). (Adapted from Halliday NL, Rayan GM, Tomasek JJ: Distribution of ED-A and ED-B containing fibronectin isoforms in Dupuytren's disease. *J Hand Surg [Am]* 19:428, 1994; with permission.)

ported to be present in myofibroblasts, but its expression is variable and in only a low percentage of the myofibroblasts.⁴⁷

Intermediate filaments are another class of cytoskeletal proteins. These do not play a role in generation of force; rather, they appear to be involved in maintaining cell shape. Fibroblasts express the intermediate filament protein vimentin, whereas smooth muscle cells may express only vimentin or both vimentin and desmin.⁴¹ Myofibroblasts in granulation tissue express only vimentin, not desmin.⁴¹ In Dupuytren's diseased tissue, expression of intermediate filament proteins is variable. The majority of the myofibroblasts express vimentin, but desmin expression may be observed

in a subpopulation of the myofibroblasts.^{41, 42} The presence of this desmin-expressing subpopulation of myofibroblasts in Dupuytren's diseased tissue is also variable, being present in only some nodules.

Based on the expression of cytoskeletal proteins, it is possible to define at least two cytoskeletal phenotypes for myofibroblasts—phenotype VA, represented by myofibroblasts positive for vimentin and α -smooth muscle actin; and phenotype VAD, represented by myofibroblasts positive for vimentin, α -smooth muscle actin, and desmin.⁴¹ Whether smooth muscle myosin can be expressed by both phenotypes or only the VAD phenotype still needs to be determined. In Dupuytren's

disease, both VA and VAD phenotypes are present, but the VA phenotype is the most prevalent and the VAD phenotype is not observed in all nodules.⁵¹

Cellular Derivation of Myofibroblasts in Dupuytren's Disease Tissue

As already described, myofibroblasts share morphologic features with both fibroblasts and smooth muscle cells. Two questions arise from these observations. From where do myofibroblasts originate—fibroblasts or smooth muscle cells? Which of these two cell types do myofibroblasts resemble functionally? Although myofibroblasts contain bundles of actin microfilaments similar to those observed in smooth muscle, this does not establish that myofibroblasts originate from smooth muscle. Fibroblasts, when placed in tissue culture, acquire bundles of actin microfilaments (stress fibers) that ultrastructurally resemble myofibrils in smooth muscle cells. In fact, fibroblasts from normal palmar aponeurosis develop stress fibers and appear similar to myofibroblasts observed in Dupuytren's disease.⁴⁵ The stress fibers present in fibroblasts derived from palmar aponeurosis are even capable of generating contractile force.⁴⁵ Fibroblasts therefore are capable of acquiring both the morphology and function of myofibroblasts.

A number of studies have examined the cytoskeletal proteins present in myofibroblasts as a means of determining their origin. As discussed earlier, myofibroblasts express the actin isoform found in smooth muscle cells— α -smooth muscle actin. Fibroblasts can be induced to express α -smooth muscle actin if treated with TGF- β 1.^{12, 61} In addition to promoting the expression of α -smooth muscle actin in fibroblasts, TGF- β 1 promotes fibronectin fibril formation and the formation of the fibronexus (discussed subsequently).⁶² These results suggest that fibroblasts have the potential to become myofibroblasts if stimulated with TGF- β 1. The presence of TGF- β 1 has been demonstrated in Dupuytren's diseased nodules, where it could promote myofibroblast formation from fibroblasts.^{1, 7}

Intermediate filament proteins have been used to determine cellular origin because they have a tissue-specific distribution that is retained during neoplastic conditions.^{46, 47} Vimentin is present in most nonmuscle cells,

such as fibroblasts. Desmin is present in parenchymal smooth muscle cells and some vascular smooth muscle cells, but also has been found in a number of nonmuscle mesenchymal cells, such as endothelial cells, podocytes, and stromal cells from various locations.⁵¹ In Dupuytren's disease, desmin immunoreactivity in myofibroblasts is variable from patient to patient and, in most Dupuytren's diseased nodules, the number of myofibroblasts expressing vimentin exceeds the number expressing desmin.^{51, 52} These results suggest that some myofibroblasts in Dupuytren's disease may arise from vascular smooth muscle cells but the majority appear to arise from fibroblasts in the palmar aponeurosis, under the influence of TGF- β 1.

DOES THE MYOFIBROBLAST GENERATE CONTRACTILE FORCE?

Evidence that Myofibroblasts are Contractile

The question that arises when considering a role for the myofibroblast in Dupuytren's disease is whether this cell can generate contractile force. The morphology of the myofibroblast is consistent with this hypothesis. The distinguishing morphologic characteristic of the myofibroblast is the presence of intracellular bundles of microfilaments. As discussed previously, these bundles of microfilaments are composed of actin, myosin, and associated proteins and resemble the myofibrils observed in smooth muscle cells. This organization of actin and myosin is necessary for generation of contractile force. In addition, intracellular bundles of actin microfilaments terminate in a specialized adhesion complex—the fibronexus—through which contractile force could be transmitted to the extracellular matrix and surrounding tissues. In addition, myofibroblasts are present in tissues undergoing contraction, such as granulation tissue and Dupuytren's diseased tissue, which is consistent with the idea that they can generate contractile force.

Further evidence that myofibroblasts can generate contractile force comes from studies measuring force generation in granulation tissue. Granulation tissue on the dorsum of a rat can generate isometric force as measured by a force transducer attached directly to the tissue.²⁶ Isolated granulation tissue also can develop contractile force in response to spe-

cific agonists and this force generation is correlated with the presence of myofibroblasts in the tissue.¹⁷ Similar studies examining contractility of Dupuytren's diseased tissue have not been done.

Models that Demonstrate Myofibroblasts are Contractile

Although studies demonstrate that tissues containing myofibroblasts can generate contractile force, they do not provide direct evidence that myofibroblasts are contractile. In vitro models have been developed to determine directly whether myofibroblasts are capable of generating contractile force. The authors' laboratory has used two different in vitro models in which isotonic or isometric contraction of myofibroblasts can be meas-

ured.^{41, 42, 44, 45, 55, 56} In these models, myofibroblasts obtained from Dupuytren's diseased palmar fascia are cultured within three-dimensional type I collagen lattices. Cells were cultured within three-dimensional type I collagen lattices because these lattices model the in vivo environment. The type I collagen lattice is stabilized by attachment to either the bottom of a tissue culture dish or to glass tubes coated with hook-and-loop fasteners. Myofibroblasts cultured for 5 days within collagen lattices appear similar to myofibroblasts in Dupuytren's diseased tissue. They have bundles of actin microfilaments, assemble fibronectin into fibrils, and form fibronexus attachment complexes at their surfaces (Fig. 5). In these models, it is important that the collagen lattice be stabilized because myofibroblasts cultured with floating collagen lattices do not have bundles of actin filaments,

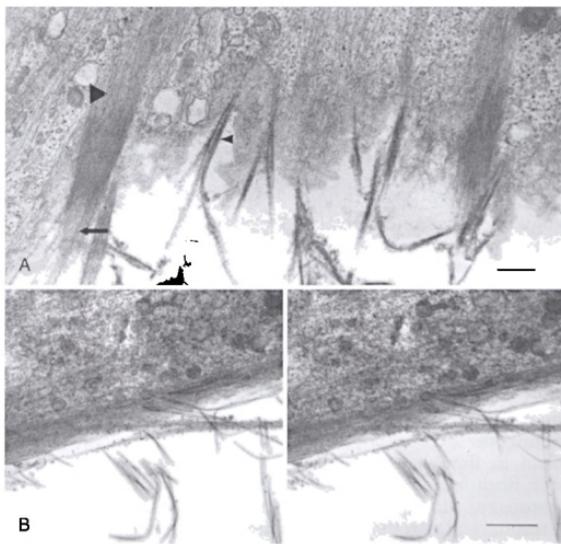


Figure 5. Transmission electron micrograph of fibroblasts cultured within stabilized collagen lattices. *A*, Large bundles of actin microfilaments (*large arrowhead*) overlap with extracellular filaments at the cell surface forming a fibronexus adhesion complex. Collagen fibrils (*small arrowhead*) also are closely associated with the cell surface. Bar = 0.5 μm . *B*, Stereo pair of high-voltage electron micrographs of lattice labelled with antifibronectin antibody followed by secondary antibody conjugated to colloidal gold. Gold particles are restricted to fibronectin fibrils extending from the cell surface. Bar = 0.5 μm . (From Tomasek JJ, Haakma CJ, Eddy RJ, et al: Fibroblast contraction occurs on release of tension in attached collagen lattices. Dependency on an organized actin cytoskeleton and serum. *Anat Rec* 232:359, Copyright \textcopyright 1992. Reprinted by permission of Wiley-Liss, Inc., a division of John Wiley & Sons, Inc.)

do not assemble fibronectin into fibrils, and do not form fibronexus attachments.²⁷

The amount of contractile force generated by the myofibroblasts cultured within these three-dimensional collagen lattices can be determined. Isotonic contraction can be measured by releasing the collagen lattice from its attachment to the culture dish and measuring changes in lattice diameter over time.²⁷ After release, the diameter of the collagen lattice is reduced by as much as 50% within 10 minutes, after which little change in lattice diameter occurs (Fig. 6). This rapid reduction in lattice diameter is the result of myofibroblast contraction and the transmission of force to the surrounding collagen matrix. Isometric contraction can be measured by attaching the glass tube stabilized collagen lattices to a force transducer.¹² Myofibroblasts increase isometric force generation in response to specific agonists that reach a plateau within 10 minutes. The amount of isometric force generated by myofibroblasts is approximately one-tenth that generated by smooth muscle cells.¹² These experiments demonstrate that myofibroblasts can generate isotonic and isometric force and can transmit this force to the surrounding collagen matrix.

Regulation of Myofibroblast Contraction

The processes that regulate the contraction of myofibroblasts in Dupuytren's disease are

just beginning to be understood. Using the previously described collagen lattice contraction models, the authors' laboratory has been investigating the regulation of myofibroblast contraction. Similar to smooth muscle cell contraction, myofibroblast contraction has been found to depend upon the presence of specific agonists. Lysophosphatidic acid (LPA), a simple phospholipid, has been identified as a potent agonist promoting the contraction of myofibroblasts isolated from Dupuytren's diseased tissue.¹⁴ LPA is released by platelets upon activation.¹⁶ In addition, LPA is a crucial intermediate in de novo lipid biosynthesis. LPA, like prostaglandins, can be released into the extracellular environment by cells after growth-factor stimulation. Whether LPA is present in Dupuytren's diseased tissue, where it could promote myofibroblast contraction, remains to be investigated.

Thrombin also has been identified as an agonist for myofibroblasts, although it is only about 70% as potent as LPA.^{11, 12} Thrombin promotes myofibroblast contraction through the enzymatic activation of its cell-surface receptor.¹¹ Other studies have demonstrated that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) can promote myofibroblast force generation.²⁸ The authors have found that LPA is over 1000 times more potent than $PGF_{2\alpha}$,¹⁴ suggesting that it may be a more important agonist for contraction of myofibroblasts in Dupuytren's diseased tissue.

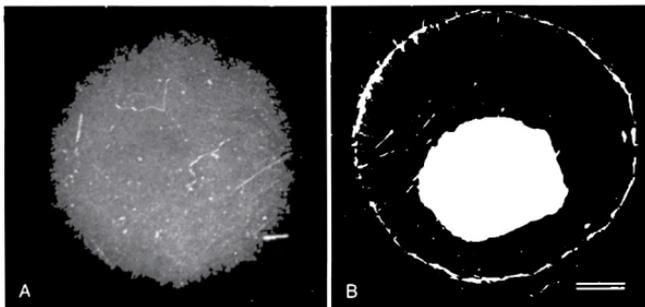


Figure 6. Dark-field photomicrograph illustrating rapid contraction of collagen lattice. *A*, Stabilized collagen lattice cultured for 5 days round and symmetrical in outline. *B*, Ten minutes after release, the diameter of the same lattice is dramatically reduced. The circumference of the lattice prior to release is visible as a white line due to the scraping of the plastic surface with a scalpel to mechanically free the attached lattice. Bar = 2 mm. (From Tomasek JJ, Haaksma CJ, Eddy RJ, et al: Fibroblast contraction occurs on release of tension in attached collagen lattices: Dependency on an organized actin cytoskeleton and serum. *Anat Rec* 232:359, Copyright © 1992. Reprinted by permission of Wiley-Liss, Inc., a division of John Wiley & Sons, Inc.)

Understanding the intracellular mechanisms that regulate myofibroblast contraction may provide improved methods of management of palmar fascia contraction. LPA may promote myofibroblast contraction by activation of specific intracellular signaling cascades through binding to its cell-surface receptor. LPA binding of its receptor will decrease cyclic adenosine monophosphate (cAMP) levels³⁰ and increase intracellular calcium levels,³² both of which can increase the activity of myosin light-chain kinase³³ (Fig. 7). Myosin light-chain kinase is an enzyme that phosphorylates myosin light chain. This phosphorylation is required for myosin-actin interaction and the generation of contractile force.³³ LPA therefore would be expected to increase the activity of myosin light-chain kinase, resulting in an increase in myosin light-chain phosphorylation and a subsequent increase in myofibroblast generation of contractile force. Prostaglandins E1 and E2 increase intracellular levels of cAMP and have been demonstrated to inhibit LPA-promoted myofibroblast contraction.³⁴ Similarly, nifedipine and verapamil can inhibit the intracellular rise in calcium and can inhibit LPA-promoted myofibroblast contraction.³⁴ Lee and coworkers³¹ recently showed that direct injection of verapamil into hypertrophic burn scars resulted in scar size reduction. The use of prostaglandins E1 or E2 and verapamil or

nifedipine needs to be investigated further for possible clinical use to control Dupuytren's contracture. Hopefully, with further study, other intracellular signaling pathways will be identified that may provide alternative methods to improve management of Dupuytren's contracture.

Generation of Contractile Force Correlates with Expression of α -Smooth Muscle Actin

One characteristic of myofibroblasts *in vivo* is the expression of α -smooth muscle actin. It has been proposed that increased expression of α -smooth muscle actin in myofibroblasts may be related to their ability to generate contractile force.^{46, 53, 56, 61} There is a positive correlation between tissues undergoing contraction and the presence of α -smooth muscle actin-expressing myofibroblasts. The authors' *in vitro* studies of myofibroblasts from Dupuytren's diseased tissue have found a positive correlation between level of expression of α -smooth muscle actin and generation of contractile force.⁵⁶ The authors also have found that treatment of myofibroblasts in collagen lattices with TGF- β 1 results in an increased expression of α -smooth muscle actin and a correlated increase in contractile force generation.⁶¹ Other studies have demon-

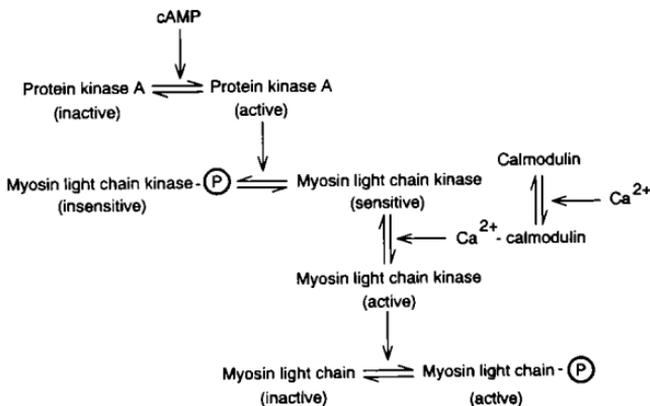


Figure 7. Intracellular signaling cascade proposed to regulate contraction of myofibroblasts in Dupuytren's disease. (From Rayan GM, Parizi M, Tomasek JJ. Pharmacologic regulation of Dupuytren's fibroblast contraction *in vitro*. *J Hand Surg [Am]* 21:1065, 1996, with permission.)

strated that decreasing the expression of α -smooth muscle actin in cultured myofibroblasts results in the loss of the contractile phenotype and acquisition of a more motile phenotype.⁴⁶ Future studies directly correlating the level of α -smooth muscle actin expression and contractile force generation are needed to confirm that the expression of this contractile protein plays a role in the contraction of the palmar fascia observed in Dupuytren's disease.

WHAT REGULATES MYOFIBROBLAST DIFFERENTIATION IN DUPUYTREN'S DISEASE?

Transforming Growth Factor- β 1 Promotes Myofibroblast Differentiation

Transforming growth factor- β 1 may play an important role in promoting the differentiation of the myofibroblast. The subcutaneous administration of TGF- β 1 to rats results in the formation of a granulation tissue in which α -smooth muscle actin-expressing myofibroblasts are abundant.¹² Other cytokines and growth factors, such as platelet-derived growth factor and tumor necrosis factor- α , despite their profibrotic activity, do not induce α -smooth expression in the resulting tissue.¹² Furthermore, the expression of α -smooth muscle actin protein and mRNA is induced by TGF- β 1 in both growing and quiescent cultured fibroblastic populations.¹² TGF- β 1 also can promote the expression of α -smooth muscle actin in cultured Dupuytren's myofibroblasts and normal palmar fascia fibroblasts.^{61, 62} In addition, when these cells are cultured within stabilized collagen lattices and treated with TGF- β 1, they increase their assembly of fibronectin fibrils and dramatically increase the size of fibronexus adhesion complexes at their surfaces.⁶² These results suggest that the TGF- β 1 present in Dupuytren's diseased tissue may play an important role in promoting the differentiation of myofibroblasts from palmar fascia fibroblasts.

Transforming Growth Factor- β 1 Promotes Generation of Contractile Force

It has been proposed that increased generation of contractile force is a functional conse-

quence of myofibroblast differentiation. To determine directly whether TGF- β 1 can increase the generation of contractile force, palmar fascia fibroblasts and Dupuytren's myofibroblasts were cultured in stabilized collagen lattices in the presence or absence of TGF- β 1. As already discussed, TGF- β 1 treatment promotes the phenotypic differentiation of the myofibroblast. TGF- β 1 treatment significantly increased the generation of contractile force in both fibroblasts and myofibroblasts as determined via the released collagen lattice contraction assay.^{61, 62} These results are consistent with the authors' previous study demonstrating a correlation between expression of α -smooth muscle actin and generation of contractile force in cultured Dupuytren's myofibroblasts.³⁶ The increased generation of contractile force in response to TGF- β 1 suggests that the presence of this growth factor in Dupuytren's diseased tissue may be responsible for the resulting pathological contracture.

Mechanical Stress Regulates Myofibroblast Differentiation

Along with growth factors, such as TGF- β 1, mechanical stress may play an important role in myofibroblast differentiation.^{22, 25} Fibroblasts cultured within a mechanically stressed collagen lattice form bundles of actin microfilaments, assemble fibronectin into fibrils, and form fibronexus adhesion complexes.⁵⁵ These cells are also responsive to TGF- β 1, as demonstrated by increased expression of α -smooth muscle actin, increased assembly of fibronectin fibrils, and increased formation of fibronexus adhesion complexes.⁶² In contrast, fibroblasts cultured within mechanically relaxed collagen lattices do not organize actin into bundles; rather, actin microfilaments are present as a cortical meshwork. In addition, fibroblasts in this mechanically relaxed environment do not assemble fibronectin into fibrils and do not form fibronexus adhesion complexes.²⁵ A mechanically stressed environment along with TGF- β 1 appears to be necessary for the differentiation of fibroblasts to myofibroblasts *in vitro*. How these mechanical forces influence fibroblasts and myofibroblasts *in vivo* remains to be determined.

Interferon- γ Suppresses Myofibroblast Differentiation

Interferon- γ (IFN- γ), a cytokine produced by T-helper lymphocytes, can decrease α -smooth muscle actin protein and mRNA expression in cultured fibroblasts.¹⁴ The authors' recent studies found that IFN- γ can block the TGF- β 1-promoted changes observed in cultured palmar fascia fibroblasts and Dupuytren's myofibroblasts.^{61, 62} This includes blocking expression of α -smooth muscle actin, assembly of fibronectin fibrils, and formation of fibronexus adhesion complexes. In addition, IFN- γ reduces the generation of contractile force by palmar fascia fibroblasts and Dupuytren's myofibroblasts in response to TGF- β 1. Preliminary *in vivo* results have shown that IFN- γ treatment decreases the symptoms and the size of hypertrophic scars and Dupuytren's nodules.⁶³ In addition, the expression of α -smooth muscle actin was decreased in myofibroblasts in IFN- γ -treated hypertrophic scars. These results suggest that IFN- γ , by suppressing myofibroblast differentiation, could represent a useful adjunct to the nonsurgical therapy of Dupuytren's disease.

SUMMARY

Numerous studies support the idea that the myofibroblast is a key cell responsible for the tissue contraction in Dupuytren's disease. *In vitro* models have been developed to study the underlying cellular basis of myofibroblast differentiation and contraction. Studies suggest that the growth factor TGF- β 1 combined with mechanical stress can promote the differentiation of fibroblasts into myofibroblasts. Agonists, such as LPA and thrombin, can promote the contraction of myofibroblasts through specific intracellular signaling pathways that regulate levels of phosphorylated myosin light chain. Agents that can affect these intracellular signaling pathways hold promise as a means to decrease contraction of the myofibroblast and of the palmar fascia in Dupuytren's disease. Finally, the recent finding that IFN- γ can suppress both the differentiation of the myofibroblast and the generation of contractile force, together with preliminary clinical results using IFN- γ , suggest the potential use of IFN- γ for nonsurgical therapy of Dupuytren's disease. Future studies into the cellular basis of tissue contraction should provide alternative methods to im-

prove management of Dupuytren's contracture.

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