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8 Cellular structure and interconnections

INTRODUCTION

Dupuytren's disease (DD) is characterized by the presence of a nodule and/or fibrous band which may ultimately produce a flexion deformity of the fingers. A specialized cell present in the diseased palmar fascia has been termed the 'myofibroblast' by Gabbiani & Majno in 1972 because of its morphological similarities to fibroblastic and smooth muscle cells. The structural similarities of this cell to the smooth muscle cell in its contractile apparatus and its presence in the clinical nodule during the active stage of contraction have led to the proposal that the myofibroblast is responsible for the digital contracture (Chiu & McFarlane 1978).

The cellular mechanisms underlying the digital flexion characteristic of Dupuytren's disease are still unclear. Studies by Brickley-Parsons and co-workers (1981) have suggested that the contracture of the palmar fascia is due to an active cellular process that progressively draws the extremities of the affected tissue together and at the same time replaces the original tissue. The result of these two processes is a shorter, smaller piece of tissue fabric. In order for a cell to be the active agent responsible for bringing together the extremities of affected tissue it must be able to generate an intracellular contractile force and secondly, it must be able to transmit this intracellular force to the surrounding tissue.

The generation of intracellular contractile force requires a specialized cytoskeleton, whereas the transmission of this contractile force to the surrounding tissues demands an adherent link between adjacent cells as well as other surrounding tissues. To understand these mechanisms one must have a knowledge of the intracellular contractile elements, namely the proteins present in the cytoskeleton of the cell, and the adhesion glycoproteins found at the cell's surface.

The structural similarities of the myofibroblast to smooth muscle cells and fibroblasts have stimulated research to determine which of these two cell types the myofibroblast more closely resembles and from which it is derived. As illustrated by Gabbiani & Majno (1972) and Chiu & McFarlane (1978), myofibroblasts contain numerous mitochondria and large amounts of rough endoplasmic reticulum typical of fibroblasts. They also contain large bundles of actin microfilaments, which are similar in appearance to the myofibrils of smooth muscle. Furthermore, myofibroblasts have an interrupted, amorphous extracellular layer at their surface, which resembles the basement membrane around smooth muscle cells.

Fibroblasts and smooth muscle cells can be more accurately distinguished by the proteins present in their cytoskeleton and extracellular matrix than by their appearance. Smooth muscle cells contain smooth muscle myosin and are associated with the extracellular glycoprotein, laminin (Burridge 1974; Foidart et al 1980). On the other hand, fibroblasts also contain an intracellular myosin — non-muscle myosin — and are associated with fibronectin in their extracellular matrix. Recent immunocytochemical studies, discussed below, indicate that Dupuytren's myofibroblasts are more akin to fibroblasts than smooth muscle cells. They were found to contain non-muscle myosin but no smooth muscle myosin or laminin and thus no muscle elements (Tomasek

In: McFarlane RM, McGrouther DA, Flint MH (eds):
Dupuytren's Disease Biology and Treatment
Churchill Livingstone Edinburg 1990.

et al 1986). Immunocytochemical studies by Schürch and co-workers (1984) demonstrated that the cytoskeleton of myofibroblasts contains the intermediate filament protein vimentin and lacks desmin, further indicating that these cells are distinct from smooth muscle cells.

These studies indicate that Dupuytren's myofibroblasts closely resemble fibroblasts. In addition to the fact that they contain non-muscle myosin, the large bundles of actin microfilaments present in these cells are similar to stress fibres seen in cultured fibroblasts. Furthermore, the presence of fibronectin at the surfaces of Dupuytren's myofibroblasts and the transmembrane association between actin microfilaments and extracellular fibrils are also similar to observations in cultured fibroblasts. These findings further indicate that the Dupuytren's myofibroblast is a modified fibroblast with a specially organized cytoskeleton with large amounts of fibronectin accumulated at its surface. In view of this we feel that the term myofibroblast is rather misleading and prefer to call this cell a Dupuytren's specialized contractile fibroblast, or more descriptively a 'tractofibroblast', from the Latin *tractare* — to pull. In this chapter we have occasionally interchanged the terms myofibroblast and tractofibroblast, depending on the circumstances.

Cytoskeleton of the myofibroblast

The myofibroblast, felt to be the basic cell of DD, structurally resembles both fibroblasts and smooth muscle cells and thus derives its name. The most prominent feature of the myofibroblast is the fibrillar system present within its cytoplasm. Unlike the meshwork organization of actin microfilaments seen at the periphery of normal fibroblasts in vivo, myofibroblasts contain large bundles of actin microfilaments (Fig. 8.1). These bundles parallel the long axis of the myofibroblast and terminate at the cell surface in a specialized association with extracellular fibrils (see section on interconnecting bonds, below). This ultrastructure is similar to either that of myofibrils in smooth

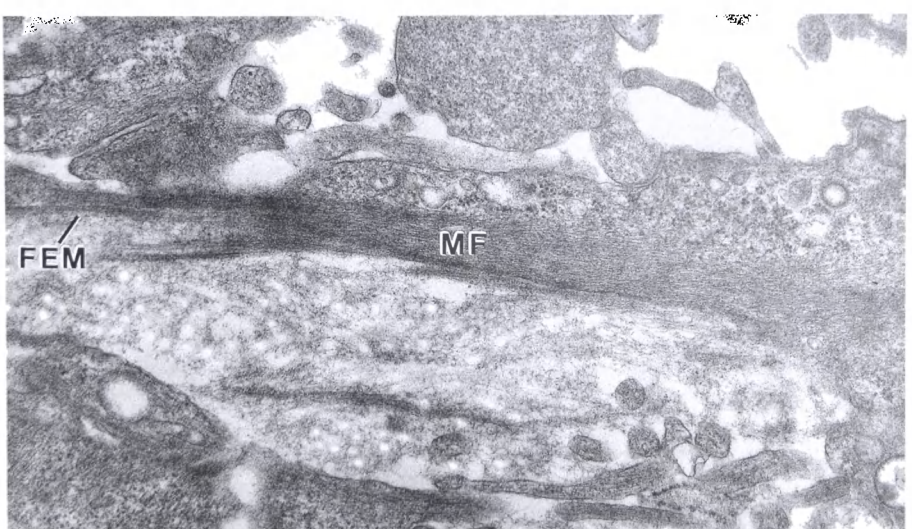

Fig. 8.1 Electron micrograph of a Dupuytren's myofibroblast. A large bundle of actin microfilaments (MF) with electron-dense regions along its length is present. This bundle terminates at the cell membrane in a well defined end-to-end transmembrane association with filamentous extracellular material (FEM). Magnification × 21 000.

muscle or stress fibres which form in fibroblasts under culture conditions (Fig. 8.2). To understand the cellular mechanism of force generation in the myofibroblast one must consider whether the large bundles of actin microfilaments within myofibroblasts more closely resemble myofibrils or stress fibres.

In 1986 we demonstrated by immunocytochemistry that Dupuytren's myofibroblasts stain intensely with a monoclonal anti-actin antibody (Fig. 8.3a; Tomasek et al 1986). It should be stressed that this antibody recognizes both non-muscle and smooth muscle actin. The fibroblasts in normal palmar fascia and in the surrounding band tissue show little if any staining with this anti-actin antibody (Fig. 8.3b). The intense staining pattern in Dupuytren's myofibroblasts and smooth muscle cells exists since the actin in both these cells is organized into bundles of microfilaments. The actin in fibroblasts is organized into a filamentous meshwork which stains only faintly.

Another important protein in DD is myosin. Myosin is a cytoskeletal protein that interacts with actin to generate contractile force in either muscle or non-muscle cells. The presence of myosin in Dupuytren's myofibroblasts was first demonstrated by Meister and co-workers in 1979; utilizing immunocytochemistry, these authors found that myosin was present in substantial

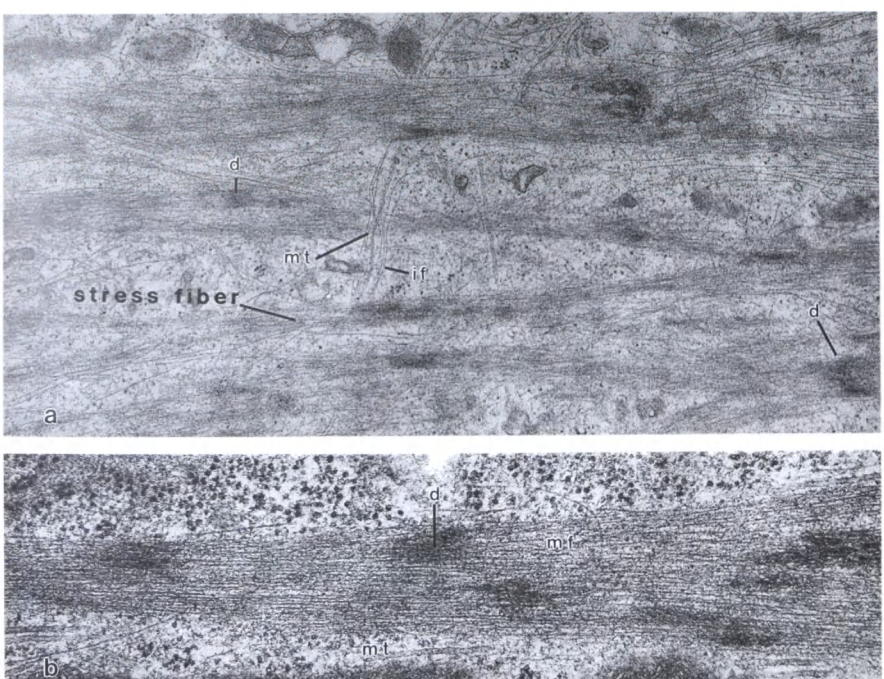

Fig. 8.2 Electron micrographs of fibroblasts cultured on coverslips a Bundles of actin microfilaments (stress fibres) with periodic densities (d) along their length are present throughout the cytoplasm near the substratum. Intermediate filaments (if) and microtubules (mt) are present. b High magnification electron micrograph of stress fibre. Periodic densities (d) are present. Magnification A × 24 0000; B × 55 000.

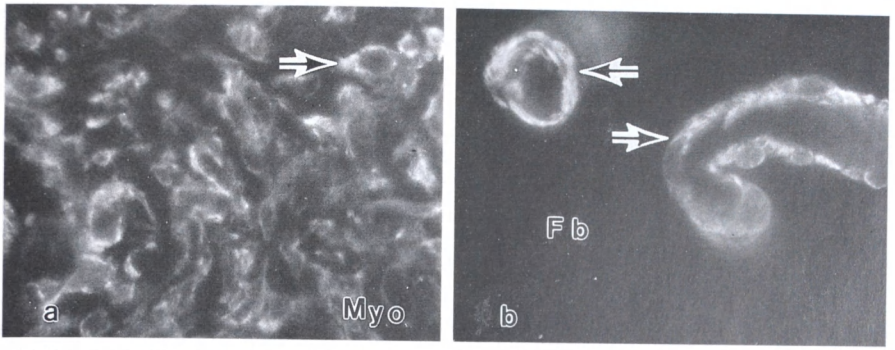

Fig. 8.3 Fluorescent micrographs of cryosections of (a) Dupuytren's nodular tissue and (b) normal palmar fascia stained with anti-actin antibody. a Bright staining for actin is seen in the cytoplasm of the cells in the stroma (arrow), showing the presence of a large population of myofibroblasts (Myo). b The fibroblasts (Fb) in the normal palmar fascia do not stain for actin. Vascular smooth muscle cells around blood vessels stain intensely for actin (arrows). Magnification × 800.

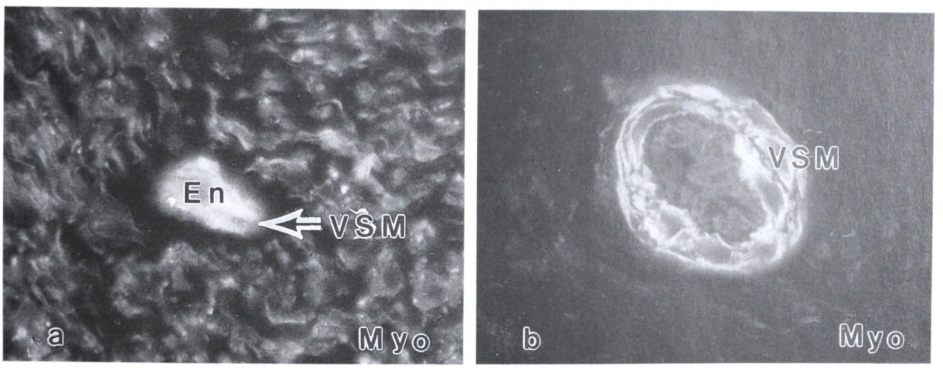

Fig. 8.4 Fluorescent micrographs of cryosections of Dupuytren's nodular tissue stained with either (a) anti-non-muscle myosin antibody or (b) anti-smooth muscle myosin antibody. a The myofibroblasts (Myo) in the stroma of the nodule stain heavily for non-muscle myosin. Vascular smooth muscle (VSM) does not stain with anti-non-muscle myosin antibody, but vascular endothelium (En) does. Myofibroblasts (Myo) present in the stroma of the Dupuytren's nodule do not stain with anti-smooth muscle myosin antibody. As expected, vascular smooth muscle (VSM) stains intensely with this antibody. Magnification × 800.

quantities in Dupuytren's myofibroblasts during the involutional phase of the disease. It should be emphasized that the myosin found in smooth muscle cells is distinct from that found in non-muscle cells. Although most anti-myosin antibodies do not distinguish between these two types of myosin, it is possible to prepare antibodies specific for the myosin in smooth muscle and non-muscle cells. These antibodies can identify these two distinct types of myosin in a variety of mammalian tissues examined by immunofluorescence microscopy. The anti-non-muscle myosin antibodies specifically stain non-muscle cell types, while anti-smooth muscle myosin antibodies stain only smooth muscle cells (Larson et al 1984).

Utilizing these specific antibodies in our 1986 study we found that Dupuytren's myofibroblasts contain non-muscle myosin and do not contain

smooth muscle myosin (Fig. 8.4). In these preparations only the vascular smooth muscle cells stained with anti-smooth muscle myosin antibodies (Fig. 8.4b). These results demonstrated that the Dupuytren's myofibroblast, with regard to its myosin, is a fibroblastic-type cell and not a smooth muscle-like cell; its cytoskeleton is that of an altered fibroblast.

STRESS FIBRES

If fibroblasts are cultured on a planar substratum such as plastic they can develop large bundles of actin microfilaments within their cytoplasm (Fig. 8.2). These intracellular bundles have been termed stress fibres and are found running parallel to the long axis of the cell (for review see Byers et al 1984). The distinguishing features of stress fibres are the organization of actin microfilaments into bundles, and the presence of various cytoplasmic proteins in an alternating pattern along their length. These proteins include non-muscle myosin, α-actinin, and tropomyosin. When examined by electron microscopy, stress fibres have electron-dense regions separated by an electron-translucent region along their length. Stress fibres terminate at the cell membrane, forming a specialized contact with the underlying substratum.

In DD the large actin bundles of microfilaments present in myofibroblasts appear similar to the stress fibres that form in cultured fibroblasts. These bundles of actin microfilaments parallel the long axis of the myofibroblast, have alternating electron-dense and electron-translucent regions along their length and terminate at the cell surface in specialized adhesion sites (see Figs 8.1, 8.9, 8.12 and 8.13). In addition our immunocytochemical studies demonstrated that myofibroblasts in Dupuytren's nodules contain large amounts of actin and non-muscle myosin but no smooth muscle myosin, similar to stress fibres.

The presence of stress fibres in myofibroblasts leads one to believe that this fibrillar system is contractile. The non-muscle myosin in stress fibres of cultured fibroblasts is organized in the form of bipolar filaments (Langanger et al 1986). In this configuration the myosin has the potential to mediate a sliding actomyosin movement. The addition of Mg^{2+}-ATP to isolated stress fibres causes these fibrils to contract (Isenberg et al 1976). Whether this will occur in cells is still unclear, although studies have suggested that stress fibres in cells can contract and exert tension upon the substratum (Harris et al 1980). Once formed, the contraction can be held for long periods of time.

This ability of stress fibres to contract and maintain tension is consistent with their proposed role in DD. It has been suggested that the active cellular process in DD involves contraction of the affected tissue and replacement of the contractile tissue with a new shorter tissue structure. The contraction of stress fibres in Dupuytren's myofibroblasts would bring together the tissue extremities.

The conditions leading to the development or visualization of stress fibres within Dupuytren's myofibroblasts are unknown. Fibroblasts will form stress fibres when cultured on a rigid planar substratum. The formation of stress fibres is dependent upon the adhesion of the cultured fibroblast to the underlying substratum. It has been demonstrated by Ali et al (1977) and Willingham et al (1977) that cells which adhere weakly to the substratum will not form stress fibres; however, when adhesion is increased by the addition of the extracellular glycoprotein fibronectin, stress fibres develop. Burridge (1981) proposed an explanation as to why tight adhesion to a substratum might induce the formation of stress fibres. Stress fibres may arise within a cell because it attempts to pull against a point of tight adhesion. If the substratum is resistant to deformation and the adhesion is strong enough so as not to break, tension will develop. Microfilaments would tend to line up along the line of force, forming large bundles of actin microfilaments. Thus strong adhesion to a substratum resistent to deformation would impose isometric conditions on a contraction, resulting in the formation of stress fibres. Once formed, stress fibres have the potential to contract and maintain tension upon the substratum. Dupuytren's myofibroblasts appear to be strongly adherent to the surrounding tissue, as demonstrated by the presence of fibronectin at their surfaces and filamentous extracellular material connecting them to adjacent myofibroblasts and surrounding col-

lagen fibres (see below). Whether the stress fibres present in these cells form in response to isometric tension remains to be determined.

THE EXTRACELLULAR MATRIX

To produce clinical contraction the myofibroblast of DD, in addition to being inherently contractile, must also be able to transmit its intracellular force to the surrounding cells and extracellular matrix. In order for myofibroblasts to produce pathological effects they must be attached strongly to one another, and also to the surrounding collagen.

There are a group of adhesive glycoproteins that link cells to one another and to the surrounding extracellular matrix; these glycoproteins could fulfil the role of transmitting the necessary contractile force. The best understood of these anchoring molecules are the proteins known as fibronectins or, collectively, as fibronectin (*fibre* and *nectere* — to bind, tie; for review see Hynes 1986). The major function of fibronectin is as an adhesive protein. Fibronectin acts as a ligand attaching cells to various biological substrata including collagen and fibrin. The ability of fibronectin to link collagen and fibrin with the cell surface is related to its structure. The molecule is subdivided into functional domains that bind to collagen, fibrin and the cell surface (for review see Yamada 1983).

Laminin is another adhesion glycoprotein (for review see Kleinman et al 1984). Similar to fibronectin, laminin binds cells to surrounding collagen. Laminin however is specific in terms of its location and function, being found in basement membranes at the basal surface of epithelial cells and surrounding muscle cells.

We have demonstrated by immunocytochemistry the presence of increased amounts of fibronectin and the absence of laminin in Dupuytren's diseased palmar fascia. Cryosections of palmar nodules, which contained myofibroblasts as screened by anti-actin immunostaining and electron microscopy, were stained with anti-fibronectin antibody and anti-laminin antibody. These tissue cryosections demonstrated intense staining with anti-fibronectin antibody, whereas no staining was observed with anti-laminin antibody in the extracellular matrix surrounding myofibroblasts (Figs 8.5 and 8.6). Laminin was localized only in the basement membranes surrounding endothelial cells and smooth muscle cells in blood vessels (Fig. 8.6).

Fibronectin is present in increased amounts in diseased tissue but only in association with the presence of myofibroblasts (Fig. 8.7a). Little if any fibronectin is present around fibroblasts in the

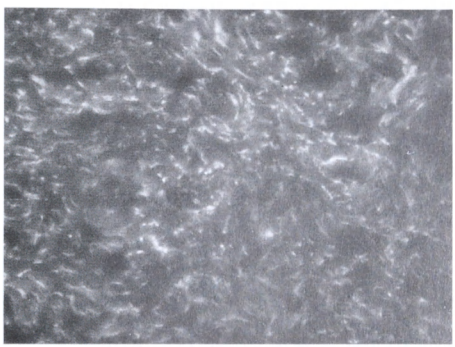

Fig. 8.5 Fluorescent micrograph of a cryosection of Dupuytren's nodular tissue stained with anti-fibronectin antibody. Bright fibrillar staining for fibronectin can be seen in the extracellular matrix around myofibroblasts. Magnification × 800.

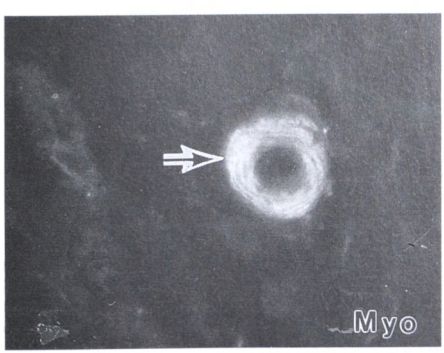

Fig. 8.6 Fluorescent micrograph of a cryosection of Dupuytren's nodular tissue stained with anti-laminin antibody. The basement membranes surrounding blood vessels and vascular smooth muscle stain brightly for laminin (arrow). No staining for laminin is observed around myofibroblasts (Myo) present in the stroma of the nodule. Magnification × 800.

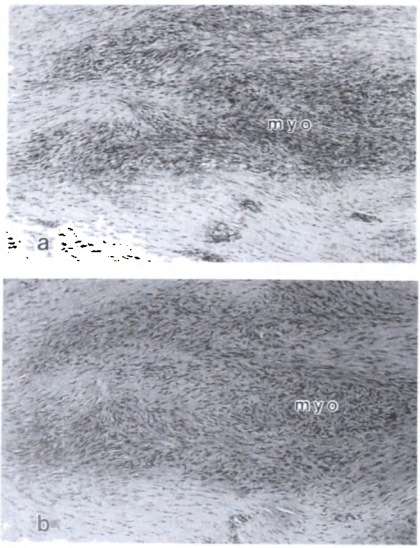

Fig. 8.7 Light micrographs of adjacent cryosections of Dupuytren's diseased palmar fascia stained by immunoperoxidase with either (a) anti-actin antibody or (b) anti-fibronection antibody followed by staining with haematoxylin & eosin. a The myofibroblasts (myo) organized into a nodule stain intensely with anti-actin antibody. The fibroblasts in the surrounding band tissue do not stain. Vascular smooth muscle cells around blood vessels in the surrounding band tissue also stain. b The extracellular matrix around myofibroblasts stains intensely with anti-fibronectin antibody. The extracellular matrix around fibroblasts in the surrounding band tissue does not stain. Magnification × 80.

surrounding band tissue (Fig. 8.7b). Similarly, little if any fibronectin is present around fibroblasts in normal palmar fascia.

The fact that laminin does not appear to be present in DD, and therefore does not play a role in linking Dupuytren's myofibroblasts to the surrounding tissue, further demonstrates that the myofibroblast is not a smooth muscle cell.

For this reason we feel that the term myofibroblast may be misleading, and prefer to regard this cell as a 'specialized contractile fibroblast'. In view of this for the rest of the chapter we will refer to this cell as a 'tractofibroblast'.

As yet we have not determined whether the

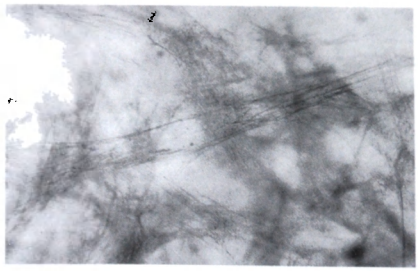

Fig. 8.8 Light micrograph of a Dupuytren's tractofibroblast cultured within a hydrated collagen lattice and stained by immunoperoxidase with anti-human fibronectin antibody. Fibrillar staining for fibronectin is present at the surface of tractofibroblasts. Since this antibody only recognizes human fibronectin, these fibronectin fibrils must have been produced by the cultured tractofibroblasts. Magnification × 400.

fibronectin present at the surfaces of Dupuytren's tractofibroblasts is synthesized by these cells. However, utilizing a monoclonal antibody specific for human fibronectin we have found that Dupuytren's tractofibroblasts cultured within hydrated collagen lattices can synthesize and accumulate fibronectin fibrils at their surfaces (Fig. 8.8). This suggests that they may be able to do the same in vivo.

Fibronectin probably plays a similar role in granulation tissue and hypertrophic scars. It has been localized by immunocytochemistry in the extracellular matrix surrounding myofibroblasts in granulation tissue and hypertrophic scars (Grinnell et al 1981; Kischer & Hendrix 1983). In addition, an immunocytochemical study from our laboratory has demonstrated the presence of fibronectin and the lack of laminin around myofibroblasts in these tissues (Eddy et al 1987), similar to our observations in DD.

THE INTERCONNECTING BONDS

Two features must be present to produce contracture: a contractile cell and the presence of an interconnecting link for the contracting cell to pull on. Ultrastructural studies by Gabbiani & Majno (1972), Chiu & McFarlane (1978) and Tomasek et

al (1987) have demonstrated that there are physical connections between individual tractofibroblasts and collagen fibres in Dupuytren's nodules. By electron microscopy we have been able to identify further the nature of these physical connections.

Structure of filamentous extracellular material

Our ultrastructural studies have demonstrated that the extracellular connections are composed of two types of fibrils — fine fibrils with a diameter of 3–5 nm and, of lesser frequency, large fibrils, 10–13 nm in diameter (Fig. 8.9). These fibrils, found only associated with tractofibroblasts, intermingle with each other, making a complex which we refer to as filamentous extracellular material.

This extracellular material at the surfaces of Dupuytren's tractofibroblasts has been described by numerous investigators as basal lamina-like or basement membrane-like (Gabbiani & Majno 1972; Chiu & McFarlane 1978; Meister et al 1979; Gelbermann et al 1980). Although in some ways this filamentous extracellular material does resemble a basal lamina and therefore appears basal lamina-like, it is however ultrastructurally and biochemically (see above) distinct from other basal laminae located at the basal surfaces of epithelia, endothelia, and surrounding skeletal and smooth muscle cells.

The filamentous extracellular material at the surface of Dupuytren's tractofibroblasts is probably in part fibronectin. Preliminary results from our laboratory have demonstrated that Dupuytren's tractofibroblasts cultured in hydrated collagen lattices will form large bundles of actin microfilaments and associated filamentous extracellular material, identical to tractofibroblasts in vivo (Fig. 8.10). Immunoelectron microscopy has demonstrated that this filamentous extracellular material will stain with anti-fibronectin antibody (Fig. 8.11).

Location of filamentous extracellular material

The filamentous extracellular material associated with tractofibroblasts is present only in close transmembrane apposition to intracellular bundles of 5 nm actin microfilaments at the tractofibroblast's

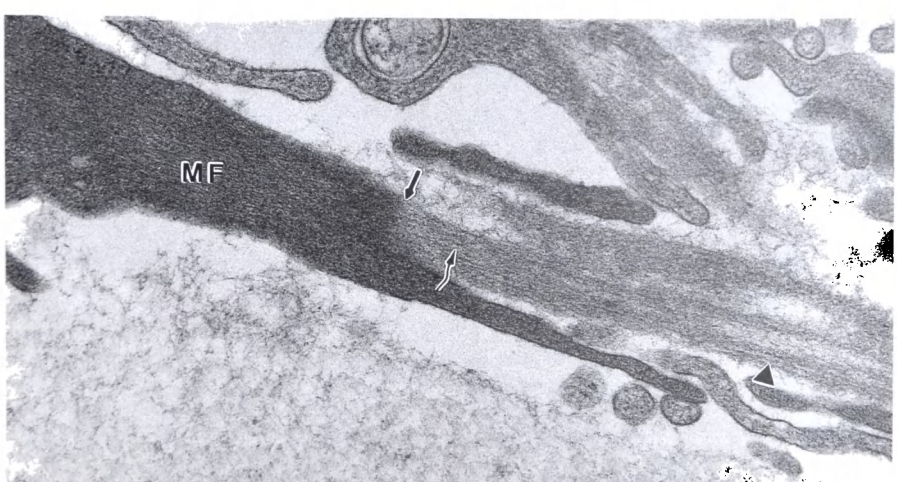

Fig. 8.9 Electron micrograph of a Dupuytren's tractofibroblast. Filamentous extracellular material is composed of fine filaments (curved arrow), 3–5 nm in diameter, and larger filaments (arrowhead) 10–13 nm in diameter. Intracellular actin microfilaments (MF) and extracellular filaments appear to be colinear at the cell surface in an end-to-end association (arrow). Note the absence of a morphologically distinct cell membrane in the region of this transmembrane association. Magnification × 35 000.

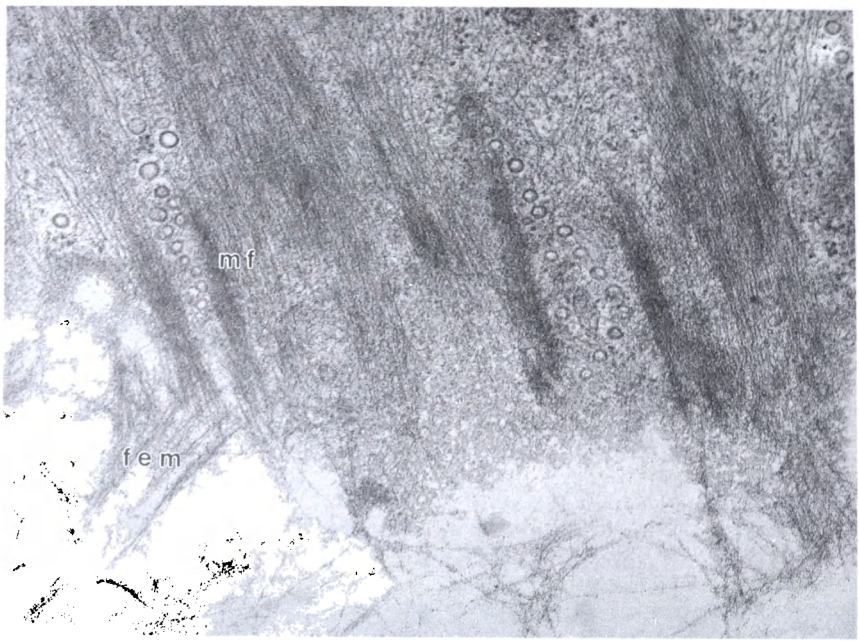

Fig. 8.10 Electron micrograph of a Dupuytren's tractofibroblast cultured within a hydrated collagen lattice. These cultured cells form large bundles of actin microfilaments (mf) and closely associated filamentous extracellular material (fem). Magnification × 38 000.

surface. Here there are two types of transmembrane relations between actin microfilaments and filamentous extracellular material: end-to-end and lateral associations. End-to-end associations consist of 5 nm actin microfilaments and filamentous extracellular material which appear to be colinear across the cell membrane (see Figs 8.1, 8.9, 8.12 and 8.13). Lateral associations are composed of bundles of 5 nm actin filaments and filamentous extracellular material which parallel the intervening cell membrane (Figs 8.12 and 8.13).

The filamentous extracellular material may extend from transmembrane associations at the tractofibroblast's surface to transmembrane associations at the surface of adjacent tractofibroblasts (Fig. 8.14), or to terminate in the surrounding extracellular matrix (Fig. 8.15). Thus filamentous extracellular material participating in transmembrane associations can link the actin cytoskeleton of one tractofibroblast with that of surrounding tractofibroblasts and also with the extracellular matrix.

Although at the time of writing, the exact function of the filamentous extracellular material is unclear, it is possible to speculate on its role in producing flexion deformity in DD. In its specific location the filamentous extracellular material is in a key position to transmit the contractile force generated by the tractofibroblast from cell to cell and to the surrounding tissue. The glycoprotein fibronectin is ideally suited for being the connecting macromolecule since it can bind to the surfaces of cells and collagen fibres and in addition, will form fibrils at the surface of cells. Thus we feel that this filamentous extracellular material is the

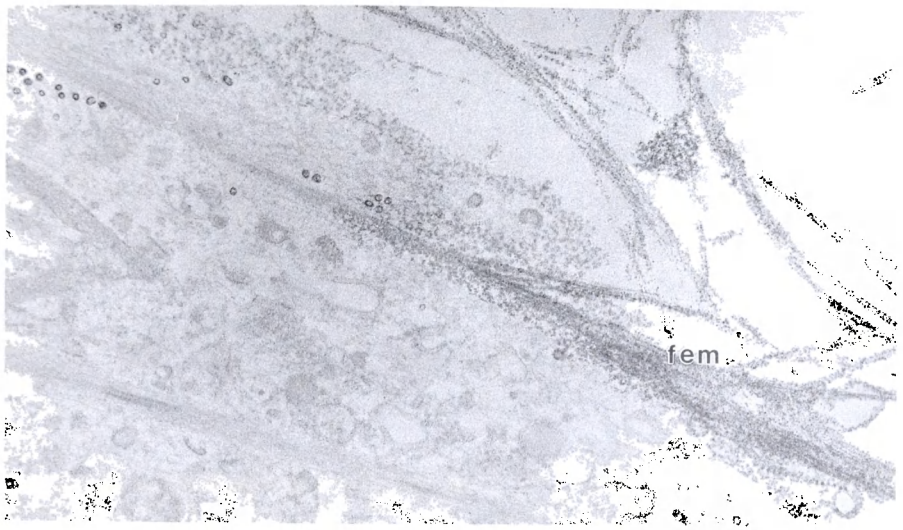

Fig. 8.11 Electron micrograph of a Dupuytren's tractofibroblast cultured within a hydrated collagen lattice and stained by immunoperoxidase with anti-fibronectin antibody. The filamentous extracellular material (fem) present at the surface of the tractofibroblast stains intensely for fibronectin. Magnification × 17 000.

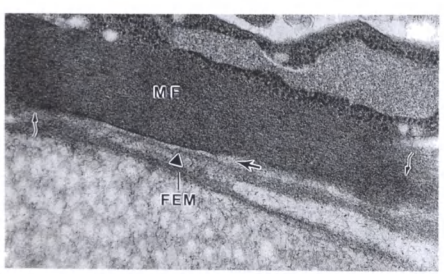

Fig. 8.12 Electron micrograph of a Dupuytren's tractofibroblast. Filamentous extracellular material (FEM) associated with this tractofibroblast is composed only of fine filaments 3–5 nm in diameter. Both end-to-end (curved arrows) and lateral (arrowhead) associations between actin microfilaments (MF) and filamentous extracellular material are present at the surface of this tractofibroblast. An electron translucent space (arrow) separates the cell membrane and filamentous extracellular material in the lateral association. Magnification × 24 000.

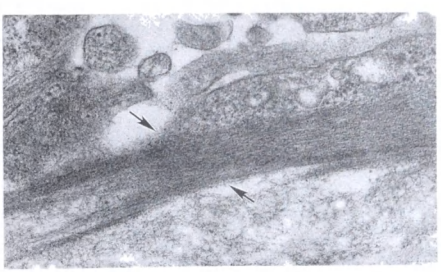

Fig. 8.13 Electron micrograph of a Dupuytren's tractofibroblast. A well defined end-to-end transmembrane association of a bundle of actin microfilaments (MF) and filamentous extracellular material (FEM) is present at the surface of this tractofibroblast. The cell membrane in the region of the end-to-end transmembrane association is not distinct (arrows). Actin microfilaments and filamentous extracellular material appear to be colinear across this transmembrane association. Magnification × 21 000.

key link in attaching tractofibroblasts in Dupuytren's diseased tissue to surrounding tractofibroblasts and collagen fibres.

Connections between bundles of actin microfilaments and filamentous extracellular material are not restricted to Dupuytren's diseased tissue. Fibronectin-rich filamentous extracellular material with the same ultrastructure and similar end-to-

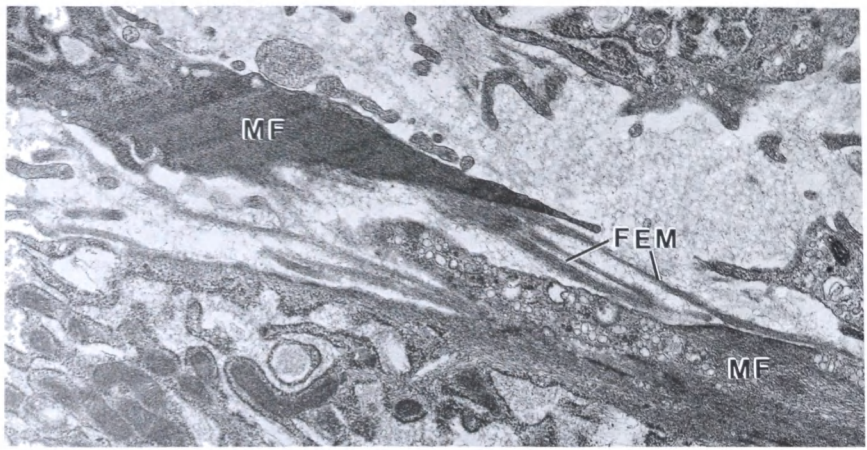

Fig. 8.14 Electron micrograph of a Dupuytren's tractofibroblast. Bundles of filamentous extracellular material (FEM) traverse the surrounding extracellular matrix connecting adjacent tractofibroblasts. Bundles of actin microfilaments (MF) are present within tractofibroblasts. Magnification × 14 000.

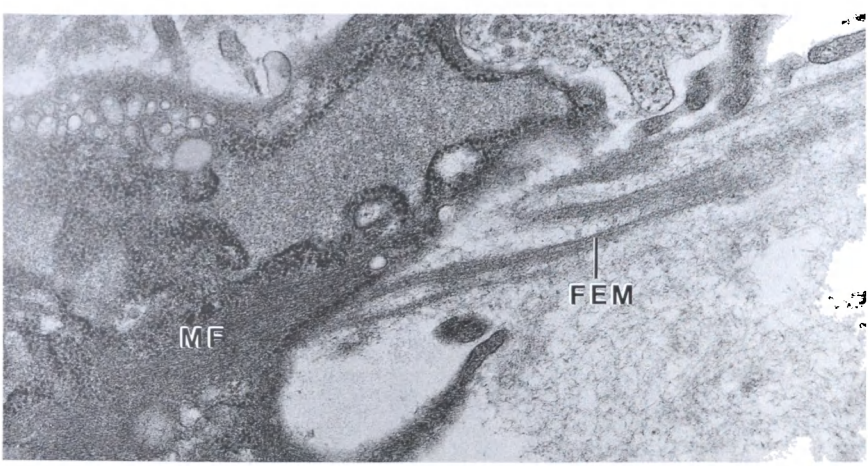

Fig. 8.15 Electron micrograph of a Dupuytren's tractofibroblast. Bundles of filamentous extracellular material (FEM) extend from the surface of a Dupuytren's tractofibroblast into the surrounding extracellular matrix. The filaments composing these bundles appear to disperse and interdigitate with the fibrous matrix surrounding these cells. This filamentous extracellular material is closely associated with an intracellular bundle of actin microfilaments (MF). Magnification × 21 000.

end transmembrane associations with actin microfilaments have been described at the surfaces of cultured fibroblasts by Singer (1979). This transmembrane association between extracellular fibronectin and intracellular actin has been termed the 'fibronexus'. The formation of fibronexus was suggested to occur as a result of attachment by fibronectin to the underlying substratum.

Fibronexus have been described at the surface of myofibroblasts in granulation tissue (Singer et al 1984). The presence of fibronexus in granulation tissue strongly suggests that they are an important in vivo cell surface adhesion site functioning in wound repair. The ultrastructural similarity between the fibronexus seen at the surfaces of myofibroblasts in granulation tissue and the transmembrane associations described at the surfaces of tractofibroblasts in DD suggest they may be the same structure.

TRANSMEMBRANE LINKAGE

The intimate relationship of filamentous extracellular material and intracellular actin microfilaments at the surfaces of tractofibroblasts in Dupuytren's diseased tissue suggests they are physically connected. Since the cell membrane is interposed between them they cannot be in direct contact. If they are to be physically joined there must be a transmembrane linkage connecting them.

This transmembrane linkage may be formed in part by integrin, a complex of cell surface receptors that connect the extracellular matrix and the cell's cytoskeleton (for review see Hynes 1987). This complex spans the cell membrane and contains an extracellular domain, a transmembrane segment and a cytoplasmic domain (Tamkun et al 1986). The extracellular domain can bind directly to fibronectin. The intracellular domain can bind to talin, a cytoskeletal protein that can bind indirectly to actin (Horwitz et al 1986). In addition, integrin has been localized at the surface of cultured cells where extracellular fibrils containing fibronectin and intracellular bundles of actin are closely associated (Chen et al 1985).

It would seem that integrin is a crucial component of the transmembrane linkage between fibronectin-rich extracellular matrices and the cytoskeleton. It is possible to speculate as to the role of such a linkage in the adhesion of Dupuytren's tractofibroblasts to the surrounding tissue. A simplified model of the connection of bundles of actin microfilaments within a Dupuytren's tractofibroblast to the surrounding tractofibroblasts and collagen fibres via such a transmembrane linkage and extracellular fibronectin fibrils is illustrated in Figure 8.16. As indicated in the diagram, actin microfilaments and associated non-muscle myosin could bind either directly or indirectly through actin-binding proteins to the transmembrane linkage. A transmembrane linkage such as integrin could span the cell membrane coupling intracellular actin microfilaments with extracellular fibronectin fibrils. Extracellular fibrils could extend from these surface associations attaching tractofibroblasts to either adjacent tractofibroblasts or surrounding collagen fibres. That the bundles of actin microfilaments in Dupuytren's tractofibroblasts are connected to surrounding tractofibroblasts and collagen fibres is clear; whether this connection involves integrin as the transmembrane linkage and fibronectin as the filamentous extracellular material remains to be determined.

CONCLUSIONS

The myofibroblast described so well by Gabbiani & Majno (1972) has the morphological appearance of both smooth muscle cells and fibroblasts. Analysis of proteins of the intracellular cytoskeleton and extracellular matrix by immunocytochemistry failed to demonstrate any muscle elements; only proteins characteristic of a fibroblastic type cell were observed. Thus the specialized contractile cell in DD appears to be a fibroblast which has altered its cytoskeleton and surrounding extracellular matrix. Based upon these results we suggest that the term 'myofibroblast' may be misleading for this specialized contractile fibroblast and prefer to call this cell a 'tractofibroblast' from the Latin *tractare* — to pull.

The altered cytoskeleton of the tractofibroblast

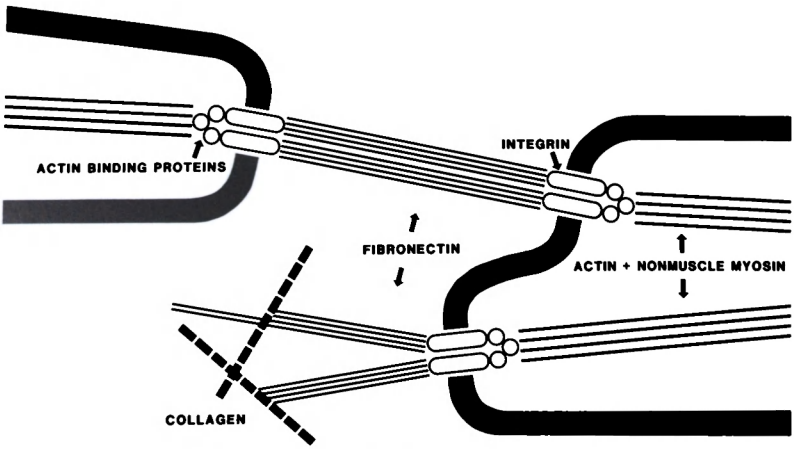

Fig. 8.16 The interconnection of tractofibroblasts with each other and the surrounding collagen fibres. See text for description.

is composed of large bundles of actin microfilaments with associated non-muscle myosin. These bundles of microfilaments resemble stress fibres in cultured fibroblasts. The generation of intracellular contractile force by the tractofibroblast could come about by an interaction of the actin and non-muscle myosin organized into these bundles.

Alterations in the extracellular matrix of tractofibroblasts occur in the form of increased amounts of fibronectin, along with the presence of filamentous extracellular material. Bundles of actin microfilaments terminate at specific locations at the tractofibroblast's surface in close association with filamentous extracellular material. At these specific locations the intracellular bundles of actin microfilaments are connected to filamentous extracellular material through a transmembrane linkage. Extracellular filamentous material extends from these transmembrane linkages on adjacent tractofibroblasts, connecting the contractile cytoskeletons of these cells to each other. In addition, filamentous extracellular material extends from these transmembrane linkages into the surrounding extracellular matrix, connecting the cytoskeleton with surrounding collagen fibres. This filamentous extracellular material, composed of fibronectin, forms a physical connection that could transmit intracellular contractile force to the surrounding tissue.

Acknowledgements

We thank Carol Haaksma and Charles Episalla for their excellent technical assistance in the experiments described in this chapter. We also thank the members of the New York Society for Surgery of the Hand for their contributions of Dupuytren's tissue. The original research reported in this chapter was supported by a grant from the Orthopedic Research and Education Foundation.