Extracellular matrix-cytoskeletal connections at the surface of the specialized contractile fibroblast (myofibroblast) in Dupuytren disease

JJ Tomasek, RJ Schultz and CJ Haaksma

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Extracellular Matrix-Cytoskeletal Connections at the Surface of the Specialized Contractile Fibroblast (Myofibroblast) in Dupuytren Disease*

BY JAMES J. TOMASEK, PH.D.†, ROBERT J. SCHULTZ, M.D.†, AND CAROL J. HAAKSMA, B.S.†, VALHALLA, NEW YORK

From the Departments of Orthopaedic Surgery and Anatomy, New York Medical College, Valhalla

ABSTRACT: **The cellular basis of contracture of the palmar fascia in patients who have Dupuytren disease involves the generation of intracellular force and the transmission of this force to the surrounding tissue. A specialized cell, the myofibroblast, supposedly generates this intracellular force. Recently published studies from our laboratory demonstrated that the cytoskeleton of the myofibroblast contains non-muscle myosin and not smooth-muscle myosin, suggesting that it utilizes a non-muscle contractile system. In addition, these studies identified the extracellular glycoprotein fibronectin, not the basal-lamina-specific glycoprotein laminin, at the surface of myofibroblasts, suggesting that the transmission of the intracellular force to the surrounding tissue also occurs by a non-muscle mechanism. Because of the lack of proteins that are specific to smooth muscle in the specialized cell in Dupuytren disease, we prefer the term specialized contractile fibroblast to describe this type of cell. To determine the mechanism by which the intracellular force may be transmitted to the surrounding tissue, we examined the ultrastructure of the connection of the cytoskeleton of the specialized contractile fibroblast to the surrounding extracellular matrix. By electron microscopy, extracellular filamentous material was identified at the surface of the specialized contractile fibroblast. These extracellular fibrils were found to be in close association with intracellular bundles of actin microfilaments, resulting in specialized transmembranous associations at the surface of the specialized contractile fibroblast. Bundles of filamentous extracellular material were found to extend from the surface of the specialized contractile fibroblast, connecting it with the surrounding matrix and also with adjacent specialized contractile fibroblasts. The filamentous extracellular material is ultrastructurally distinct from basal laminae and thus is consistent with a non-muscle mechanism of force transmission. Through this transmembranous connection of filamentous extracellular material to actin microfilaments, the contractile cytoskeleton is brought in contact with the surrounding tissue.**

CLINICAL RELEVANCE: **Contracture of the palmar fascia in patients who have Dupuytren disease depends on the transmission of intracellular contractile forces to the surrounding extracellular matrix and neighboring contractile cells. Identification of this connection is essential to understanding the pathophysiology of Dupuytren disease as well as other contracture problems. Interruption of these connections may inhibit the development of contractures.**

Dupuytren disease is characterized by the presence of a specialized cell that was termed the myofibroblast by Gabbiani and Majno because of its morphological similarities to fibroblastic and smooth-muscle cells[6]. The presence of this specialized cell in the nodules of Dupuytren disease has led to the proposal that the myofibroblast is responsible for digital flexion in Dupuytren contracture[1,4,6,7,14]. If we are to understand how the myofibroblast can act as the agent of contracture of the palmar fascia, we must understand how it generates intracellular force and transmits this force to the surrounding tissue.

Hypothetically, the myofibroblast could generate and transmit contractile force using either a smooth-muscle or a non-muscle mechanism, since structurally and functionally it resembles both of these types of cells[1,4,6,7,14]. To determine the mechanism that is utilized by the Dupuytren myofibroblast, we studied the cytoskeletal and extracellular matrix proteins that are associated with it[21].

Myosin is a cytoskeletal protein that interacts with actin to generate contractile force in both muscle and non-muscle cells[8]. The myosin that is found in smooth-muscle cells is distinct from that found in non-muscle cells[8]. In previous studies, large amounts of actin and myosin have been identified in Dupuytren myofibroblasts; however, the researchers

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† Basic Sciences Building, New York Medical College, Valhalla, New York 10595.

did not determine whether the actin or myosin that is present in these cells is of the smooth-muscle or non-muscle type[6,14]. Using antibodies that can distinguish between smooth-muscle and non-muscle myosin[13], we determined by indirect immunofluorescence that the myofibroblasts in the nodules of Dupuytren disease contain only non-muscle myosin[21].

Smooth-muscle cells are surrounded by a highly organized layer of extracellular material that is called basal lamina[16]. Characteristic of this basal lamina is the extracellular glycoprotein laminin, which binds smooth-muscle cells to the surrounding layer of type-IV collagen[5,19]. In contrast, fibroblasts lack basal lamina and laminin. The extracellular glycoprotein fibronectin attaches fibroblasts to the surrounding type-I and type-III collagen[11]. We examined the extracellular matrix macromolecules that are associated with Dupuytren myofibroblasts, employing antibodies that are specific for laminin or fibronectin in conjunction with indirect immunofluorescence[21]. That study demonstrated that these cells are surrounded by a matrix that is rich in fibronectin but lacks laminin[21]. Thus, these studies have demonstrated that the myofibroblast in Dupuytren disease is not a smooth-muscle-type cell but rather a specialized

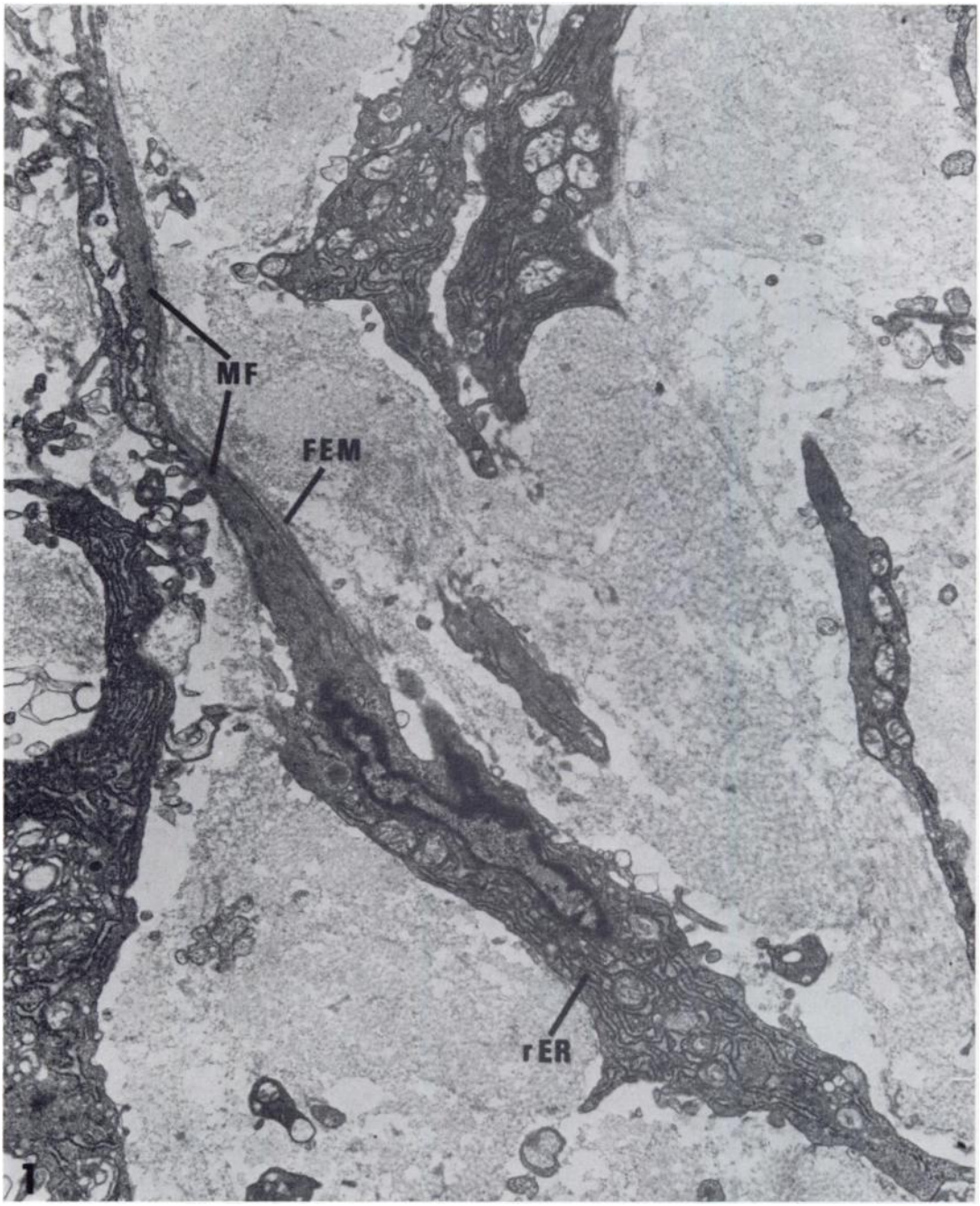

FIG. 1

A typical specialized contractile fibroblast in a Dupuytren nodule. This cell contains actin microfilament bundles (MF) and well developed rough endoplasmic reticulum (rER). A layer of filamentous extracellular material (FEM) covers part of the surface of the myofibroblast (× 9,000).

non-muscle connective-tissue cell that has a cytoskeletal structure with the potential to undergo contraction[21]. Because of the lack of smooth-muscle-specific proteins and to avoid associating this cell with a smooth-muscle cell, we prefer the term specialized contractile fibroblast.

The process by which specialized contractile fibroblasts participate in the contracture of the palmar fascia in Dupuytren disease is unclear. Contractile intracellular force may be generated in the specialized contractile fibroblast by the interaction of bundles of actin microfilaments, the enzyme calcium adenosine triphosphatase[1], and, based on our studies, large quantities of non-muscle myosin[21]. For contracture of the palmar fascia to occur, contractile intracellular forces must be transmitted to the surrounding extracellular matrix and cells; otherwise there would be contraction only of isolated cells. Previous ultrastructural studies of myofibroblasts in the nodules of Dupuytren disease described a discontinuous layer of basal-lamina-like or basement-membrane-like material at their surface; it was postulated that this material was involved in the transmission of intracellular contractile force[4,6,7,14]. However, as already noted, the results of our immunocytochemical studies indicated that a basal lamina is not present at the surface of the specialized contractile fibroblast in Dupuytren disease[21].

In this study, electron microscopy was used to examine the structure and location of the extracellular material at the surface of specialized contractile fibroblasts in Dupuytren nodules. The structure of the material was not found to be similar to that of basal laminae. It is composed of filamentous material that lies close to intracellular bundles of actin microfilaments, resulting in specialized transmembranous associations at the surface of the specialized contractile fibroblast. This filamentous material was found to connect the specialized contractile fibroblasts with the surrounding extracellular matrix as well as with one another. Because of its location and structure, the filamentous extracellular material that is associated with specialized contractile fibroblasts may play an important role in the pathogenesis of Dupuytren contracture, linking the contractile intracellular cytoskeleton with the surrounding tissue.

Materials and Methods

Palmar fascia from thirty-four patients who had Dupuytren contracture was removed at operation and was transported to the laboratory in ice-cold balanced saline solution. The surrounding cord and apparently normal fascia were dissected away from the nodular tissue, which was defined as a hard, fusiform thickening in the palmar fascia. Pieces of nodular tissue were examined by two different methods: light-microscopic immunocytochemistry and electron microscopy.

Pieces of tissue were prepared for immunocytochemical staining as has been previously described[21]. Briefly, pieces of tissue were fixed in 4 per cent paraformaldehyde in 0.1-molar sodium phosphate buffer, pH 7.4; infiltrated with 30 per cent sucrose; and frozen in OCT compound (Lab-Tek Products, Miles Laboratories, Naperville, Illinois) by rapid immersion in liquid nitrogen. Cryostat-cut sections (four to six micrometers) were thaw-mounted on glass slides and allowed to air-dry at room temperature. The sections were stained with a monoclonal anti-actin antibody[3,15] and then with a secondary antibody (goat anti-mouse IgA + IgG + IgM-rhodamine; Cappel Laboratories, West Chester, Pennsylvania) and were examined by incident-light fluorescence on a Leitz Orthoplan microscope.

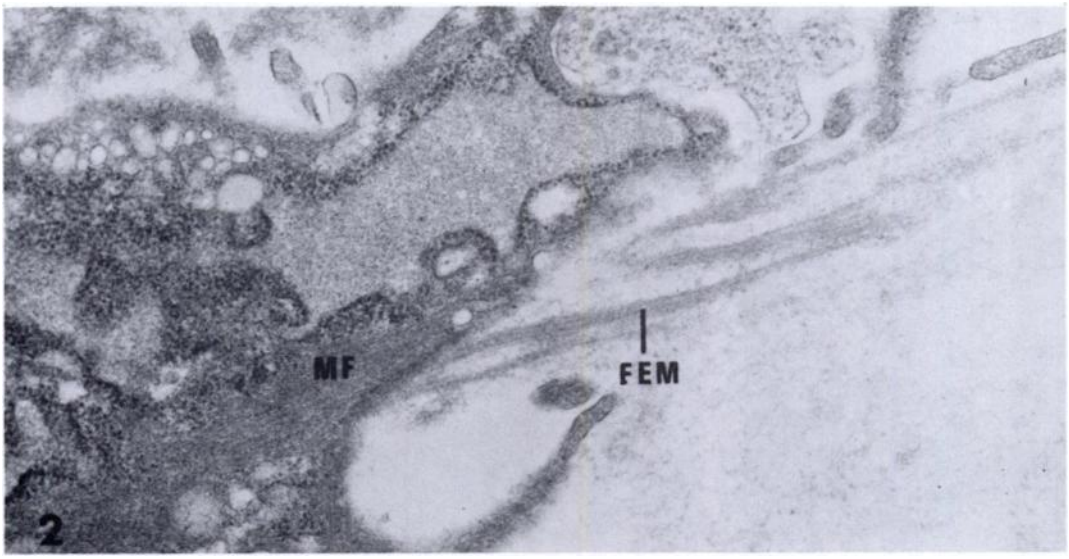

FIG. 2

Bundles of filamentous extracellular material (FEM) extend from the surface of a specialized contractile fibroblast into the surrounding extracellular matrix. The filaments composing the 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) (× 20,000).

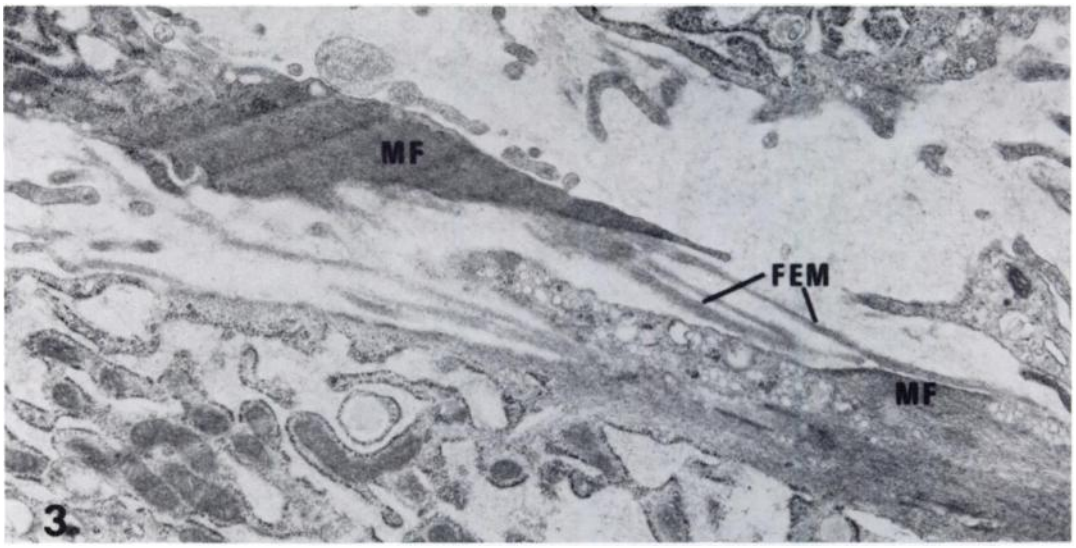

FIG. 3

Bundles of filamentous extracellular material (FEM) traverse the surrounding extracellular matrix, connecting adjacent specialized contractile fibroblasts. Bundles of actin microfilaments (MF) are present within specialized contractile fibroblasts (× 13,000).

Pieces of tissue were prepared for electron microscopy as has been previously described[21]. The tissue was placed in half-strength Karnovsky fixative[12] for one hour at room temperature and then was rinsed rapidly, three times, in 0.1-molar sodium cacodylate buffer, pH 7.4. The tissue was post-fixed in 1 per cent osmium tetroxide in 0.1-molar sodium cacodylate buffer, rinsed in water, stained *en bloc* with 1 per cent aqueous uranyl acetate, dehydrated in a graded series of ethanol, and embedded in Polybed 812 (Polysciences, Warrington, Pennsylvania). Ultra-thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined on a Japanese JEOL 100C transmission electron microscope.

Results

This study was an examination of the ultrastructure of the myofibroblasts — or, as we prefer to call this type of cell, the specialized contractile fibroblasts — that are present in the nodules of excised diseased palmar fascia in patients who have Dupuytren disease. The palmar fascia that was to be studied by electron microscopy was examined first for the presence of specialized contractile fibroblasts by indirect immunofluorescence with anti-actin antibody. This procedure permits screening large quantities of surgically excised tissues, as specimens that do not stain by this technique do not contain specialized contractile fibroblasts[21]. Thirty-four surgical specimens were examined. Nine of them stained intensely with anti-actin antibody and were studied by electron microscopy. Our inability to detect large numbers of specialized contractile fibroblasts in all thirty-four specimens of diseased palmar fascia is consistent with the results of previous studies that suggested that the specialized contractile fibroblast is a transient type of cell that is present in the nodule during only the active stage of the disease[4,7,14,21]. Each nodule that was determined by light-microscopic immunocytochemistry to contain large numbers of specialized contractile fibroblasts also was found to contain these cells when it was studied by electron microscopy. The specialized contractile fibroblasts were easily identified by their intracellular bundles of actin microfilaments, well developed rough endoplasmic reticulum and Golgi apparatus, and a layer of extracellular material covering part of the surface of the cell (Fig. 1).

The layer of extracellular material that is associated with the specialized contractile fibroblast was localized by electron microscopy. The other types of cells within the nodule — such as fibroblasts, vascular smooth-muscle cells, and endothelia — do not have a similar layer of extracellular material associated with them. This layer of extracellular material not only is closely related to specialized contractile fibroblasts but also extends from their surfaces (Figs. 2 and 3). Bundles of extracellular material were observed to extend either into the surrounding extracellular matrix to terminate or from the surface of one specialized contractile fibroblast to the surface of an adjacent one (Figs. 2 and 3). The bundles that appear to terminate in the matrix disperse into individual filaments and interdigitate with the surrounding extracellular matrix (Fig. 2). The question of whether these bundles actually terminate in matrix connections cannot be determined by conventional electron microscopy and it is currently under investigation using high-voltage electron microscopy. Bundles of extracellular material also span the matrix surrounding specialized contractile fibroblasts, linking them together (Fig. 3). These bundles have been observed to extend in length for as much as ten micrometers, terminating in close association with the surfaces of specialized contractile fibroblasts.

The extracellular material that is associated with specialized contractile fibroblasts is composed of fibrils (Fig. 4). Fine fibrils that are three to five nanometers in diameter are present, as are larger fibrils that are ten to thirteen nanometers in diameter. The larger fibrils are not always present

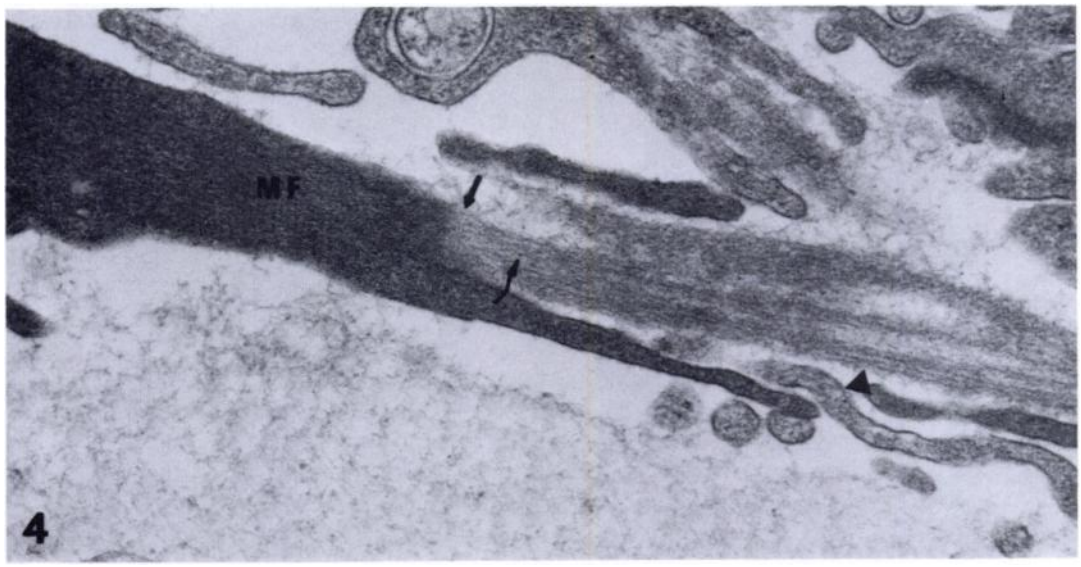

FIG. 4

Filamentous extracellular material is composed of fine filaments (curved arrow), three to five nanometers in diameter, and larger filaments (arrowhead), ten to thirteen nanometers in diameter. Intracellular actin microfilaments (MF) and extracellular filaments appear to be co-linear at the cellular surface in an end-to-end association (arrow). Note the absence of a morphologically distinct cellular membrane in the region of this transmembranous association (× 32,000).

(Fig. 5), but when they are they appear to be intermixed with the fine fibrils (Fig. 4). In previous studies, this layer of extracellular material was termed basal-lamina-like or basement-membrane-like[1-4]. In view of the morphological appearance of this layer of extracellular material, we think that filamentous extracellular material would be a better term.

The filamentous extracellular material that is associated with specialized contractile fibroblasts is not a continuous layer; it is present only at certain regions of the surface of the specialized contractile fibroblast. Where this material is present, it is in close transmembranous apposition to intracellular bundles of five-nanometer actin microfilaments (Figs. 2 through 8). Other cytoskeletal components, such as ten-nanometer intermediate filaments (Fig. 6) or microtubules (not shown), were not found to be closely associated with the filamentous extracellular material.

Two types of transmembranous relations between actin microfilaments and filamentous extracellular material were observed: lateral and end-to-end associations. The lateral associations are composed of bundles of five-nanometer actin microfilaments and filamentous extracellular material that parallel the intervening cellular membrane (Figs. 5 and 6). An electron-translucent layer is present between the cel-

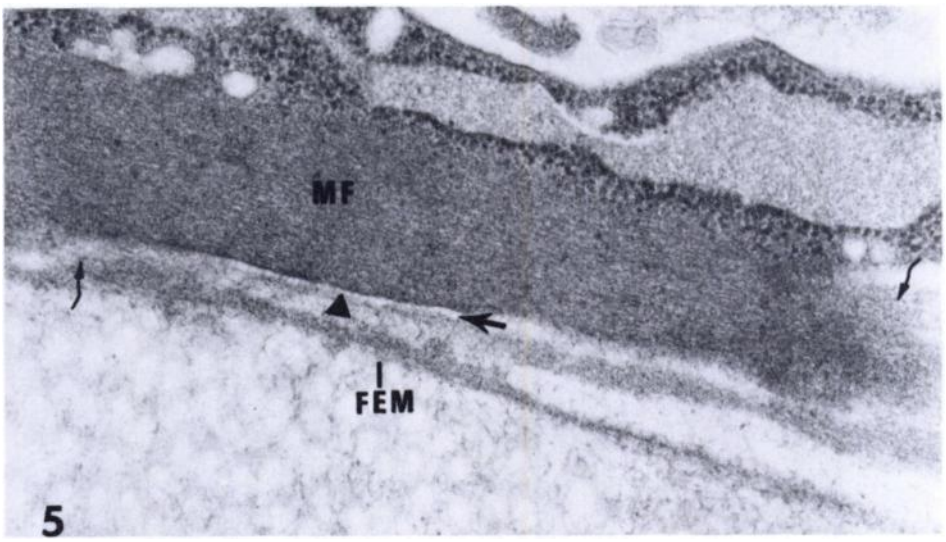

FIG. 5

Filamentous extracellular material (FEM) that is associated with this specialized contractile fibroblast is composed only of fine filaments, three to five nanometers in diameter. Both end-to-end associations (curved arrows) and lateral associations (arrowhead) between actin microfilaments (MF) and filamentous extracellular material are present at the surface of this specialized contractile fibroblast. An electron-translucent layer (arrow) separates the cellular membrane and the filamentous extracellular material in the lateral association (× 40,000).

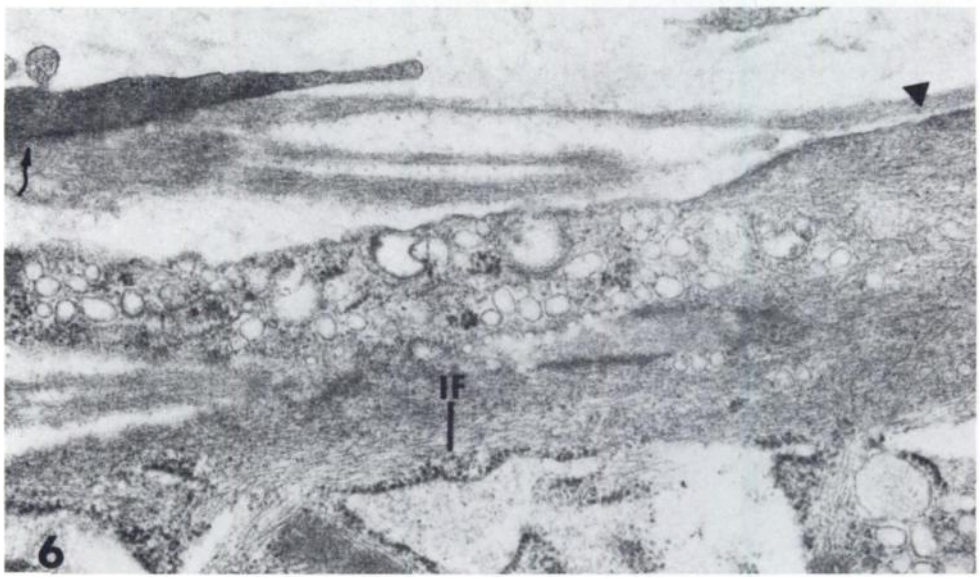

FIG. 6

This higher-magnification electron micrograph of Fig. 3 shows bundles of filamentous extracellular material connecting two adjacent specialized contractile fibroblasts. A bundle of this material ends on one specialized contractile fibroblast in an end-to-end association (curved arrow), while it ends on the other specialized contractile fibroblast in a lateral association (arrowhead). Ten-nanometer intermediate filaments (IF) are not found closely associated with filamentous extracellular material (× 27,000).

lular membrane and the filamentous extracellular material. The order of arrangement of these structures from within the cell outward is: actin microfilaments, cellular membrane, electron-translucent layer, and filamentous extracellular material. The width of the electron-translucent layer ranges from twenty to thirty nanometers.

End-to-end associations consist of five-nanometer actin microfilaments and filamentous extracellular material that appear to be co-linear across the cellular membrane (Figs. 4, 7, and 8). The structure of the cellular membrane in the region of end-to-end associations is not distinct. Neither actin nor extracellular macromolecules such as fibronectin or collagen are transmembranous proteins. Therefore another component, a transmembranous protein or proteins linking actin and the filamentous extracellular material, must be present at the interface. The close association of the actin microfilaments, the filamentous extracellular material, and a transmembranous protein or proteins may obscure the structure of the cellular membrane.

In both types of associations, the filamentous extracellular material appears to link the cytoskeleton with the surrounding specialized contractile fibroblasts and extracellular matrix. The same bundle of filamentous extracellular material can participate in both a lateral and an end-to-end association on the surface of a specialized contractile fibroblast (Fig. 5) or adjacent specialized contractile fibro-

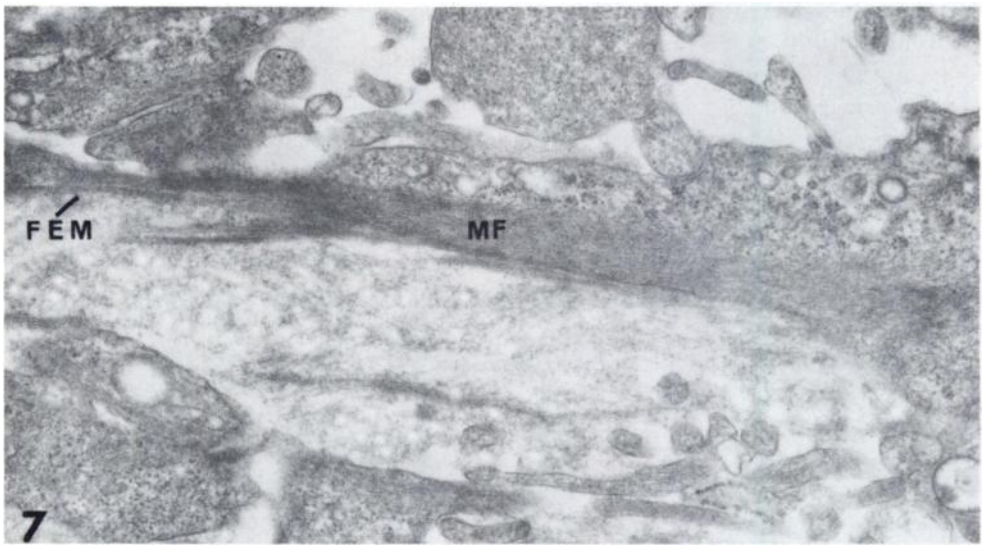

FIG. 7

A well defined end-to-end transmembranous association of a bundle of actin microfilaments (MF) and filamentous extracellular material (FEM) is present at the surface of this specialized contractile fibroblast (× 18,000).

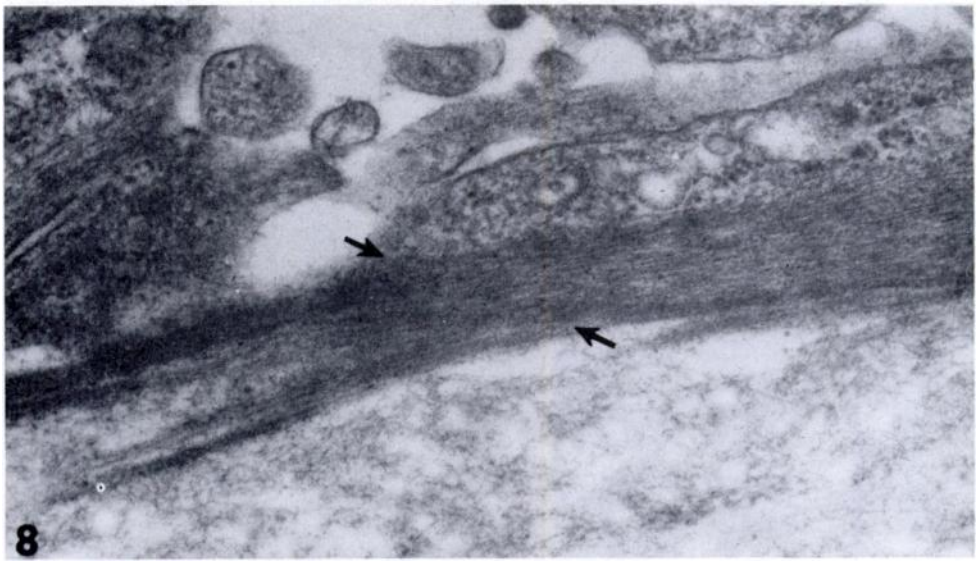

FIG. 8

This higher-magnification electron micrograph of Fig. 7 shows that the cellular membrane in the region of the end-to-end transmembranous association is not distinct (arrows). Actin microfilaments and filamentous extracellular material appear to be co-linear across this transmembranous association (× 36,500).

blasts (Fig. 6). Therefore, although these associations appear to be morphologically distinct, the same bundle of filamentous extracellular material can participate in both types of association.

Discussion

In this study of the extracellular material of specialized contractile fibroblasts in nodules of Dupuytren disease, we found that the extracellular material is composed of fine fibrils, three to five nanometers in diameter, and of occasional larger fibrils, ten to thirteen nanometers in diameter. These fibrils intermingle, making a complex of filamentous extracellular material. Within the nodule, the filamentous extracellular material is associated only with the specialized contractile fibroblast. The bundles of filamentous extracellular material were found to extend from specialized contractile fibroblasts connecting these cells to one another and to the surrounding extracellular matrix.

A distinguishing characteristic of the specialized contractile fibroblast is the presence of large intracellular bundles of actin microfilaments. The filamentous extracellular material that is present at the surface of the specialized contractile fibroblast was found to be associated with the intracellular bundles of actin microfilaments. Two types of associations between intracellular and extracellular filamentous bundles were observed: a lateral type, in which intracellular actin microfilaments and extracellular fibrils are parallel to one another, with the cellular membrane juxtaposed; and an end-to-end association, in which intracellular actin microfilaments and extracellular fibrils appear to be co-linear through the cellular membrane. These results suggest a transmembranous connection between bundles of actin microfilaments and filamentous extracellular material.

Gabbiani and Majno, as well as other investigators[4,7,14], have described the extracellular material that is present at the surface of these specialized cells as basal-lamina-like or basement-membrane-like. Our studies appear to be of the same extracellular material. Although this filamentous extracellular material does resemble a basal lamina and is therefore basal-lamina-like, we have demonstrated that it is ultrastructurally distinct from basal laminae. An immediate difference is that the basal lamina is a continuous structure that is located at the basal surfaces of epithelia and endothelia, as well as completely surrounding skeletal and smooth-muscle cells. The filamentous extracellular material at the surface of the specialized contractile fibroblast, however, is a discontinuous layer that is present only in close association with actin microfilaments. In addition, this filamentous extracellular material, unlike basal laminae, extends from the surface of specialized contractile fibroblasts into the surrounding matrix.

We previously demonstrated that the extracellular matrix surrounding specialized contractile fibroblasts lacks laminin and therefore is biochemically distinct from basal lamina[21]. We think that the terms basal-lamina-like or basement-membrane-like are not accurate descriptions of the filamentous extracellular material that is associated with these specialized contractile fibroblasts. The use of those terms could lead to confusion of these cells with the basal lamina that is present at the surfaces of smooth-muscle cells. The differences between specialized contractile fibroblasts and smooth-muscle cells are highly relevant if mechanisms for the actions of this specialized cell are to be sought.

The biochemical composition of the filamentous extracellular material around specialized contractile fibroblasts in the nodules of Dupuytren disease is currently unknown. Recent studies on granulation tissue have demonstrated that ultrastructurally similar filamentous extracellular material that is present at the surface of myofibroblasts stains, by electron microscopic immunocytochemistry, with anti-fi-

bronectin antibody[2,18]. We previously demonstrated by light microscopic immunocytochemistry that the extracellular matrix around specialized contractile fibroblasts stains intensely with anti-fibronectin antibody[21]. These results suggest that the filamentous extracellular material at the surface of specialized contractile fibroblasts may be composed, at least in part, of fibronectin. We are currently examining the composition of the filamentous extracellular material by immunoelectron microscopy. Preliminary studies from our laboratory have demonstrated that cultured specialized contractile fibroblasts will form bundles of actin microfilaments, as well as surface-associated filamentous extracellular material. This extracellular material stains with anti-fibronectin antibody[20].

The exact function of the filamentous extracellular material is unclear, but it is possible to speculate on its role in producing digital flexion in patients who have Dupuytren contracture. If the specialized contractile fibroblast is the contractile agent, it must be capable of generating intracellular contractile force and transmitting this force to the surrounding tissue. Intracellular forces may be generated by the interaction of actin microfilaments and non-muscle myosin. The filamentous extracellular material is in a key location to play a role in transmitting this force. Fibronectin is ideally suited to be the connecting macromolecule, as it is divided into functional domains that can bind both to the surface of cells and to surrounding collagen[22]. Fibronectin and actin cannot be in direct contact, because the cellular membrane is between them and neither is a transmembranous protein. Recently, a transmembranous protein has been described that has the capability of binding fibronectin extracellularly and the cytoskeleton intracellularly[10]. Such connections between the cytoskeleton and filamentous extracellular material are not restricted to the nodules of Dupuytren disease. Extracellular material that has the same ultrastructure and a similar association with actin microfilaments has been described at the surface of myofibroblasts in granulation tissue[2,18]. Thus, this filamentous extracellular material may play a role in attaching potentially contractile cells to surrounding tissues not only in Dupuytren contracture but also in other contractile tissues and diseases. It is clear, however, that the evaluation of these hypotheses must involve functional tests in addition to ultrastructural analyses such as those reported here.

Finally, we suggest, as in our previous study[21], that the term myofibroblast may be misleading when it is applied to this type of cell in the nodule of Dupuytren disease. This cell appears to be a specialized fibroblast that has the potential to generate and transmit contractile forces to the surrounding tissues.

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