Myofibroblasts Are Not Specific to Dupuytren's Disease

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Introduction

The etiology of Dupuytren's contracture has confounded clinicians and scientists since Dupuytren's first description in 1834. While the light and electron microscopic appearance of Dupuytren's contracture has been frequently described (MacCallum and Hueston 1962; Bazin et al. 1980; Millesi 1985; Nëzelof 1985; Gabbiani and Majno 1972; Gokel and Hübner 1977; Iwasaki et al. 1984; Kischer and Speer 1984; Tomasek et al. 1987), little attention has been paid to palmar fascia sampled from patients without Dupuytren's contracture (Legge et al. 1981; Gabbiani and Montandon 1985).
We have compared the fine structure of palmar fascia from patients with Dupuytren's contracture and carpal tunnel syndrome and have been able to highlight significant morphological changes. These findings contradict previously held assumptions regarding the uniqueness of Dupuytren's contracture fibroblasts.

Methods

Patients

Palmar fascia was obtained from patients undergoing either fasciectomy for Dupuytren's contracture or carpal tunnel release for carpal tunnel syndrome. Skin for culture was obtained from a forearm biopsy. Informed consent was obtained from all patients prior to operation.
For the light microscopic studies, tissues were taken from nine patients with Dupuytren's contracture (seven men, two women; age range 45–73) and five with carpal tunnel syndrome (all women; age range 50–63); for electron microscopy, from eight patients with Dupuytren's contracture (six men, two women; age range 52–73) and six with carpal tunnel syndrome (all women; age range 50–65); and for tissue culture from six patients with Dupuytren's contracture (three men, three women; age range 53–70) and six with carpal tunnel syndrome (three men, three women; age range 53–90).

Light Microscopy

Samples were fixed in buffered formalin saline (pH 7.4) for 24 h, dehydrated and embedded in paraffin wax. Sections were cut at 5 μm and stained with hematoxylin and eosin. Fibroblasts and blood vessels were counted using a 21 mm eyepiece graticule (Graticules Ltd, Kent, UK) and a Cooke 100 1 mm graduated slide. Mann-Whitney U nonparametric statistical tests were used for all data.

Electron Microscopy

Tissue Samples. Tissue was immediately dissected into 1 mm cubes and placed in 3% glutaraldehyde, buffered in 0.1 *M* sodium cacodylate (pH 7.3), postfixed in 2% osmium tetroxide in the same buffer, dehydrated in ethanol, and embedded in Epikote 812 (Emscope Ltd, UK). Ultrathin sections, stained on formvar coated copper grids with 2% uranyl acetate and Reynold's lead citrate, were examined in a JEOL T8 electron microscope (Japan Electron Optics Laboratory, Tokyo, Japan). Analysis of the diameter of collagen fibrils was performed by tracing the outlines of 288 collagen fibrils with a Zeiss modulator system for quantitative digital image analysis (MOP AM02) (Oberkochen, West Germany).

In Vitro Culture. Tissue was immediately placed in 100% Dulbecco's modified Eagle's medium, pH 7.35 (Flow Laboratories Ltd, UK), with 10% foetal calf serum (Flow) and dissected into 1 mm cubes. Two cubes of tissue were placed into 25 cm^2 tissue culture flasks (Nunclan Ltd, Denmark) and cultured at 37°C. Media were changed every 3 days throughout the culture period. At confluence cells were harvested and frozen in 9:1 (v/v) foetal calf serum:dimethyl sulfoxide in a Kryo 10 series cell freezer (Planer Products Ltd, Sunbury-on-Thames, UK). Two weeks prior to electron microscopic examination appropriate cell lines were thawed and cultured, thus each cell line was passaged three times prior to electron microscopy. Flasks (25 cm^2) seeded with 25×10^4 cells were incubated for 4 days until cells were near confluence. Media were discarded, cell layers washed with phosphate buffered saline (pH 7.4) and fixed in 3% glutaraldehyde, buffered with 0.1 M sodium cacodylate (pH 7.3), postfixed with 2% osmium tetroxide in the same buffer and dehydrated in an ascending series of ethanol. The specimens were preliminarily infiltrated in 1:1 hydroxypropyl methacrylate:Epikote, then 100% Epikote before baking in a 60°C oven. Embedded cell layers were separated from flasks after immersion in liquid nitrogen. Appropriate zones were trimmed, reembedded and sectioned parallel to the culture surface. The staining and subsequent characterization was as for tissue samples.

Results

Carpal Tunnel Syndrome

Palmar fascia from patients with carpal tunnel syndrome consisted of a tough, thin (<1 mm thick), pale, shining, translucent membranous structure that arose from beneath the flexor retinaculum and extended as a sheet across the palm.

The microscopic appearance was one of infrequent, well-spaced, elongated and spindle-shaped fibroblasts lying parallel to the encompassing matrix of well-organized collagenous fibers (Fig. 1). Microvessels were occasionally observed, usually at the periphery of bundles of collagenous fibers. These vessels had patent lumina of greater diameter than vessel wall thickness. Hyalinization of vessel walls was rarely encountered as was any perivascular cellular accumulation.

Electron microscopic examination revealed collagen fibrils to be grouped into well-organized fibers (2–20 μm in diameter) and arranged in a regular lattice, the majority having a diameter of either 60 nm or 110 nm (ratio 1:3) with an overall density of 100 fibrils per μm^2.

Fibroblasts contained numerous microfilaments (6–8 nm) that coursed in bundles from the perinuclear region to or close to the plasmalemma and were

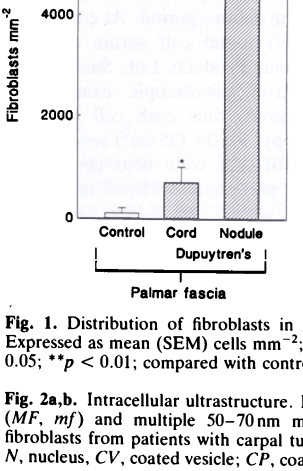
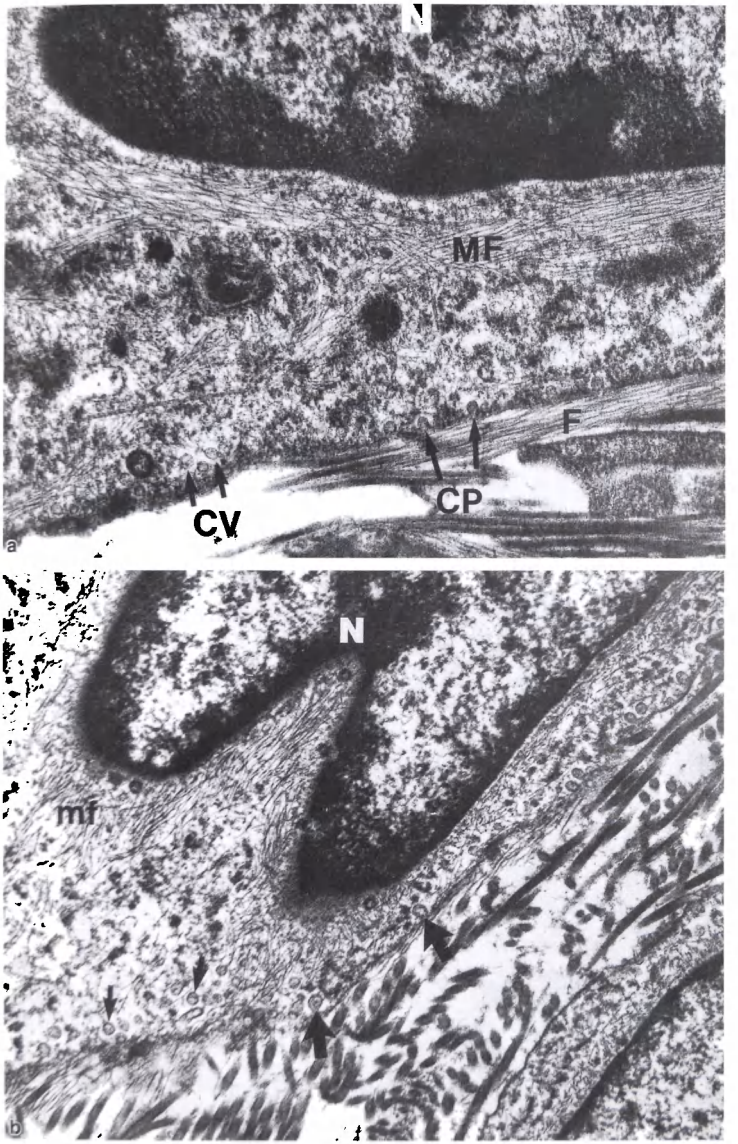


Fig. 1. Distribution of fibroblasts in Dupuytren's contracture and control palmar fascia. Expressed as mean (SEM) cells mm^{-2}; control (n = 5), cord (n = 9), nodule (n = 8). *p < 0.05; **p < 0.01; compared with control

Fig. 2a,b. Intracellular ultrastructure. Regular arrays of perinuclear, 6–8 nm microfilaments (*MF*, *mf*) and multiple 50–70 nm micropinocytotic vesicles (*arrows*) are prominent in fibroblasts from patients with carpal tunnel syndrome (**a**) and Dupuytren's contracture (**b**). *N*, nucleus, *CV*, coated vesicle; *CP*, coated pit; *F*, fibrils. (From Murrell et al. 1989) ×33 000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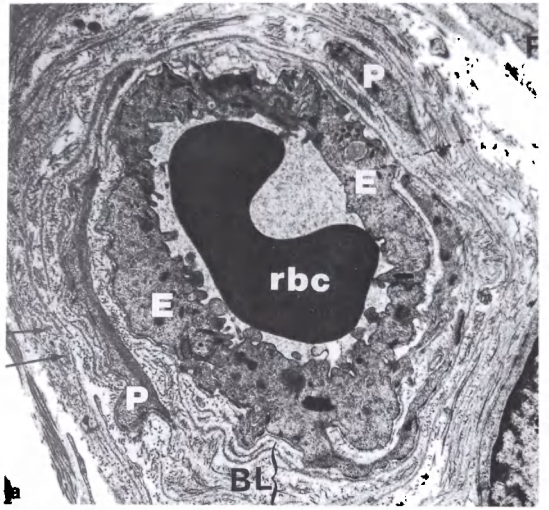
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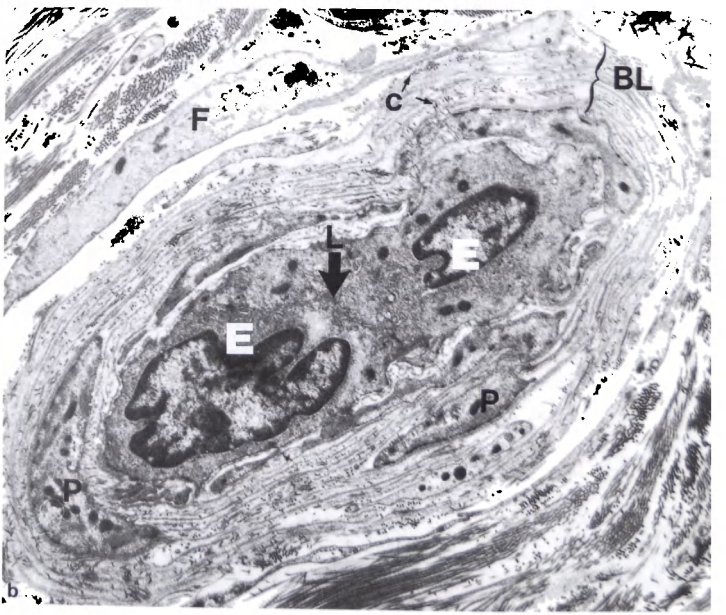
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generally aligned in the long axis of the cell (Fig. 2a). Micropinocytic vesicles (50–70 nm in diameter) were abundant, particularly associated with the cell membrane. The nuclear membrane contained several large smooth indentations. No intercellular junctional complexes were observed.
Capillaries with luminal diameters of 4–6 μm were lined by a single continuous layer of endothelial cells and enveloped with one to six (mean three) circumferential layers of basal lamina. Pericytes and collagen fibrils lay circumferentially between layers of basal laminae (Fig. 3a).

Dupuytren's Contracture

Palmar fascia from patients with Dupuytren's contracture consisted of a variety of longitudinally arranged, glistening, opaque, yellow-white, firm fibrous cords (2–5 mm in diameter) often extending from nodules (5–10 mm in diameter).
The microscopic appearance was heterogeneous, with cord, nodular and intermediate forms observed in all sections (as described by Nézelof 1985). Scattered within and immediately peripheral to the nodular areas were a number of small vessels. The majority of these vessels coursed parallel to the long axis of collagen fibers and were surrounded by an irregular swirling proliferation of fibroblasts. Many vessels were markedly narrowed and their walls grossly thickened and hyalinized. Leukocytes and pigmentary deposits were not encountered.
Electron microscopic examination showed the fibrillary matrix to be markedly disorganized, being composed of haphazardly arranged fibrils with varying diameters between 60 and 100 nm. Clustered around these fibrils were smaller swirls of 8–10 nm filaments.
Within the fibroblast cytoplasm were numerous perinuclear and longitudinally arranged microfilaments (6–8 nm diameter) extending to the cell surface. Abundant swollen rough endoplasmic reticula were frequently filled with moderately electron dense material. Micropinocytic vesicles (40–80 nm) were also numerous. Nuclei were invariably indented (Fig. 3b).
Completely or partially occluded capillaries (lumina 0–4 μm in diameter) were consistently observed, particularly in the nodular areas. These vessels were lined by continuous, bulging endothelial cells and five to ten (mean seven) layers of basement membrane, interspersed by collagen fibrils and hypertrophied pericytes. The outermost layers of basal lamina were loosely textured, folded and frequently disrupted (Fig. 3b). Peripheral to these vascular walls were fibroblasts and associated fibrillary bundles of collagen.

Fig. 3a,b. Capillaries from the palmar fascia of patients with carpal tunnel syndrome (**a**) and Dupuytren's contracture (**b**). Note the narrowing of the lumen (*L*) and lamination of the basal lamina (*BL*), collagen fibrils (*C*) and thickened pericytes (*P*) envelope the endothelial cells in (**b**). *rbc*, red blood cell; *E*, endothelial cells; *P*, pericytes; *small arrows*, collagen fibrils; *F*, fibrob-last. (From Murrell et al. 1989) ×8500

Cultured Fibroblasts

Cells cultured from each set of skin and of palmar fascia from patients with carpal tunnel syndrome or Dupuytren's contracture had a similar fine structure. The prominent subcellular feature was longitudinally arranged microfilaments (6–8 nm), coursing parallel to the long axis of the cell. Similar cytoskeletal components arose from within the cytoplasm and tapered outward to the surface of the flask forming apparent focal contacts (Fig. 4). In essence, all the cells in culture had features of myofibroblasts and their origin could not be distinguished by ultrastructural criteria.

Discussion

Although the fine structure of palmar fascia from patients with Dupuytren's contracture has been reported on extensively, palmar fascia from patients without Dupuytren's contracture (e.g., carpal tunnel syndrome) has rarely been examined. In this study the major difference between palmar fascia from patients with Dupuytren's contracture and carpal tunnel syndrome was *not* a difference in the ultrastructural characteristics of the fibroblasts, as previously suggested (Gabbiani and Majno 1972; Gokel and Hübner 1977; Iwasaki et al. 1984; Gabbiani and Montandon 1985). *All* fibroblasts examined had ultrastructural features of myofibroblasts. In addition *all* cultured cells derived from skin and palmar fascia from both groups of patients had prominent myofibroblastic characteristics including anchoring strands, features previously reported in other fibroblast cell lines (Abercrombie and Heaysman 1966; Goldman et al. 1976).

Fibroblast density was sixfold and 40-fold greater in cords and nodules from Dupuytren's contracture than in carpal tunnel syndrome. These observations and an increase in proliferation of perivascular fibroblasts (Mohr and Vossbeck 1985) suggest that the crucial phenomenon of fibroblast proliferation begins around narrowed microvessels.

Narrowing of capillary lumina and thickening and lamination of basal laminae were particularly pronounced in palmar fascia from patients with Dupuytren's contracture, changes consistent with those found by Kischer and Speer (1984). One of the striking features in Dupuytren's contracture capillaries was the hypertrophied and swollen, electron lucent endothelial cells; the latter appearance may represent damage to the integrity of the endothelial membrane. Swollen endothelial cells and occluded lumina have also been noted in hypertrophic scars and keloids (Kischer et al. 1982). Vracko (1974) proposed that noxious conditions cause pericytic necrosis in the microvasculature with subsequent regeneration within the old basal lamina tube. Repetition of this cycle adds further layers, progressively narrowing the lumen and trapping collagen fibers in a thickening wall. As the basal lamina widens, older outer layers become looser in texture, increase in width and electron lucency. Our observations are in keeping with such a hypothesis. We

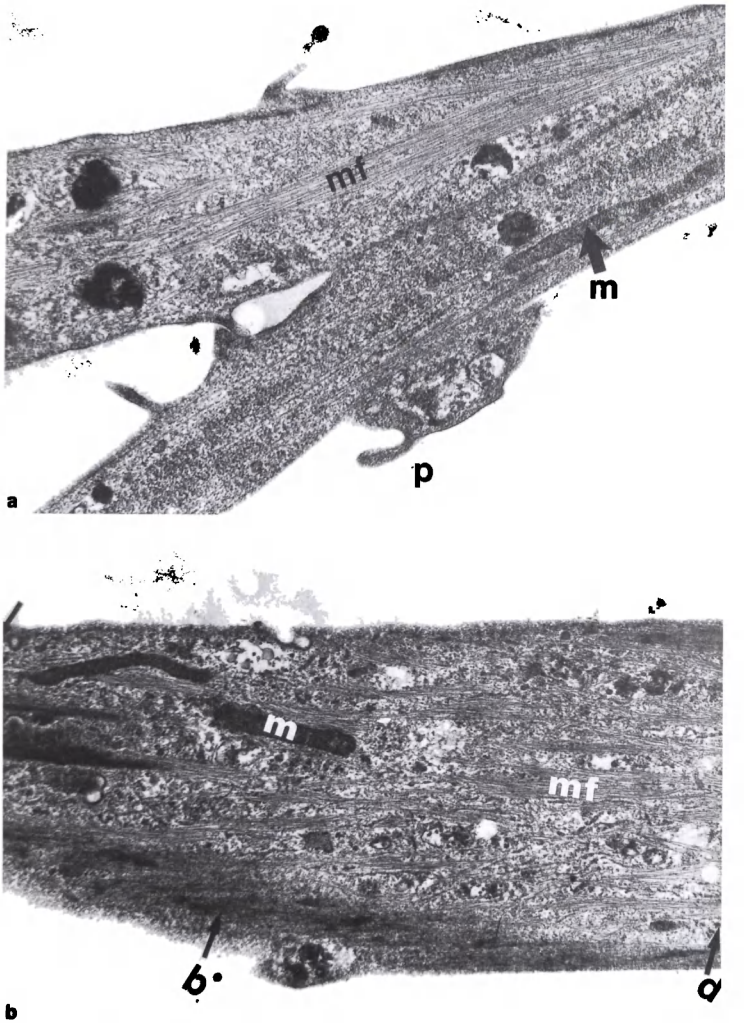

Fig. 4a,b. Cultured fibroblasts from palmar fascia of a patient with carpal tunnel syndrome (**a**) and from a patient with Dupuytren's contracture (**b**). Bundles of 6–8 nm microfilaments (*mf*) extend through the cytoplasm. *M*, mitochondria. (From Murrell et al. 1989) ×14 500 (**a**), ×5700 (**b**)

propose that one source of noxious stimuli is oxygen free radicals (Murrell et al. 1987a,b, 1988, 1989, 1990; Murrell and Hueston 1990). The source and effects of these species are outlined in our chapter titled Oxygen Free Radicals and Dupuytren's Disease. Vascular narrowing may occur both prior to the onset and during the progression of Dupuytren's contracture.

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