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Dupuytren's Disease: Comparative Growth Dynamics and Morphology Between Cultured Myofibroblasts (Nodule) and Fibroblasts (Cord)

*Jerry S. Vande Berg, †Richard H. Gelberman, ‡Ross Rudolph, †Duane Johnson, and *Paula Sicurello

**Core Clinical and Research Electron Microscopy Laboratory, Veterans Administration Medical Center; and Divisions of †Orthopaedic Surgery and ‡Plastic Surgery, University of California at San Diego Medical Center, San Diego, California*

Summary: The excised palmar fascia of 11 patients with Dupuytren's disease was separated clinically into nodules and cords. Myofibroblasts were seen by light and electron microscopy in each of the nodules, but the cords generally lacked myofibroblasts. Only one cord specimen had microscopic features that were intermediate between nodule and cord. Electron microscopy demonstrated that *in vivo* differences between myofibroblasts from nodules and fibroblasts from cords and control skin samples could be preserved *in vitro*. Growth studies showed slower growth of cultured myofibroblasts (mean ± SD generation time 68.7 ± 15 h) than cord-derived fibroblasts (mean ± SD generation time 51.5 ± 0.9 h). These data suggest that the life cycle of the myofibroblasts from Dupuytren's disease nodules differs from that of fibroblasts found in cordlike tissues. These myofibroblasts have biological characteristics nearly identical to those of myofibroblasts found in other contracting tissues, such as granulating wounds and breast cancer. **Key Words:** Dupuytren's disease—Cord—Nodule—Tissue culture.

The active contractile force in Dupuytren's disease appears to be the myofibroblast, a contractile cell that has been found in a variety of tissue contracture states (2). Several authors (1–5,8) have found myofibroblasts in the nodules of the palmar aponeurosis, but not in the cordlike tissues.

To date, research in this area has focused on the electron microscopic identification of myofibroblasts in Dupuytren's disease tissue, but questions about the biological function of these cells have received scant attention from the research community. Tissue culture studies of myofibroblasts from granulating wounds in both humans and animals have suggested that these cells are derived from fibroblasts that have modulated under special conditions (6,9,10). This study was designed to determine if myofibroblast cell lines from Dupuytren's disease nodules can be established in tissue culture, and to determine the growth characteristics of nodule myofibroblasts compared with cord and control fibroblasts.

Address correspondence and reprint requests to Dr. Vande Berg at Core Clinical and Research Electron Microscopy Laboratory, Veterans Administration Medical Center, 3350 La Jolla Village Drive, San Diego, California 92161.

MATERIALS AND METHODS

Clinical Procedures

Tissue for electron microscopic examination and culture was obtained from the palms of 11 male patients with Dupuytren's contracture on the Hand

Surgery Service of the Veterans Administration Medical Center and the University Hospital, San Diego, CA. The principal indication for operation was a digital contracture of >30° that was causing significant functional disability. Patients with painful faciitis without digital contracture were not considered candidates for the procedure. In eight patients, areas of palpable fusiform thickening within the fascia were labeled nodules. In the three other patients, no nodules were found, and firm, bandlike tissue that was excised was labeled cord. Normal palmar skin obtained from one patient undergoing surgery for an unrelated problem served as a source of normal fibroblasts. All tissues were studied following approval of the Human Subjects Committee of the University of California Medical Center, San Diego.

Biopsy Examination

Random portions of each control, cord, and nodule biopsy specimen were examined by light and electron microscopy to determine general tissue organization and to approximate the presence and number of myofibroblasts in cord and nodule specimens. The remaining portions of each biopsy specimen were used for explanting in culture.

Each specimen, cut into four to six pieces (1–3 mm^3), was fixed in 3% glutaraldehyde buffered with 0.2 *M* sodium cacodylate (pH 7.4) at 4°C for 2 h. Following three buffer washes, the tissue was postfixed in buffered 2% OsO_4 for 1 h. Following dehydration in a graded series of ethanol, the specimens were embedded in Epon 812. After polymerization, a portion of each block was cut into thick sections (0.25 μm) and viewed by light microscopy to determine cell types within each specimen and their distribution within the collagen matrix. Thin sections (60 nm) from the same blocks were mounted on unsupported 300-mesh grids, stained in saturated ethanol-uranyl acetate and lead citrate, and examined in a Zeiss EM-10 electron microscope.

All specimens were examined in the same way by the same electron microscopist to assure uniformity of examination. Constant reference was made to thick sections for identification of cells. Immediately after each grid was studied, the electron microscopist recorded impressions of the percentage of cells that contained myofibroblast features—that is, microfilaments with electron-dense bodies, convoluted nuclei, and basal lamina.

Tissue Culture Methods

Immediately after the biopsy specimen had been obtained, specimens from the cords and nodules were minced in growth medium into small (3–4 mm^3) pieces. Eight to 10 pieces of tissue were placed into 75-cm^2 flasks. In general, at least five explants remained attached and contributed cells to the primary cell lines. Fifteen milliliters of Eagle's minimal essential medium (90%; pH 7.2) plus fetal bovine serum (10%) and penicillin (50 units/ml) was then added to the flasks. Cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37°C. At confluency and all subsequent passages, cells were fed 24 h before dispersion by pouring off the growth medium, washing the monolayer in phosphate-buffered saline (PBS; pH 7.2) for 5 min, and adding 3 ml of trypsin (0.25% in PBS). Both fibroblasts and myofibroblasts were exposed to trypsin for a maximum of 3 min. While all cells were easily removed during this interval, it appeared that nodule-derived myofibroblasts became detached in sheets from the substrate generally sooner than normal or cord fibroblasts, which separated singly from the bottom of the flasks. During trypsinization each flask was frequently agitated to facilitate detachment of the cells. Trypsin was inactivated by adding an equal amount of growth medium to each flask. Detached cells were collected and centrifuged at 500 *g* for 4 min, washed, and suspended in growth medium. An inoculum of 3×10^6 cells was then replaced into new flasks with 15 ml of culture medium for continued growth.

Preparation of Culture for Electron Microscopy

Cultured cells (5×10^6 cells/60-mm dish) for electron microscopic examination were grown in Permanox (Lux) dishes to subconfluency. Cultures were washed three times in serum-free medium and fixed in 3% glutaraldehyde buffered with 0.2 *M* sodium cacodylate (pH 7.4) for 30 min. Following three buffer washes, the cells were postfixed in buffered 2% OsO_4 for 1 h. Following dehydration in a graded series of ethanol, the cells were embedded in a thin layer (approximately one-fourth the depth of the dish) of Epon 812. After polymerization of the Epon, the hardened plastic containing the cells was removed from the dish and floated for 15 s in a near-boiling solution of 1% toluidine blue in 1% sodium borate. Using this procedure all cells picked up the stain and could be selected as desired

in a dissecting microscope. Those cells chosen were then cut out from the embedded plate of plastic and glued to blank blocks of Epon 812. Thin sections were cut and mounted on unsupported 300-mesh grids. Sections were stained with ethanol-uranyl acetate and lead citrate before viewing in a Zeiss EM-10B electron microscope.

Growth Studies

Cells for growth studies were fed with fresh growth medium 24 h before trypsinization. Passage 5 cells were inoculated into 60-mm Petri dishes (Falcon) at a concentration of 5×10^4 cells/5 ml of culture medium, and three replicate dishes per cell type were harvested at alternate days and counted in a Coulter counter. All cultures were fed every third day during the growth study.

RESULTS

Light Microscopy of Cord and Nodule Biopsy Specimens for Culture Growth

Cord

From each biopsy specimen, random pieces of tissue were examined by light microscopy to observe the approximate number and organization of cells within the connective tissue matrix. Most cord specimens, as exemplified by DC-1, displayed an increased number of cells among mature collagen fibers (Fig. 1). This was in agreement with our studies (3,8) showing that most cord tissues demonstrated a banded pattern of collagen fibers with intermittent cells. All three cord biopsy specimens displayed a collagen matrix that was dense, interrupted, and sometimes irregular.

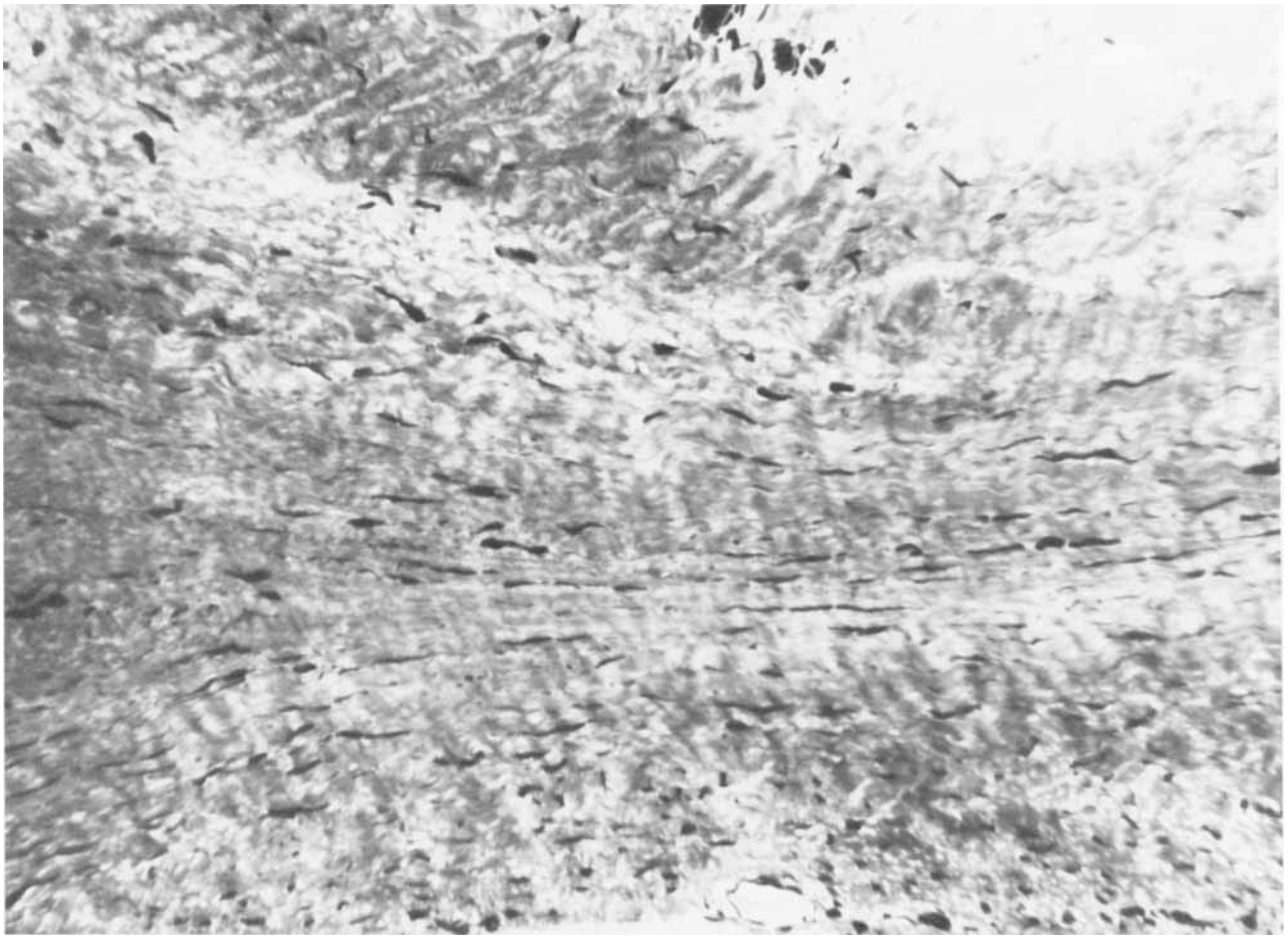

FIG. 1. This cord specimen, DC-1, shows few cells in a parallel pattern within a band of dense collagen fibers. The histologic pattern is reminiscent of tendon, which cords can clinically resemble (×425).

Nodule

In all nodule biopsy specimens (DC-4 through DC-11), a marked increase in cell numbers was observed. Six of the eight nodule specimens displayed an elliptical arrangement of cells, as exemplified by DC-10 (Fig. 2). The remaining two biopsy specimens showed cells that were arranged in layers forming an irregular banded pattern. All nodule biopsy specimens demonstrated a collagen matrix composed of dense fibers.

In the clinical examination of one patient, the palmar fascia displayed a well-defined nodule. The light microscopic examination of the nodule showed an elliptical pattern (Fig. 2) of tightly packed parallel layers of cells. These cells appeared to converge at opposite ends of the ellipse into two polar areas, similar to the arrangement of spindle fibers converging into centrioles during cell division (Fig. 3).

Electron Microscopy of Cord and Nodule Biopsy Specimens for Culture Growth

Cord

Electron microscopic examination of cord specimens demonstrated numerous classic fibroblasts that appeared active, with well-developed endoplasmic reticulum, Golgi apparatus, and mitochondria (Fig. 4). Plasma membranes appeared irregular and drawn out into numerous filiform processes. The intercellular space immediately surrounding many of the fibroblasts was filled with electron-dense granular material containing fine filament and

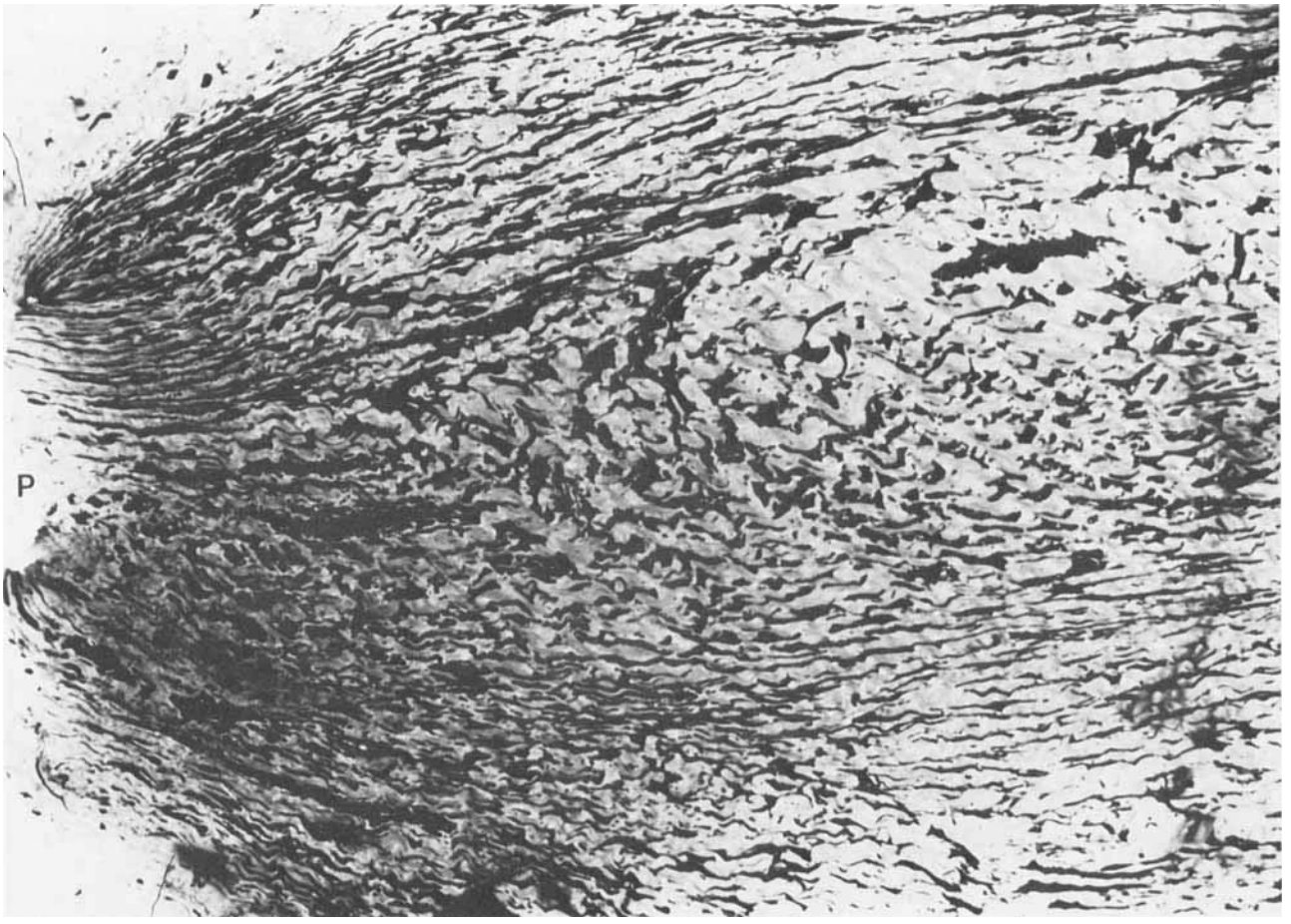

FIG. 2. All nodule biopsy specimens showed increased cell numbers above that observed in cord specimens. This nodule, DC-10, exhibited a large cell density that was arranged in an elliptical pattern. Some of these cells among dense collagen fibers appear to be drawn in a contracted state toward one of the poles (P) (×425).

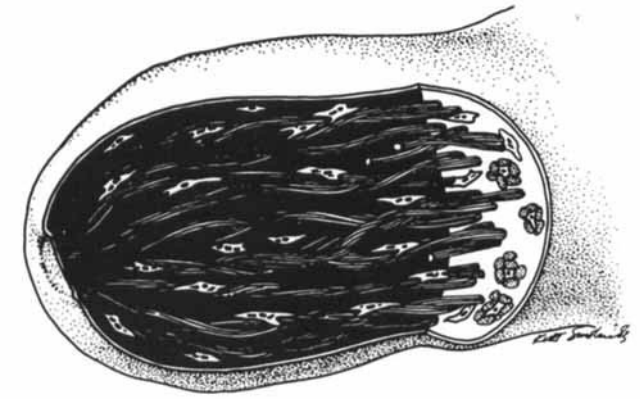

FIG. 3. Diagrammatic representation of the DC-10 nodule.

immature-appearing collagen fibers. This cellular organization closely resembled that described by Chiu and McFarlane (1) during Stage I or the early phase of Dupuytren's disease.

Nodule

Of the cells in each of five different block faces from all nodule biopsy specimens, 80–90% were myofibroblasts. These cells appeared to be active, with well-developed, dilated rough endoplasmic reticulum, Golgi apparatus, and mitochondria. Well-formed bundles of 40–80 Å microfilaments with electron-dense bodies were clearly seen near the plasma membrane, parallel to the long axis of the cell.

Electron microscopic inspection of thin sections from well-defined nodule DC-10 showed that all cells forming the elliptical pattern were myofibroblasts (Fig. 5). At both poles of this cellular configuration, collagen and myofibroblasts appeared to be contracted toward a centralized area.

Collagen in all nodules appeared to be composed of mature fibers. New collagen synthesis was less

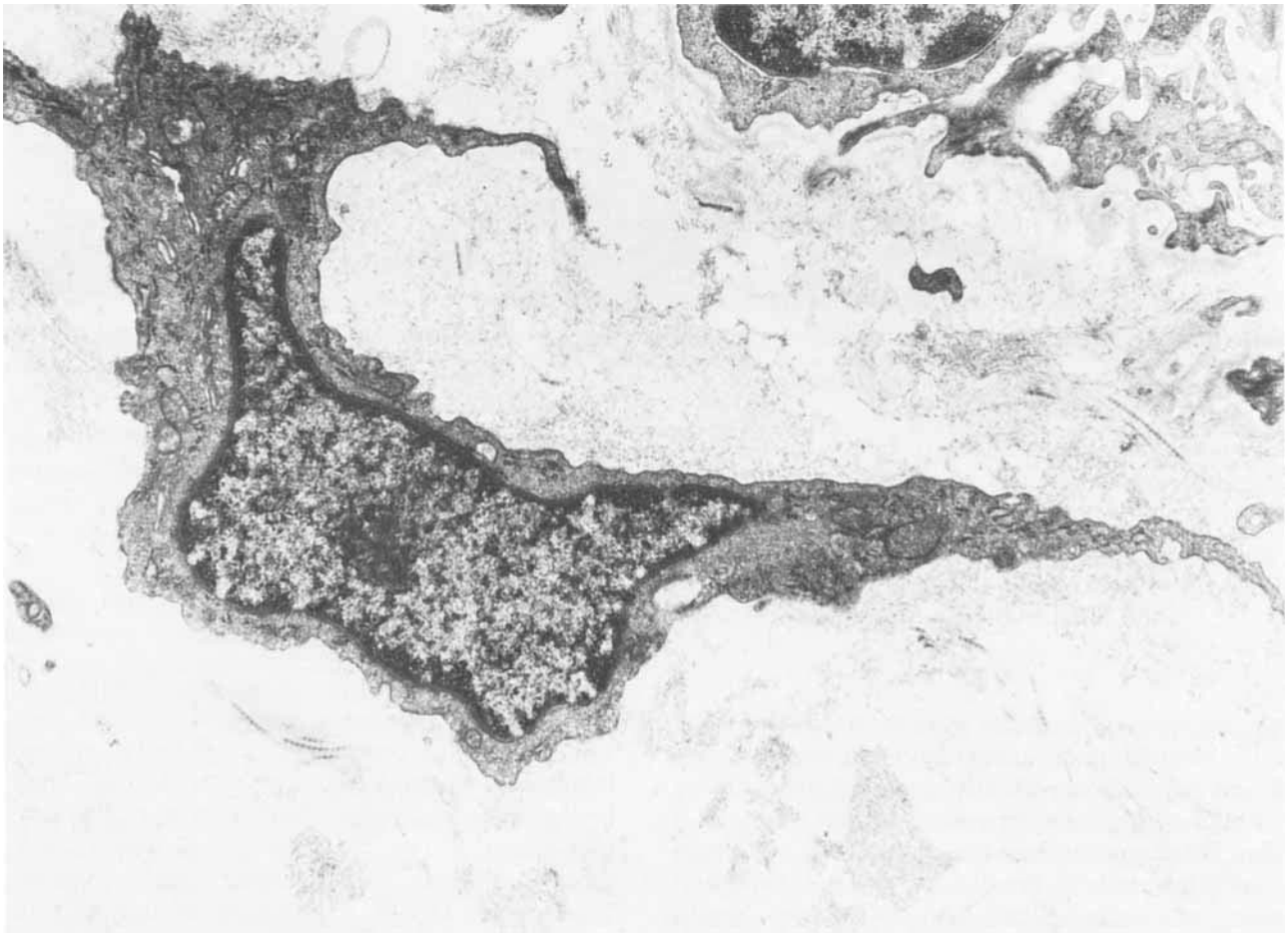

FIG. 4. All cells from cord specimens generally resembled fibroblasts, with well-developed rough endoplasmic reticulum, Golgi apparatus, and mitochondria (×17,100).

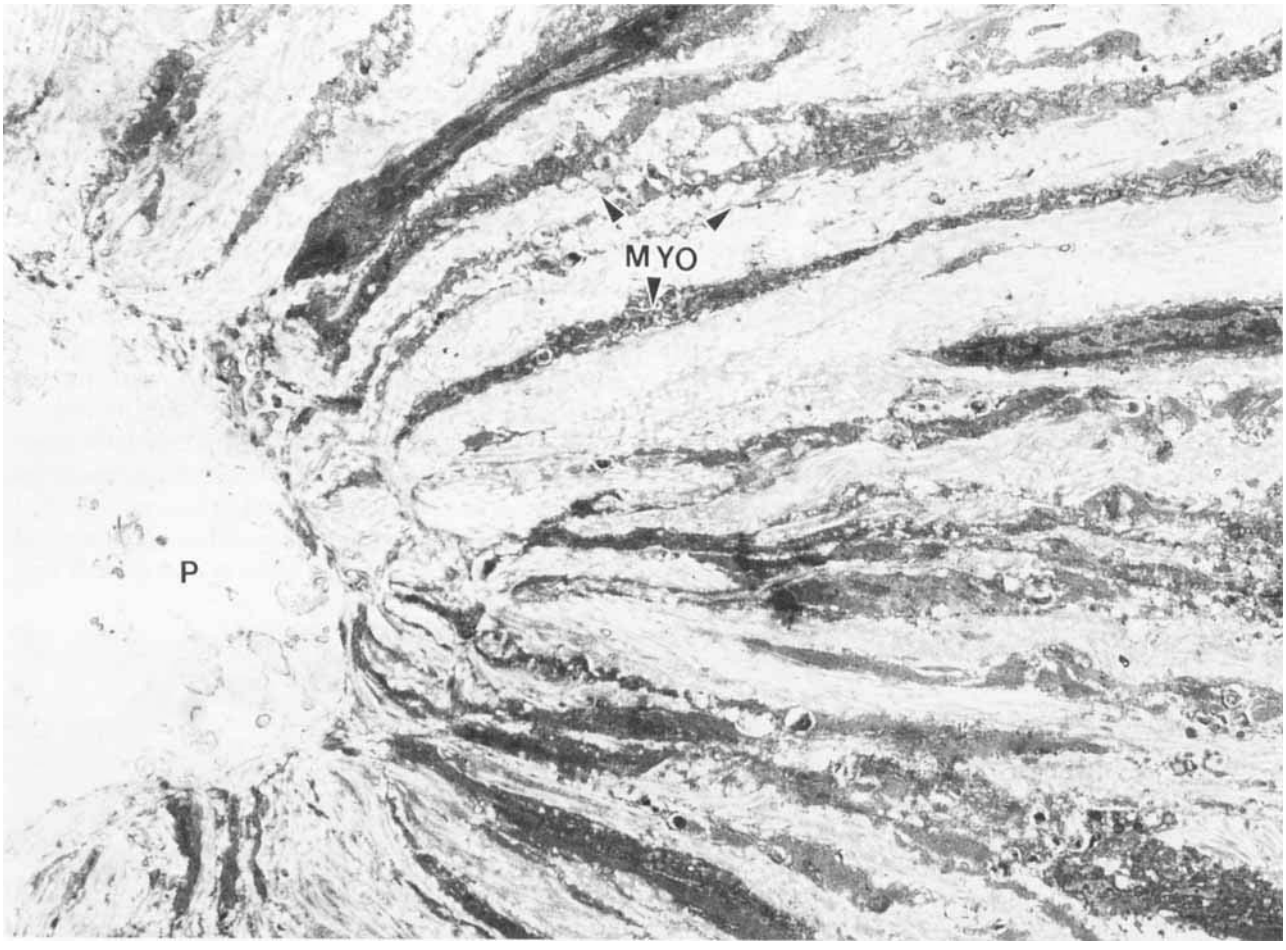

FIG. 5. Electron micrograph of DC-10 showing polar area (P) of nodule specimen. Note that all cells appear to be myofibroblasts (MYO; arrowheads). The result of probable contraction is evidenced by the drawing in of collagen to a localized area leaving space containing only cellular fragments (×10,900).

apparent than observed in cord biopsy specimens. Very little particulate material or cellular debris was noted in any of the thin sections.

Electron Microscopy of Cultured Cells from Cord and Nodule Biopsy Specimens

Cord

Cord biopsy specimens were cultured to passage 5 for electron microscopic examination. Subconfluent populations of cells appeared to be active with all intracellular organelles present as noted in their *in vivo* counterparts. Occasional cells presented intracellular bundles of 40–80-Å microfilaments along the cellular margins (Fig. 6). Neighboring cells displayed similar intracellular organelles, but without microfilament bundles. These morphological features were consistent with randomly chosen samples from cultured cell populations that originated from all cord biopsy specimens.

Nodule

Subconfluent cultures of passage 5 cells from nodule biopsy specimens displayed cells that were similar to cultured cord cells, except that prominent bundles of 40–80-Å filaments with electron-dense bodies were a consistent feature in all of the samples examined (Fig. 7). This was particularly evident in cultured cells originating from the well-defined nodule DC-10. Figure 7 shows that all cells contained well-developed bundles of 40–80-Å microfilaments with electron-dense bodies.

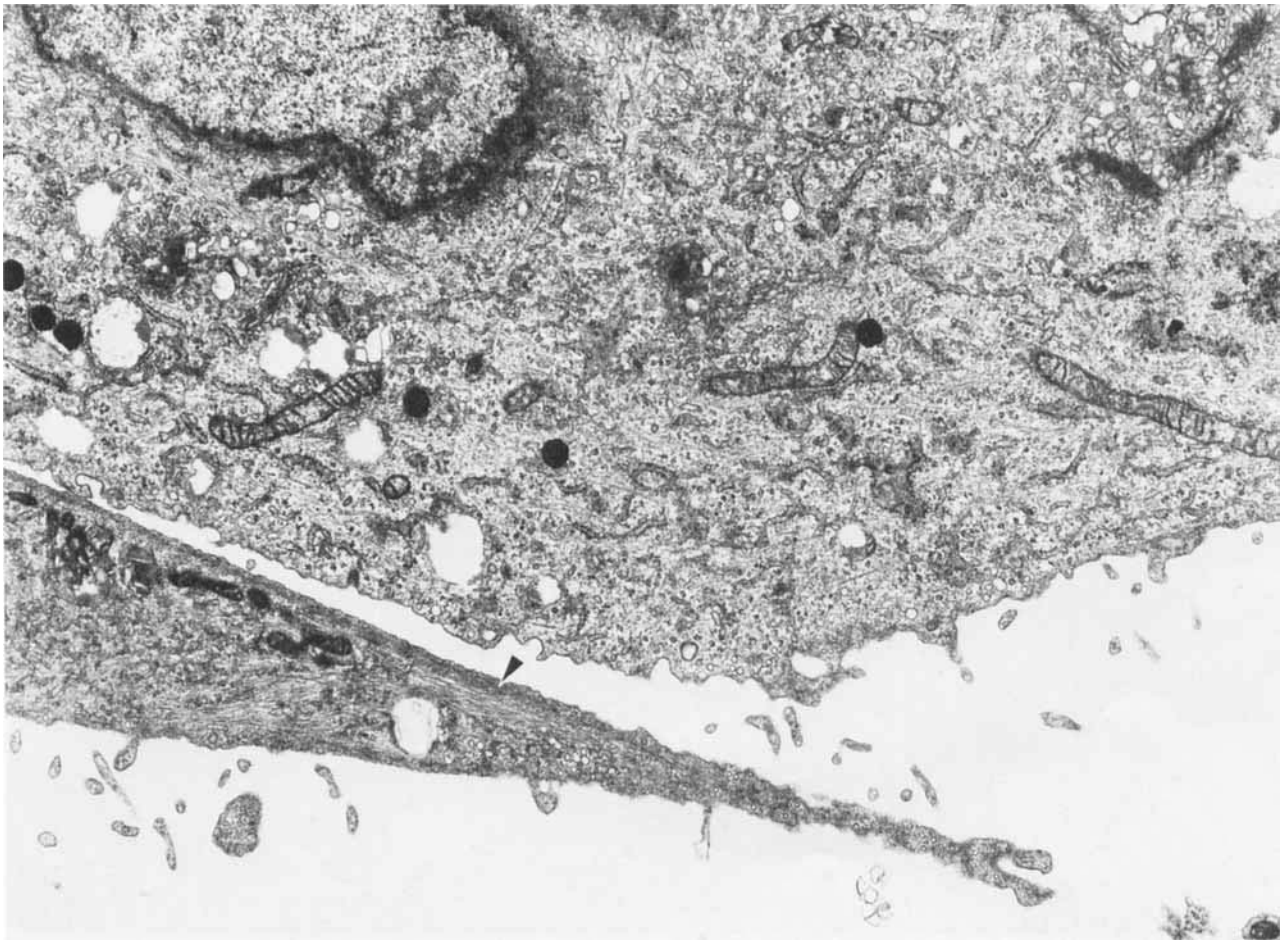

FIG. 6. Cultured fibroblasts at passage 5 from cord explant. One cell contains a bundle of microfilaments (arrowhead), while a neighboring cell does not (×15,200).

Comparison of Growth Kinetics Between Cultured Fibroblasts (Cords) and Myofibroblasts (Nodules)

Cord Cell Growth

Cells from normal skin and cord biopsy specimens were first observed 5–7 days following explanting. Epithelial cells were the first to appear, followed by fibroblasts, occasional lymphocytes, and nerve cells. After three passages 95% of the cells appeared to be fibroblast types.

At passage 5, the growth curves of fibroblasts from three different cord biopsy specimens and fibroblasts from normal palmar fascia were compared (Fig. 8). Fibroblasts from all three cord biopsy specimens grew at a similar rate during logarithmic growth through day 7. During the plateau phase (days 9–14), minor differences were observed. Of particular interest, however, was the observation that the rate of growth for fibroblast populations from cord explants was slower than that of fibroblast populations from normal palmar skin (Table 1). The mean ± SD population doubling time for cords was 51.5 ± 0.9 h.

Nodule Cell Growth

Cells grown from eight nodule biopsy specimens were first observed on the culture dish surface 7–9 days following explanting. As in the cord biopsy specimens, phase microscopic inspection showed that primary passage cells were composed of epithelial, fibroblast, lymphocyte, and nerve cell types. After three passages, only fibroblast type cells remained in culture. Electron microscopic examination of randomly chosen cells originating from

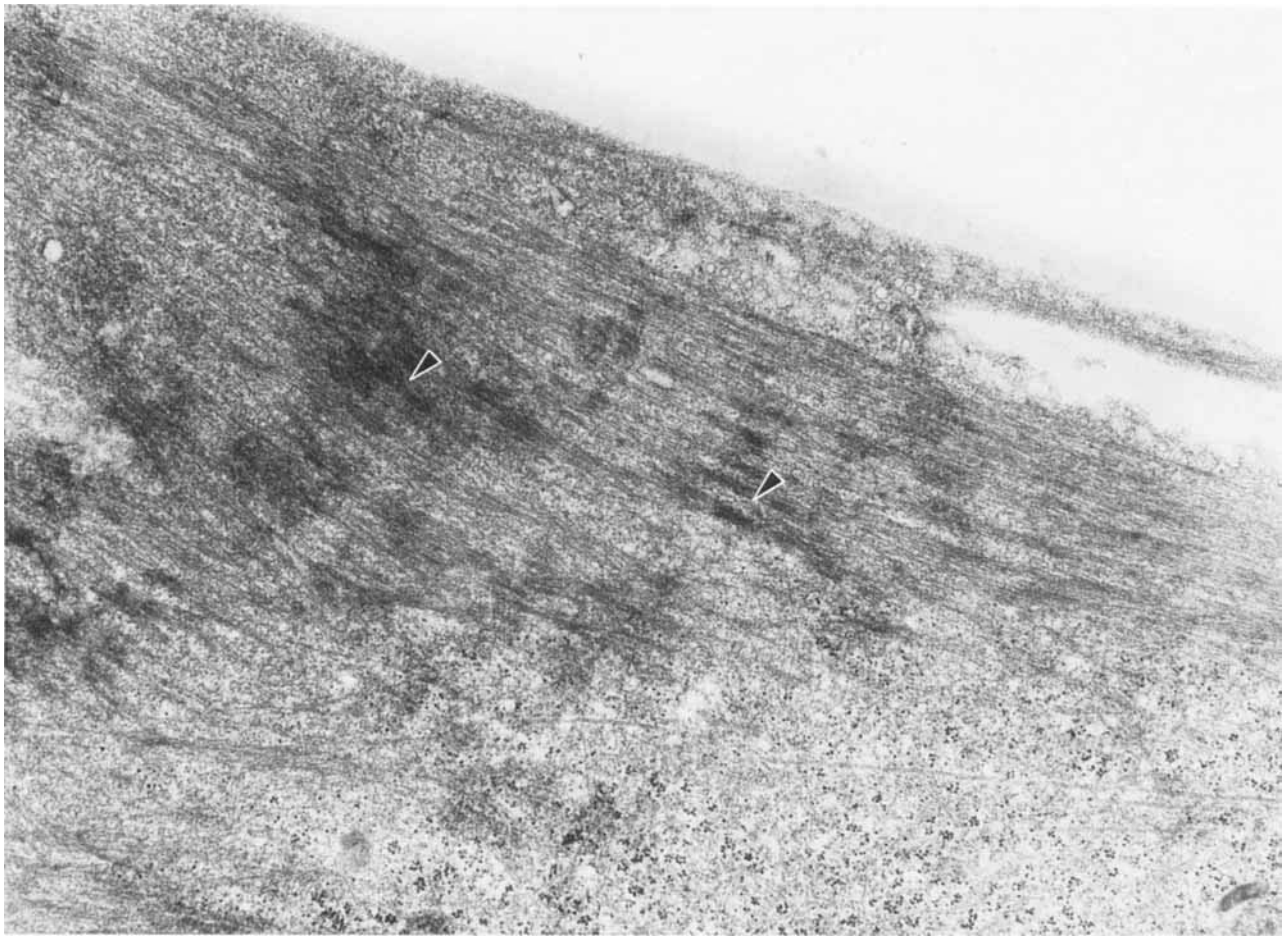

FIG. 7. Higher magnification from nodule explant DC-6 shows numerous well-developed microfilaments with electron-dense bodies (arrowheads). In this cultured myofibroblast, the bundled filaments extended not only along the cell axis but also throughout the lamellar area (×20,900).

each nodule explant showed well-developed bundles of 40–80-Å microfilaments with electron-dense bodies. Thus, these cultured cells appeared to maintain their differentiated state as myofibroblasts from the original nodule explants.

At passage 5, growth curves of myofibroblast populations from the eight different nodules and fibroblasts from normal palmar skin were compared (Fig. 9). Myofibroblast division appeared to be slow and gradual through 13 days. Logarithmic growth of these cell populations through 7 days showed some variation. However, all myofibroblast populations grew significantly slower than fibroblast populations from both normal palmar skin and cord fibroblasts (Table 1). The mean ± SD population doubling time for cells from the nodules was 68.7 ± 15 h. The population doubling time for control fibroblasts from normal palmar skin (mean ± SD 39.1 ± 0.9 h) was faster than that of control fibroblasts grown from cord fibroblast populations.

While the mean population doubling times in Table 1 of normal and cord fibroblasts and cultured myofibroblasts from nodules appear to be different, a statement of true statistical significance is complicated by the difference in cell numbers during the initial plating of the cells at the beginning of the growth study and the unequal sample sizes among the three cell lines. With this in mind, when Student's t test is applied to evaluate the differences between the means of the two samples (cord and nodule), a value of $p = 0.08$ is obtained, suggesting these differences to be borderline in terms of significance. However, the sample means are significantly different with $p < 0.05$ by the unpaired Wil-

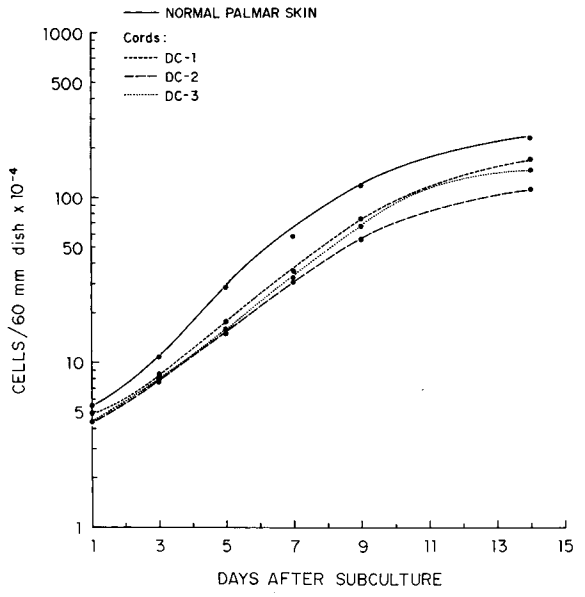

FIG. 8. Growth curve data comparing rate of population growth at passage 5 between cultured fibroblasts from cord explants and normal palmar skin. Note that the rate of growth of all cord fibroblasts was significantly slower than control cells. Each point represents the average count from three replicate dishes.

coxon rank test. Because of a single control sample, a statement of statistical significant difference could not be made relative to cultured cord fibroblasts and nodular myofibroblast populations.

TABLE 1. *Population doubling times of normal palmar skin, Dupuytren's contracture cords (fibroblasts), and nodules (myofibroblasts)*

Cell origin	Population doubling times (h)
Cord	
DC-1	50.4 ± 0.1
DC-2	52.3 ± 0.3
DC-3	51.8 ± 0.9
Nodule	
DC-4	57.9 ± 4.2
DC-5	98.5 ± 3.1
DC-6	79.6 ± 6.1
DC-7	67.1 ± 2.3
DC-8	57.3 ± 5.1
DC-9	67.5 ± 10.6
DC-10	68.2 ± 0.1
DC-11	53.8 ± 4.7
Normal palmar skin	
DC-12	39.1 ± 0.9

Doubling times were calculated from logarithmic growth measured from day 1 to day 5, and are mean ± SD values.

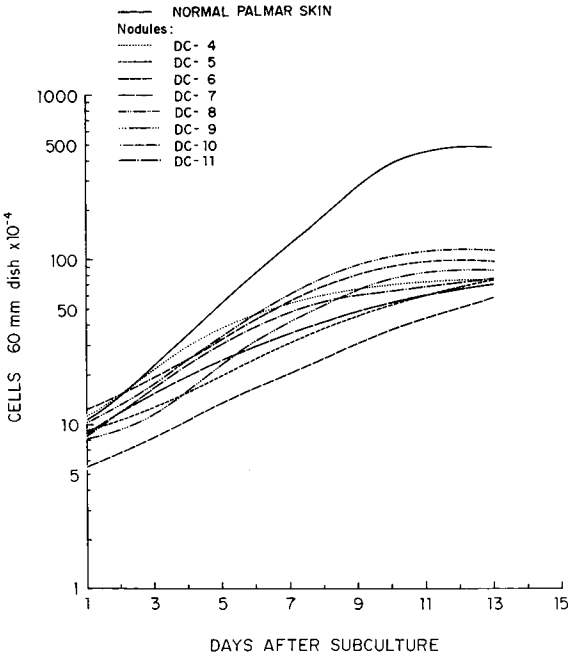

FIG. 9. Growth curve data comparing rate of population at passage 5 between cultured myofibroblasts from eight nodule explants and fibroblasts from normal palmar skin. Note that the rate of growth of all nodule myofibroblast populations was slightly varied, but was significantly slower than cord fibroblasts and control fibroblasts. Each point represents the average count from three duplicate dishes.

DISCUSSION

The cellular organization of the excised fascia of 11 patients with Dupuytren's disease was similar to that noted in previous studies of granulating wounds and human breast cancer (6,9,10). In the present study, the nodule specimens were composed almost entirely of myofibroblasts, whereas the cord tissue contained primarily fibroblasts. A consistent finding was the preservation of *in vivo* morphological differences between fibroblasts and myofibroblasts *in vitro*. This finding was characteristically evident in DC-10, a well-defined area of fascial thickening that was clinically labeled a nodule prior to surgery. Light and electron microscopic examination of the specimen showed that it was composed entirely of myofibroblasts organized in a tightly contracted elliptical pattern. All of the cultured cells originating from the nodular core displayed well-defined myofibroblast characteristics. These data demonstrate a clear correlation of clinical, electron microscopic, and tissue culture observations. This lends further support to the hypothesis that the characteristic clinical contracture

depends primarily on the contracture of cells located within this nodule (2,3). Thus, in this study, as in previous investigations of cultured myofibroblasts from granulation tissue and breast cancer, the differentiated state of the myofibroblast *in vivo* is maintained *in vitro* in Dupuytren's disease (6,9,10).

The state of cellular differentiation can be demonstrated physiologically as well as morphologically by comparing the rates of growth of the various cells. Cells from both the cords and the nodules grew slower than fibroblasts from unaffected palmar skin (Figs. 8 and 9). In addtion, the myofibroblast populations divided more slowly than the cord fibroblasts. Population doubling times for the cord fibroblasts were relatively consistent (Table 1), but the nodule myofibroblasts displayed a range of mean ± SD doubling times from 53.8 ± 4.7 to 98.5 ± 3.1 h. The slow doubling times for the myofibroblast population is consistent with the slow rates of growth noted for other well-differentiated cells.

While the slope of the growth curve typically indicates the rate at which the cell populations increase, the height of the curve indicates a cessation of growth or the end of the total replicative life span (7). Most populations of nodule myofibroblasts displayed a plateau in growth at 10–11 days, whereas the cord fibroblast populations showed an onset of declining growth at 15 days. Continuous observations of laboratory growth have shown that this feature of delimitative growth was particularly evident after 12–15 passages. The control fibroblasts from normal palmar skin showed an altered rate of growth at passages 12 and 15, at a time when the cord fibroblasts were displaying declining growth. The cultured nodule myofibroblasts showed cell degeneration and essentially no growth by passage 15.

These data show that myofibroblasts originating from nodule and fibroblasts from cord and normal palmar skin maintain distinct differences in rates of growth *in vitro*. However, two cell populations from nodule tissues DC-4 and DC-11 demonstrated a population doubling time that was similar to the doubling time of cord fibroblasts. This observation indicates that these two cell populations contained a higher ratio of undifferentiated fibroblasts to myofibroblasts. Such a cell ratio suggests that these patients, clinically determined to have nodules, may have possessed cord development or some intermediate form. This rationale would support the belief that the development of Dupuytren's disease follows a trend from nodule to mature cord.

While the clinical distinction of nodule from cord may not always be precise, our findings reveal that differences between nodule and cord biopsy specimens can be demonstrated by tissue culture, giving a clearer understanding of cell behavior in Dupuytren's disease fascia. It is also particularly significant that these differences in cell cycling between nodules and cords closely resemble differences between myofibroblasts and control fibroblasts in granulation tissue and breast cancer (6,9,10).

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