The role of skin and subcutaneous tissues in Dupuytren’s contracture: an electron microscopic observation

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Objective: To determine the relationship between the overlying tissues and recurrence of Dupuytren’s contracture.

Methods: Forty-three patients (68 hands) who accepted surgical treatment were divided into two groups according to treatment methods: partial fasciectomy or dermofasciectomy and full-thickness skin graft. Diseased palmar fascia, subcutaneous tissues and skin obtained during surgery were then assessed by electron microscopy.

Results: All patients were followed up. None of the hands which accepted dermofasciectomy and full-thickness skin grafting recurred, while 46.4% of the hands which accepted partial fasciectomy recurred. Under electron microscopic observation, myofibroblasts were found in the skin and subcutaneous tissues.

Conclusion: The overlying tissues play an important role in Dupuytren’s contracture, which may be a reason for recurrence of this condition after surgical treatment.

Key words: Dupuytren’s contracture; Recurrence; Surgery; Skin; Microscopy, electron

Introduction

Since Dupuytren’s description of palmar fasciectomy, a variety of surgical procedures have been recommended for Dupuytren’s contracture (DC), ranging from limited release of the diseased cord to dermofasciectomy and skin graft. Surgery has been regarded as the only effective form of management for DC. A common disadvantage of surgical treatments is the high rate of recurrence, which ranges from 27% to 66%¹-⁹. Fortunately, recurrence after surgical excision can be reduced by replacing the overlying skin with a skin graft, a procedure was first suggested by Piulachs in 1952¹⁰. Up until 1969, a total of 65 full thickness grafts had been used to control Dupuytren’s disease without recurrence¹¹,¹². The full thickness graft was considered to be the most important feature of a dermofasciectomy. The work of Rudolph on fibroblast maturation under skin grafts¹³ has been invoked as a possible explanation. The statement that ‘Dupuytren’s disease does not recur beneath a skin graft’ has entered textbooks¹⁴. That skin replacement reduces the risk of recurrence has become an well accepted concept¹⁵,¹⁶. Hueston has even suggested that the skin may be a mediating neurovascular organ; exerting some control on the disease process rather than simply being secondarily involved⁸. Similarly, Hoopes et al. have reported differing enzymatic activity in the dermis overlying nodules and bands as compared to that overlying normal fascia¹⁷. However, the basic nature of the pathological process and the role of the skin in this disease remain controversial.

Examination of fascia from patients with DC for electron microscopic and biochemical abnormalities has provided several new and significant findings over the past decades. Gabbiani and Majno were the first to describe the presence of myofibroblasts in the palmar fascia of DC on the basis of electron microscopic observation¹⁸. They suggested that the characteristic clinical contracture depends ultimately upon contraction of these cells in the nodules. Bailey found that the fascia of these patients contains type-III collagen, which is different from the type-I collagen present in the normal palmar aponeurosis¹⁹.

However, little literature concerned with the overlying skin of DC can be found¹⁷. In this study, in order to reveal the essential relationship between the skin and the recurrence of DC after surgery, specimens including fascia, skin and subcutaneous tissues from each hand were observed.
under the electron microscope to determine whether ultrastructural changes occur in palmar fascia and overlying tissues.

**Materials and methods**

This research was approved by the institutional review board. Informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Forty-three patients (38 men and 5 women, a total of 68 hands) with a mean age of 65 years (range, 45–75 years) who had undergone surgical treatment in our institution from 1998 to 2006 were reviewed. The mean duration of clinical symptoms prior to surgery was 3 years (range, 2–7 years). According to the staging system of Tubiana et al., 33 hands were in stage I, 23 in stage II and 12 in stage III. Patient records revealed two diagnosed epileptics, four diabetics, and eight alcoholics.

The 43 patients were divided into two groups. Group 1, 25 patients (40 hands) underwent dermofasciectomy and full-sickness skin grafting. DC was localized to the right hand in 8 patients and the left in 2, in 15 patients it was bilateral. Twenty hands were in stage I, 13 in stage II and 7 in stage III. Group 2, 18 patients (28 hands) underwent partial fasciectomy without skin grafting. Thirteen hands were in stage I, 10 in stage II and 5 in stage III. In all hands, all affected palmar tissues were removed, including 2 to 4 mm of adjacent normal palmar tissues and anchoring fibers. The incrassate chalmydate-fascias of hypothenar and thenar muscles were also resected. In group 1, a full-thickness skin graft was taken from the hairless area of the proximal volar forearm or the medial aspect of the arm (Fig. 1). In group 2, the original skin was sutured back to close the resultant defect. Postoperative management was the same in the two groups.

All patients were followed up. All operations were performed by the same surgeon with the aim of minimizing

Figure 1 Dermofasciectomy and skin graft. (a, b) The diseased skin and subcutaneous tissues were removed, and then (c, d) a normal full thickness skin graft was sutured in place to cover the wound.
the variability of the operation. During surgery, tissues for electron microscopic observation were taken from every nodule. These tissues were dissected into three parts: palmar fascia, subcutaneous tissue and dermis. Cubes about 1 mm in size were cut from selected areas, and fixed as soon as possible at room temperature in 2.5% glutaraldehyde in cacodylate buffer at pH 7.2–7.4 for 5 h. These were left in buffer at 4°C overnight, postfixed at 4°C with 1% osmium tetroxide in collidine buffer at pH 7.3–7.4, dehydrated in acetone and embedded in Epon 812 (Emicron, Shanghai, China). Sections about 1 μm thick were cut with glass knives on a Reichert (Reichert-Jung, Vienna, Austria) or LKB III ultramicrotome (Pharmacia LKB, Uppsala, Sweden), and stained with methylene blue and azur II. Thin sections were cut with diamond knives, mounted on noncoated grids, stained with uranyl acetate and lead citrate, ‘sandwiched’ with a thin layer of carbon and examined with an EM-TECNAL10 electron microscope (Philips, Eindhoven, The Netherlands). All electron microscopic specimens were examined in the same way by the same microscopist to assure uniformity of examination.

For statistical analysis of the outcomes, the χ² test provided by Microsoft Excel was used.

Results

Recurrence

True recurrence was defined as the appearance of new lesions within an operated area. Lesions outside the operated area, where no disease had been detected previously, were defined as extensions.

The mean follow-up time for all patients was 3.2 years (range, 2.0–3.5). There was a significant difference in recurrence rate of the disease between the two groups. In group 1, no recurrence was found under the graft; in only seven were extensions found beyond the graft. In group 2, 13 true recurrences with 8 extensions were detected and the rate of recurrence was 46.4%. Total recurrence rate of all hands was 19.1% (Table 2).

Electron microscopy

Cells with the following features (described by Giulio in 1972) were identified as myofibroblasts: (i) a possible

| Table 1 Number of hands in which myofibroblasts were found to be present in different tissues of the two groups |
|----------------|----------------|----------------|
| Group         | In dermis | In sub¹ | In fascia |
| Group 1       | 21        | 23      | 21        |
| Group 2       | 17        | 20      | 17        |
| Total         | 38        | 43      | 38        |

¹Sub refers to the subcutaneous tissue.

‘contracting machine’, such as thick bundles of fibrils which made the cells resemble smooth muscle; (ii) indirect evidence of contraction in a distorted shape of the nucleus, such as the accordion-type folds which have been successfully correlated with cellular contraction in at least three systems: smooth muscle, myocardial fibers and vascular endothelial cells; and (iii) devices for transmitting the contraction of individual cells to other cells as well as to the stroma (desmosomes, attachments to a basement lamina). In group 1, myofibroblasts were found in the diseased palmar aponeurosis in 21 samples (52.5%). Moreover, these cells were also found in subcutaneous tissue of 23 hands (57.5%) and skin of 21 hands (52.5%). In group 2, the 28 hands were divided into two subgroups in order to describe the outcomes exactly. Among the 13 hands in which recurrence developed, myofibroblasts were found in the palmar aponeurosis of 8 hands, dermis of 11 hands, and subcutaneous tissue of 12 hands. Among the other 15 hands, the figures were 9, 6 and 8, respectively. The difference between the two subgroups was significant for both dermis and subcutaneous tissues, but was not significant for the palmar aponeurosis (Figs. 2–6, Tables 1–3).

Cell apoptosis was found in some myofibroblasts (Fig. 5). Attachment sites between cells such as desmosomes, tight junctions, gap junctions and intermediate junctions (zonula adherens) were numerous, these are effective devices for transmitting contracture of the individual cell to other cells as well as to the stroma. In particular the gap junction, which is low ohmic, can transmit contracture information rapidly (Fig. 4). In addition, numerous collagen deposits were found surrounding the cells and an increase in the number of cross-links between collagen chains was identified (Figs. 2, 4). As myofibroblasts, the phenomenon of collagen in palmar fascia also presented in subcutaneous tissue and dermis.

Discussion

The purpose of this study was to explore the role of overlying tissues in DC. Despite many studies reporting the pathological changes of DC, most of them have been limited to changes in palmar fascia: the overlying skin and subcutaneous tissues have seldom been investigated.

Gabiani and Majno first described the presence of an unusual fibroblast, the myofibroblast, in palmar fascia obtained from six patients with DC. Reporting an electron microscopic study, Gelberman et al. reported that myofibroblasts were detected in palmar fascia in seven of twenty-four (29.2%) patients. In these studies, aponeurosis was observed, while the overlying tissues

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were neglected. However, according to our findings the ultrastructural changes in the skin should not be ignored.

Among a total of 68 hands, in 38 hands (55.9%) myofibroblasts were found to be present in the aponeurosis. This is significantly higher than the results of a previous study which reported only 29.2%21. Another new discovery of this study was that myofibroblasts were found in dermis (63.2%) and subcutaneous tissue (55.9%). Although the basic nature of the pathological process and the role of the skin and subcutaneous tissues remain controversial, despite some recurrence beyond the grafted area, the absence of recurrence under the grafts and the presence of myofibroblasts in the skin and subcutaneous tissues, suggest that these tissues may play a role in the recurrence process. This is confirmed by the findings in group 2.

In group 2, the 28 hands which underwent partial fasciectomy were divided into two subgroups. In subgroup 1, of the 13 hands which recurred postoperatively, myofibroblasts were found in the dermis of almost 85% of hands; but in subgroup 2, of the 15 hands in which no recurrence developed, only in 40% were myofibroblasts found. This difference is significant (P = 0.015), as is that for subcutaneous tissue (Table 2). Many studies have suggested that DC begins as a nodule located in the fibro-fatty tissue between fascia and skin, then secondarily affects these tissues18,22,23. Hueston believes that the skin may be a mediating neurovascular organ, and is not simply involved secondarily15. Hoopes et al. have shown different enzymatic activity in the involved, as compared to the normal, dermis17. As far as our findings are concerned, in view of the significant differences between the two subgroups of group 2 in terms of myofibroblasts and absence of recurrence under the graft, the overlying tissues may play an important role in the process of DC, especially in regard to recurrence. Myofibroblasts in these tissues continue to secrete extracellular matrix (type-III collagen) and to exert high contractile force on the tissues, ultimately causing recurrence of the disease.

In conclusion, myofibroblasts in the overlying tissues play a crucial role in recurrence of DC. The overlying tissues contain myofibroblasts and these cells may lead to recurrence of the disease. When dermofasciectomy and skin grafting removes all of the diseased fascia and overlying tissues, recurrence can be avoided.

### Table 2 Recurrence in the two groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Recurrence</th>
<th>Non-recurrence</th>
<th>Total</th>
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<tbody>
<tr>
<td>Group 1</td>
<td>0</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Group 2</td>
<td>13</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>55</td>
<td>68</td>
</tr>
</tbody>
</table>

### Table 3 Presence of myofibroblasts in group 2

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>In dermis</th>
<th>In sub†</th>
<th>In fascia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup 1</td>
<td>11</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Subgroup 2</td>
<td>6</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>20</td>
<td>17</td>
</tr>
</tbody>
</table>

†Sub refers to the subcutaneous tissue.

Figure 2 Myofibroblast in palmar fascia. Numerous collagen chains (A), dilated endoplasmic reticulum (B) and an indented nucleus (C) were detected (×15 000).

Figure 3 Cell from subcutaneous tissue. Deposition of collagen (A), dilated endoplasmic reticulum (B), indented nucleus (C), plus numerous peripheral vesicles; all features are reminiscent of myofibroblasts (×12 500).
Figure 4  Myofibroblasts aggregate in subcutaneous tissue. Gap junctions between cells (A, B) can transmit the retractive information from one cell to another and to the matrix rapidly. There is also much dilated endoplasmic reticulum and collagen. (a, ×3700, b, ×24000; c, ×12500; d, ×6200).

Figure 5  A myofibroblast from dermis in the process of apoptosis. The same features as other myofibroblasts except for more peripheral vesicles (B) and a smaller nucleus (C) in the collagen fiber (A) (×6200).

Figure 6  Myofibroblast with an indented nucleus. In the collagen fiber (A) of the dermis, beside a melanocyte (B) was a myofibroblast with an indented nucleus (C). Numerous collagen deposits surround the cells (×6200).
References