Mechanical Forces Induce Scar Remodeling

Study in Non-Pressure-Treated versus Pressure-Treated Hypertrophic Scars

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Reparative process of second and third degree burns usually results in hypertrophic scar formation that can be treated by pressure. Although this method is efficient, its mechanisms of action are not known. In this work, we have studied the histological organization of hypertrophic scars submitted to pressure. Skin biopsies were performed 2 to 7 months after the onset of treatment in two adjacent regions of the scar, non-pressure- or pressure-treated and analyzed by immunohistochemistry and transmission electron microscopy for extracellular matrix organization and cellular morphology. In non-pressure-treated regions, fibrillin deposits did not present the classical candelabra-like pattern under epidermis and were reduced in dermis; in pressure-treated regions the amount was increased compared to non-pressure-treated regions but the organization was still disturbed. In non-pressure-treated regions, elastin was present in patch deposits; in pressure-treated regions elastin formed fibers, smaller than in normal dermis. Tenascin was present in the whole dermis in non-pressure-treated regions, whereas in pressure-treated regions it was observed only under epidermis and around vessels, as in normal skin. α-Smooth muscle actin-expressing myofibroblasts were absent in normal skin, present in large amounts in non-pressure-treated regions, and almost absent in pressure-treated regions. The disturbed ultrastructural organization of dermal-epidermal junction observed in non-pressure-treated regions disappeared after pressure therapy; typical features of apoptosis in fibroblastic cells and morphological aspects of collagen degradation were observed in pressure-treated regions. Our results show that, in hypertrophic scars, pressure therapy restores in part the extracellular matrix organization observed in normal scar and induces the disappearance of α-smooth muscle actin-expressing myofibroblasts, probably by apoptosis. We suggest that the pressure acts by accelerating the remission phase of the postburn reparative process. (Am J Pathol 1999, 155:1671–1679)

The reparative process of second and third degree burns usually results in formation of hypertrophic scars that are clinically characterized by elevation above skin surface limited to injury borders, redness, and itching, and are frequently associated with contractures. Beyond causing bodily disfigurement, contractures may also cause disorders or even loss of the normal functions of the affected region. Histologically, hypertrophic scars are characterized by α-smooth muscle (SM) actin-expressing myofibroblasts and thin, randomly organized collagen fibers, both usually arranged in nodules. Myofibroblasts, which are the main cellular type observed in granulation tissue, are modified fibroblasts that present some features typical of SM cells. They contain bundles of microfilaments with dense bodies similar to those found in SM cells and can express, depending on situations, specific cytoskeletal proteins including α-SM actin, desmin, and SM myosin heavy chains. These features suggest that myofibroblasts are responsible for the force determining wound contraction and for the pathological contractures observed in hypertrophic scars. In the normal healing process, after re-epithelialization, the decrease in cellularity during the transition between granulation tissue and scar is mediated by apoptosis and an impressive remodeling of the extracellular matrix occurs. During excessive scarring, the mechanisms in-
volved in normal scar formation do not occur; the granulation tissue does not regress and the cells, particularly the myofibroblasts, are continually activated and producing extracellular matrix. Although some hypertrophic scars may spontaneously regress, others remain active for years. Of all of the treatments available, pressure exerted with elastic bandages in such a manner that the enforced pressure (24 mm Hg) exceeds the inherent capillary pressure gives significant results. Although it is an efficient method, its exact mechanisms of action are not known. Previous studies concerning pressure-treated hypertrophic scars have focused mainly on the role of hypoxia. It has been shown that granulation tissue is oxygen-poor, a condition which could stimulate fibroblast proliferation and collagen production. It has been suggested that the application of pressure increases an already present condition of hypoxia, resulting in resolution of the scar.

Extracellular matrix components are involved in growth, differentiation, migration, and death of many different cellular types. During wound healing, the pattern of expression of the extracellular matrix components is different from that usually present in normal skin, and various extracellular matrix components play significant roles in the different stages of wound healing. Among extracellular matrix components, some of them participate in skin resistance (eg, collagens), whereas others allow skin elasticity (eg, elastin). In the context of hypertrophic scars, which show a high tendency to develop contractures, the study of the elastic system is relevant. The elastic system is formed by three types of fibers: oxytalan, elaunin, and elastic. The oxytalan fibers are formed exclusively by microfibrils, the elaunin fibers by microfibrils and patches of amorphous material (elastin), and the elastic fibers by a large amount of elastin with microfibrils. In a recent study, it was shown that elastin and fibrillin (a component of microfibrils) are present 7 days after injury in human experimental full-thickness wounds, showing that elastic system fibers are present in the early phases of wound healing. However, few data concerning the detailed organization of elastic system fibers in scars are available. Among other components of extracellular matrix present in dermis, glycoproteins such as tenascin are suggested to play an important role in wound healing. Tenascin is a glycoprotein sparsely distributed in normal skin, predominantly associated with basal laminae. In early phases of wound healing and during the formation of granulation tissue, there is a marked increase in expression of tenascin, but tenascin returns to normal levels after the end of wound contraction.

In the present study, we investigated by histochemistry and immunohistochemistry the expression and organization of extracellular matrix components, including fibrillin, elastin, and tenascin, and the distribution of α-SM actin-expressing myofibroblasts in non-pressure-treated and pressure-treated hypertrophic scars. We also evaluated by transmission electron microscopy the dermal-epidermal junction modifications and the presence of apoptotic features during the pressure-induced scar remodeling.

### Materials and Methods

#### Patients and Sample Processing

Nine patients were included in this study. Their ages ranged from 18 to 54 years (average 32.7 years) and they included one woman (Table 1). All patients had hypertrophic scars that arose after burn injuries. The clinical diagnosis of hypertrophic scar was done based on the standard clinical criteria as described by Sahl and Clever, such as elevation above the skin surface limited to injury borders, redness, and itching. The age of scars at the beginning of the treatment ranged from 3 to 11 months (average 7 months) and all these scars showed criteria of active hypertrophic scars. The biopsies were taken 2 to 7 months (average 4.1 months) after the beginning of the pressure treatment. Each patient had two 3-mm punch biopsies, one in the pressure-treated region and the other in an adjacent, non-pressure-treated region.

#### Table 1. Source of Human Tissues Used in this Study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Biopsy site</th>
<th>Age of scar (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>20</td>
<td>leg</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>35</td>
<td>abdomen</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>18</td>
<td>arm</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>54</td>
<td>leg</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>43</td>
<td>leg</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>19</td>
<td>arm</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>41</td>
<td>shoulder</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>37</td>
<td>shoulder</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>28</td>
<td>chin</td>
<td>7</td>
</tr>
</tbody>
</table>

Figure 1. Gomori's silver impregnation in non-pressure- and pressure-treated regions. In non-pressure-treated region (a), bundles of reticular collagen fibers are perpendicular to epidermis and there are a large amount of reticular collagen fibers in deep dermis. In pressure-treated region of the same patient (b), the reticular collagen fibers present a random arrangement without forming large bundles, similarly to that observed in normal skin. Scale bar, 50 μm.
region. The distance between the two biopsies was about 7 cm and we can exclude an effect between non-pressure- and the neighboring pressure-treated biopsy site. Normal skin biopsies from three mammaplasties were used as controls. Tissue samples were fixed in Bouin’s liquid and embedded in paraffin, cryopreserved in OCT compound (Sakura, Torrance, CA) and snap-frozen in liquid nitrogen, or fixed in 2% glutaraldehyde/0.1 mol/L Na-cacodylate/HCl, pH 7.4, postfixed in 1% osmium tetroxide/0.15 mol/L Na-cacodylate/HCl, pH 7.4, and embedded in Epon. The size of the biopsies obtained from burn patients was obviously limited and did not allow the use of other analytical methods (eg, biochemical).

All patients enrolled in this study gave written and informed consent to participate under protocols approved by the University of Lyon Institutional Review Board.

Histology and Immunohistochemistry

Tissue sections of material embedded in paraffin (5 μm) were stained with hematoxylin-eosin, Gomori’s silver impregnation, or orcein. The Gomori’s silver impregnation contrasted reticular collagen fibers present in the dermis and the orcein staining pointed out the elastic fibers.

Cryostat sections (6 μm) were labeled using the following primary antibodies: a mouse monoclonal anti-human fibrillin-1 (Neomarkers, Fremont, CA), a rabbit polyclonal anti-human elastin (Institut Pasteur de Lyon, Lyon, France), a mouse monoclonal anti-human tenasin (Sigma, St. Louis, MO), a rabbit polyclonal anti-human laminin, type IV and type VII collagens (Institut Pasteur de Lyon), a mouse monoclonal anti-human type VII collagen (Gibco BRL, Gaithersburg, MD), and a mouse monoclonal anti-α-SM actin.23 These antibodies have been previously well characterized, are very specific, and have been used extensively in other experimental and clinical conditions. The secondary antibodies were cyanine 3-conjugated goat anti-mouse IgG (Jackson Immunoresearch Lab, West Grove, PA) or fluorescein-conjugated goat anti-rabbit IgG (Jackson Immunoresearch). The sections were examined in a Leitz Laborlux S microscope (Wild-Leitz, Heerbrugg, Switzerland) equipped with epi-illumination and specific filters for fluorescein and cyanine 3.

The histological grading system used for α-SM actin immunostaining was: 0, expression of α-SM actin only in vessels; 1+, discreet; 2+, moderate; and 3+, impressive amount of myofibroblasts expressing α-SM actin. The nonparametric Mann-Whitney test was used to compare the scores in non-pressure-treated and pressure-treated regions.

Morphometry

Epidermis thickness was evaluated in hematoxylin-eosin stained sections, using a computerized image analysis system (Histo 200; Biocom, Les Ullis, France). The station included an Ortoplan photomicroscope (Wild-Leitz), a CCD camera (WV-CD 52; Panasonic, Osaka, Japan), and a Pentium Biocom S/X computer (Biocom). Ten measurements were taken for each field, and five fields were analyzed in each biopsy using a 20× objective. The slides were evaluated blindly by two independent observers and no difference was found in their data. Results are presented as mean ± SD. The two different conditions (non-pressure-treated or pressure-treated) were compared using Student’s t-test, and the result was considered statistically significant when P < 0.05.

Transmission Electron Microscopy

Semithin sections were stained with toluidine blue. Thin sections were contrasted with methanolic solution of uranyl acetate and lead citrate and observed with a Philips CM120 transmission electron microscope (Philips SA, Zurich, Switzerland).

Results

Histology and Histochemistry

The presence of inflammation was not impressive in any biopsy. In non-pressure-treated regions, the epidermis thickness (130.5 ± 35.5 μm) was increased compared...
with pressure-treated regions (106.9 ± 26.8 μm; P < 0.01); furthermore, the epidermis in both non-pressure-
and pressure-treated regions was thicker compared with
normal skin (44.8 ± 17.5 μm).

In the dermis, non-pressure-treated regions showed
the typical organization of hypertrophic scar with an
important cellularity and numerous vessels surrounding
nodule-like structures as previously described.\(^2\) In
non-pressure-treated regions, the classical dermal/epidermal
interface characterized by prominent elongated rete
ridge was not observed. In both non-pressure-treated
and pressure-treated regions, it was not possible to de-
fine the papillary and the reticular dermis. In pressure-
treated regions, the number of vessels was reduced
compared with non-pressure-treated regions. Furthermore,
in non-pressure-treated regions, the vessels were
localized mainly in superficial dermis, whereas in pres-
sure-treated regions most vessels were localized in the
deep dermis, as in normal skin.

In normal skin, Gomori’s silver impregnation showed
that reticular collagen fibers were randomly organized in
the dermis without forming bundles (data not shown). In
the non-pressure-treated regions, some bundles of retic-
ular collagen fibers anchored perpendicular to the der-
mal-epidermal junction (Figure 1a). By contrast, in the
pressure-treated regions the reticular collagen fibers
were thinner, with an arrangement rather parallel to skin
surface (Figure 1b), thus resembling normal skin.

In normal skin, orcein-stained elastic fibers were local-
ized in reticular dermis, arranged, preferentially, parallel
to epidermis. In non-pressure-treated regions, these fi-
bers were absent in most cases; when present, they were
located in the deep dermis, where they were short, thin,
and curly. In pressure-treated regions, elastic fibers were
present in superficial and deep dermis; the fibers had a
random arrangement that was different from that ob-
served in a normal dermis in that they did not form a
network and had a fragmented appearance (data not
shown).

**Immunofluorescence Staining**

The results of the immunofluorescence study are summa-
rized in Table 2. Using antibodies against fibrillin and
elastin, the detailed organization of the elastic system fibers was studied. In normal skin, fibrillin of the papillary dermis was present in brushlike fibers inserted into the basal lamina (oxytalan fibers), showing the so-called candelabra-like configuration; more deeply, thicker fibers were observed, continuous with the superficial ones, thus forming a fibrillin network (Figure 2a). In non-pressure-treated regions, fibrillin arrangement was disturbed. In the superficial dermis, the candelabra-like pattern disappeared and the amount of fibrillin was reduced compared with normal skin or with pressure-treated regions; in the deep dermis, discreet fibrillin deposits resembled fragmented fibers (Figure 2b). In the pressure-treated regions, the fibers localized under the dermal-epidermal region were better organized compared with non-pressure-treated regions. However, they were thicker than normal ones and the typical candelabra-like pattern was not completely restored; in deep dermis fibrillin deposits were nearly normal although the fibers looked smaller (Figure 2c). In normal skin, elastin was absent in the oxytalan fiber region, beneath epidermis, and present as long aggregates forming fibers arranged mainly horizontally in the dermis (Figure 2d). In non-pressure-treated regions, elastin was present in patch deposits, not forming fibers (Figure 2e). In pressure-treated regions, some elastin was observed in the superficial dermis near the dermal-epidermal junction; the fibers were shorter and thinner compared with normal dermis (Figure 2f).

In normal skin, tenascin was observed in the superficial papillary dermis near the basal lamina of sweat glands and vessels (Figure 3a), as described by Lightner et al.\textsuperscript{20} In the non-pressure-treated regions, tenascin was observed in all dermis (Figure 3b), but the staining was not homogeneous: it was more intense under epidermis, weaker in the region just below, and then intense again (Figure 3b). In pressure-treated regions, tenascin was restricted to a fine deposit within dermal papilla and...
of vessels. In non-pressure-treated regions, the proportion as previously described. In contrast, pressure-treated (average 3 regions exhibited a marked and significant decrease of fibers and elaunin fibers containing many microfibrils and surrounding filaments and fibrils, as described by McMillan et al,25 were observed (Figure 5a). In non-pressure-anchoring filaments and fibrils, as described by McMillan treated, and pressure-treated regions were compared (data not shown).

In normal skin, α-SM actin was observed exclusively in vessels. In non-pressure-treated regions, the proportion of α-SM actin-expressing myofibroblasts was impressive (average 3+) and localized mainly in nodules (Figure 4a), as previously described.2 In contrast, pressure-treated regions exhibited a marked and significant decrease of α-SM actin-expressing myofibroblasts (average 0, \( P < 0.01 \), Figure 4b).

**Transmission Electron Microscopy**

The results of the transmission electron microscopy analysis are summarized in Table 3. Significant morphological changes were observed within the dermal-epidermal junction. In normal skin, the typical organization of subepithelial basal lamina (lamina lucida and lamina densa) accompanying the microfoot processes of the keratinocytes, and regularly distributed hemidesmosomes and anchoring filaments and fibrils, as described by McMillan et al,25 were observed (Figure 5a). In non-pressure-treated regions (Figure 5b), the dermal-epidermal junction was smooth and the keratinocyte microfoot processes were almost absent. The lamina densa was thickened and the lamina lucida was not clearly defined. The hemidesmosomes and the anchoring fibrils were not regularly distributed. Furthermore, the outer plaque of hemidesmosomes was not well delimited and the sub-basal dense plate was missing; moreover, anchoring fibrils were thicker compared with those observed in normal skin or in pressure-treated regions. Collagen fibers immediately beneath the lamina densa were frequently observed. In pressure-treated regions, the dermal-epidermal junction resembled that observed in normal skin with well organized hemidesmosomes; however, the depth of keratinocyte microfoot processes was increased (Figure 5c) compared with normal skin.

The ultrastructural organization of the elastic system fibers was recognized in both non-pressure-treated and pressure-treated regions, with the presence of oxytalan fibers and elaunin fibers containing many microfibrils and a discrete core of elastin (Figure 6a); well formed elastic fibers (Figure 6b) were also observed. In non-pressure-treated regions (Figure 6a), bundles of thick collagen fibers were present. In pressure-treated regions (Figure 6b), collagen bundles appeared loose and numerous signs of extracellular matrix remodeling (eg, collagen fibrils with varying thickness) were observed. Moreover, a wide range in the diameter of collagen fibrils was present together with some large twisted fibrils (Figure 6b), as usually seen during collagen breakdown.

Microvessels were frequently occluded (lumen size <3 \( \mu m \)), as described by Kischer et al,27 without any obvious differences between non-pressure-treated and pressure-treated regions (data not shown).

Typical myofibroblasts were observed in non-pressure-treated regions, with long processes containing microfilament bundles and extending for long distances among collagen fiber bundles as previously described.12 In pressure-treated regions, the proportion of cells showing myofibroblastic features was reduced. Furthermore, numerous fibroblastic cells presenting apoptotic features were observed (Figure 6, c and d). The main criteria used for the identification of apoptotic cells were vesiculation, condensation and margination of the chromatin, fragmentation of the nucleus, and cytoplasmic condensation. As previously described in myofibroblasts undergoing apoptosis during granulation tissue remodeling,7 nuclei in part extruded from the cytoplasm were frequently observed (Figure 6, c and d).

**Discussion**

Little is known about the mechanisms leading to hypertrophic scar resolution in pressure-treated patients. The present study reports the structural changes in the dermis (extracellular matrix and cell components) of pressure-treated postburn hypertrophic scars and, although only descriptive, provides direct evidence for close relationships in vivo between mechanical modifications (ie, pressure treatment), cell phenotype, and matrix remodeling. A modification of the collagen fiber organization was induced by pressure. The disorganized orientation was replaced under pressure by a parallel arrangement similar to the pattern observed in normotrophic healing. This pressure-induced collagen reorganization has been observed previously by Kischer et al11 and Baur et al.28

<table>
<thead>
<tr>
<th>Structures</th>
<th>Normal skin</th>
<th>Non-pressure-treated regions</th>
<th>Pressure-treated regions</th>
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<tr>
<td>Dermal epidermal junction</td>
<td>keratinocyte microfoot</td>
<td>typical organization</td>
<td>almost absent</td>
</tr>
<tr>
<td>lamina lucida</td>
<td>well formed</td>
<td>not well defined</td>
<td>well formed</td>
</tr>
<tr>
<td>lamina densa</td>
<td>well formed</td>
<td>thickened</td>
<td>well formed</td>
</tr>
<tr>
<td>hemi-desmosomes</td>
<td>typical organization</td>
<td>disturbed organization</td>
<td>typical organization</td>
</tr>
<tr>
<td>anchoring fibrils</td>
<td>regularly distributed</td>
<td>irregularly distributed and thickened</td>
<td>regularly distributed</td>
</tr>
<tr>
<td>Elastic system fibers</td>
<td>well organized</td>
<td>well organized</td>
<td>well organized</td>
</tr>
<tr>
<td>Collagen bundles</td>
<td>typical organization</td>
<td>large, with thick fibers</td>
<td>large, with thick fibers</td>
</tr>
<tr>
<td>Microvessels</td>
<td>patent</td>
<td>frequently occluded</td>
<td>frequently occluded</td>
</tr>
<tr>
<td>Myofibroblasts</td>
<td>absent</td>
<td>present</td>
<td>signs of degradation</td>
</tr>
</tbody>
</table>

Table 3. Transmission Electron Microscopy Features
In areas treated with pressure, there was a disappearance of nodular pattern. Furthermore, we observed that in hypertrophic scars, collagen fibers perpendicular to the epidermis were anchored in dermal-epidermal zone and probably participated in the impaired mechanical properties of the scar.

The elastic system fibers are not frequently considered in the studies of skin wound healing. Using similar techniques, Bhangoo et al. showed the presence of elastic fibers in different types of human scars (atrophic, normal, hypertrophic, and keloid) that were at least 1 year old. They observed that elastic fibers in hypertrophic scars had a disturbed arrangement and were localized mainly in superficial layers of the scar; in deep regions the distribution was patchy and some areas were entirely devoid of elastic fibers. However, more recent studies using transmission electron microscopy and immunohistochemistry showed the presence of some elastic system fiber components, fibrillin and elastin, also in early phases of wound healing in skin and liver. To our knowledge the present study is the first to show the presence of fibrillin and elastin and to describe their organization in hypertrophic scars. Fleischmajer et al. studying another skin fibrotic disease, scleroderma, observed an increase in deposition of microfibrils, but not of elastin, in deep dermis. In the present study we showed the presence of both components (fibrillin and elastin) in hypertrophic scars without major alterations in amount when compared with normal skin, but with important disorders in organization. Those alterations may be associated with an elastic system still immature as described by Tsuji and Sawabe. The pressure treatment allowed a rearrangement of fibrillin and elastin, with acquisition of an almost normal pattern enabling those fibers to carry out their physiological functions as in normal dermis. This may explain the softness in scars acquired as a consequence of pressure treatment.

During normal wound healing tenascin is abundant in granulation tissue but disappears soon after re-epithelialization. Our findings showing an impressive accumulation of tenascin in non-pressure-treated regions support the hypothesis that hypertrophic scars develop as a consequence of an excessive process of healing. In pressure-treated regions, as in normal skin, tenascin was present in dermal papilla and in basal lamina of vessels. Little is known about the function of tenascin in wound healing. However, Mackie et al. have suggested that the presence of tenascin allows myofibroblasts to contract the wound; in the case of hypertrophic scars, the continual presence of tenascin may contribute to the development of contractures.

Modifications of dermal-epidermal junction have been observed in different diseases, particularly in junctional forms of epidermolysis bullosa. Here, we observed that in hypertrophic scars, keratinocyte microfoot processes are elongated, while hemidesmosomes, anchoring filaments and anchoring fibrils are well organized. Scale bar, 0.5 μm.

Figure 5. Ultrastructure of the dermal-epidermal junction. In normal skin (a), the typical architecture is represented, with keratinocyte microfoot processes, regular hemidesmosomes, lamina lucida and anchoring filaments, lamina densa and anchoring fibrils. In non-pressure-treated region (b), the keratinocytic microfoot are almost absent, the lamina lucida is indistinct and the lamina densa is thickened. The hemidesmosomes are not regularly distributed, and their subbasal dense plate is missing (arrowheads). In pressure-treated region of the same patient (c), keratinocytic microfoot processes are elongated, while hemidesmosomes, anchoring filaments and anchoring fibrils are well organized. Scale bar, 0.5 μm.

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The elastic system fibers are not frequently considered in the studies of skin wound healing. Early studies using histochemical techniques described the presence of elastic fibers only in late phases of wound healing. Using similar techniques, Bhangoo et al. showed the presence of elastic fibers in different types of human scars (atrophic, normal, hypertrophic, and keloid) that were at least 1 year old. They observed that elastic fibers in hypertrophic scars had a disturbed arrangement and were localized mainly in superficial layers of the scar; in deep regions the distribution was patchy and some areas were entirely devoid of elastic fibers. However, more recent studies using transmission electron microscopy and immunohistochemistry showed the presence of some elastic system fiber components, fibrillin and elastin, also in early phases of wound healing in skin and liver. To our knowledge the present study is the first to show the presence of fibrillin and elastin and to describe their organization in hypertrophic scars. Fleischmajer et al. studying another skin fibrotic disease, scleroderma, observed an increase in deposition of microfibrils, but not of elastin, in deep dermis. In the present study we showed the presence of both components (fibrillin and elastin) in hypertrophic scars without major alterations in amount when compared with normal skin, but with important disorders in organization. Those alterations may be associated with an elastic system still immature as described by Tsuji and Sawabe. The pressure treatment allowed a rearrangement of fibrillin and elastin, with acquisition of an almost normal pattern enabling those fibers to carry out their physiological functions as in normal dermis. This may explain the softness in scars acquired as a consequence of pressure treatment.

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observed by transmission electron microscopy in the organization of the dermal-epidermal junction between normal skin, non-pressure-treated, and pressure-treated regions suggest subtle modifications in the organization of anchoring fibrils, although immunofluorescence did not show changes of the type IV and type VII collagen distribution. The extracellular matrix reorganization, which also involves collagen degradation, together with the disappearance of myofibroblasts, may explain the improvements obtained with pressure in mechanical properties of the scar. We suggest that pressure induces the decrease in cellularity similar to that observed in the final stages of normal wound healing, where apoptosis is the mechanism through which vascular and fibroblastic cells are gradually eliminated. We failed to demonstrate a significant proportion of apoptotic cells by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) technique, probably because pressure-induced apoptosis affects target cells consecutively rather than producing a single wave of cell disappearance.

Figure 6. Ultrastructure of dermal components. In non-pressure-treated region (a), an elaunin fiber is observed near a fibroblast (arrowhead). In pressure-treated region (b), an elastic fiber (star) and some bundles of regular collagen fibers are observed together with collagen fibers showing typical features of degradation (arrows). In pressure-treated region (c and d), fibroblastic cells presenting typical apoptotic features such as chromatin condensation and nuclei in part extruded from the cytoplasm are observed. Scale bars, 1 μm.

The mechanism of action of pressure is not known. Kischer et al. suggested that hypoxia, which causes hypertrophic scarring, is increased by pressure and causes the resolution of scar by induction of fibroblast death. Baur et al. disagreed and suggested that the treatment causes an increase in collagenase activity and a consequent increase in extracellular matrix degradation. It is known that mechanical forces induce modifications in extracellular matrix organization and composition in different situations such as development or cholestatic fibrosis. Furthermore, changes in environmental mechanical forces modulate the expression of matrix remodeling enzymes and induce apoptosis in dermal fibroblasts cultured in three-dimensional collagen gels. These modifications affect the mechanical properties of involved tissues as well as the cells present in these tissues.

In conclusion, in this study, we used immunofluorescence to observe the modifications of fibrillin, elastin, tenascin, and α-SM actin expression and electron microscopy to observe the changes of dermal-epidermal
juncture in human pressure versus non-pressure-treated hypertrophic scars. We suggest that the treatment of postburn hypertrophic scars by pressure induces extracellular matrix reorganization and apoptosis in fibrogenic (i.e., myofibroblasts) and vascular cells. Further studies are necessary to clarify the mechanisms of mechanosignal transduction involved in extracellular matrix remodelling and cell death resulting in hypertrophic scar resolution.

Acknowledgments

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References