Chapter 6

Rapid Alignment of Collagen Fibers in the Dermis of Undermined and Not Undermined Skin Stretched with a Skin Stretching Device

Paris Melis, M.D.*
Maril L. Noorlander, M.Sc.*
Chantal M.A.M. van der Horst, M.D., Ph.D.*
Cornelis J. F. van Noorden, M.Sc., Ph.D**

From the Department of Surgical Research*, Plastic, Reconstructive and Hand surgery* and the Department of Cell Biology and Histology**, Academic Medical Center, University of Amsterdam, The Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital*, Amsterdam, The Netherlands.

**INTRODUCTION**

Skin defects that cannot be closed primarily are common for plastic and reconstructive surgeons. Many techniques have been developed for closure of large defects, varying from split skin grafts to free revascularised flaps. In 1993, a new technique was introduced based on the use of the Sure-Closure skin-stretching system (Life Medical Sciences, Inc., Princeton, N.J.). This skin-stretching device was designed to harness the viscoelastic properties of skin by applying controlled and evenly distributed tension along the wound margins using incremental traction. The biomechanical properties of skin, known as mechanical creep and stress relaxation, allow skin to stretch intraoperatively beyond its inherent extensibility in a short period of time. As a result of skin stretching, wound closing tension decreases, allowing primary closure of large defects. The technique eliminates donor defects and associated morbidity. It enables sensate reconstructions with good cosmetic appearance of the skin.

Most experimental studies using tissue expanders were focused on the determination of histological changes in soft tissues during gradual stretching of the skin over the course of several weeks, whereas the Sure-Closure skin-stretching system is applied to stretch skin over a period of only 30 min. Furthermore, histological changes were often described on the basis of qualitative microscopical analysis of skin sections. To obtain more objective information with respect to changes caused by stretching in epidermis and dermis, a quantitative histochemical method was developed. This quantification method was applied in a controlled study in five piglets to determine objectively histochemical changes in undermined and not undermined stretched skin. The skin was subjected to stretching using a skin-stretching device.

**MATERIALS AND METHODS**

A series of five female Yorkshire piglets weighing between 20 and 25 kg was used in this study. All experiments were approved by the ethical committee for animal experiments of the Academic Medical Center. Each animal was anaesthetized with a mixture of Sufenta Forte and ketamine in a dose of 50 mg and 25 mg per kg body weight, respectively. After intubation, anesthesia was maintained by spontaneous
inhalation of a mixture of halothane (0.8 percent), air and oxygen (fraction of inspired oxygen, 47 percent). On each flank at a standard position, a 9 x 9-cm square was marked surrounded cranially and caudally over a 10-centimeter area to indicate the area of undermining (Fig. 1). Wounds were created by excising skin and subcutaneous tissue in the indicated square down to the muscular fascia. All wounds were created at the same location on each animal. This was essential because the tension required to close a wound is greater high on the back of the piglets than nearer to the belly or, likewise, close to the shoulder rather than close to the hip.11 On one flank of each animal, the surrounding areas previously outlined were undermined between subcutis and muscular fascia. On the remaining flank, the surrounding skin was not undermined. Skin was stretched in a longitudinal direction

![Figure 1](image)

Figure 1.
Skin markings of wound location (9 cm square) and size of area to be undermined (10 cm long) on the flanks of a piglet.

with the Sure-Closure skin-stretching system. (Fig. 2). Two straight needles were inserted through the dermis opposite to each other at a distance of 0.5 cm to the wound margin. The skin-stretching device was placed in such a way that its U-shaped arms were inserted through the skin and were anchored behind the intradermal needles. In this way, the hooks on the undersurface of the U-shaped arms of the device abut against the intradermal needles, which in turn distribute the stretching force equally along the length of the wound margin. When appropriate
tension was applied, the device was locked. By rotating the tension screw of the device, skin and subcutaneous tissue approximated (Fig. 2).

The wounds were closed using the principle of load cycling onto the skin.\textsuperscript{12,13} Load cycling of skin implies an incremental increase in the length of skin. By applying load cycling, it is possible to stretch skin gradually beyond the limits of its inherent extensibility because of the phenomenon of mechanical creep.\textsuperscript{14} The procedure consisted of a 4-minute stretching interval of the skin and a 1-minute relaxation period by unlocking the system to allow tissue perfusion. The cycle was repeated six times over a period of 30 minutes. As the skin stretched over time, tension reduced because of stress relaxation.

First, control biopsies of skin were taken from the middle of each wound that was removed (time, 0 minutes). Second, biopsies of stretched skin were taken randomly from lower and upper sides perpendicularly at a 1 cm distance from the skin margin after 15 minutes of skin stretching (time, 15 minutes) and at the end of the operation (time, 30 minutes). The biopsies contained epidermis, dermis, subcutaneous fat and muscular tissue, and the size of their surface was 10 x 3 mm. The skin from the biopsy was placed on cork, embedded in a solution of 7% gelatin
in distilled water, and frozen in liquid nitrogen. Series of cryostat sections (7 mm thick) were made that were then fixed and stained with either hematoxylin and eosin, van Giesson, Giemsa, or Shoobridge staining methods. Besides these standard staining methods, picrosirius red staining was performed. The Sirius red molecules are bound to collagen fibrils in a parallel fashion, greatly enhancing the naturally occurring weak birefringence of the fibrils. When using polarization microscopy, this phenomenon results in bright red collagen fibrils against a dark background and allows determination of orientation of the dermal collagen fibrils

(Fig. 3). Contrast between collagen fibers and background was optimized for quantitative analysis using image analysis and National Institutes of Health software (version 1.57, written by Wayne Rassband and available through the internet from http://rsb.info.nih.gov). Images of serial sections were captured digitally at low magnification and were subsequently analyzed. In the images, the longest axis of each fiber was measured (Fig. 3). The mean value of the largest 10 axes in the five piglets was calculated. Each value per piglet is a mean value obtained in three serial sections. This number was representative for alignment of collagen fibers in

Figure 3.
Collagen fibers as visualized with polarization microscopy. A major axis of a collagen fiber is shown. The mean value of the largest 10 axes in each section was calculated. This number was representative for alignment of collagen fibers in the sections. Bar, 50μm.
Figure 4.
Hematoxylin and eosin staining of epidermis and dermis in not stretched (left) and stretched (right) skin. The epidermal layer (asterisk) shows no significant changes under the influence of stretching forces. G, gelatine. Bar, 100 μm.

Figure 5.
Dermis of control piglet skin shows random orientation of collagen fibers, as visualized with picrosirius red staining and routine light microscopy (above) or polarization microscopy (below). Bar, 100 μm.

Figure 6.
Stretched piglet skin shows parallel alignment of collagen fibers after staining with picrosirius red and visualization with routine light microscopy (above) or polarization microscopy (below). Bar, 100 μm.
the sections. The data were statistically analyzed using analysis of variance of paired repeated measures followed by the Tukey-Kramer multiple comparison tests. A $p$ value of 0.05 was taken as level of significance.

**RESULTS**

Epidermal morphology or thickness did not change significantly under the influence of stretching forces in both undermined and not undermined skin (Fig. 4). In contrast, the dermis showed significant changes. Control (not stretched) skin displayed a random orientation of the collagen fibers (Fig. 5). As a result of skin stretching, collagen orientation changed from a random orientation to a more aligned orientation in the direction of the stretching force, perpendicular to the wound margin (Fig. 6).

In Figure 7, mean values of the 10 largest major axes in the five piglets are shown. Control skin had the lowest mean ($\pm$ SD) major axis value ($76 \pm 14$), showing little alignment of the collagen fibers (Fig. 5). Skin stretching for a period of 15 minutes in undermined skin resulted in a higher mean major axis value of the collagen fibers ($91 \pm 30$), but the difference was not statistically significant. The

![Figure 7](image)

**Figure 7.**
Mean values of largest 10 major axes as determined quantitatively (with standard error of the mean) in three serial sections of dermis from five piglets with picrosirius red staining and polarization microscopy. *Asterisk* indicates significant difference from control value ($p < 0.05$).
### Table I.

Statistical Analyses Using Analysis of Variance of Paired Repeated Measures Followed by the Tukey-Kramer Multiple Comparisons Test* \( (p = 0.0002) \).

<table>
<thead>
<tr>
<th></th>
<th>N=5</th>
<th>Mean major axis ± SD</th>
<th>Control skin</th>
<th>Stretched skin (15 min), undermined</th>
<th>Stretched skin (15 min), not undermined</th>
<th>Stretched skin (30 min), undermined</th>
<th>Stretched skin (30 min), not undermined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control skin</td>
<td></td>
<td>76 ± 14</td>
<td>15.100</td>
<td>45.440</td>
<td>55.960</td>
<td>30.160</td>
<td>4.381*</td>
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<td>q=2.193</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Stretched skin (15 min), undermined</td>
<td></td>
<td>91 ± 30</td>
<td>6.601**</td>
<td>8.129***</td>
<td>40.860</td>
<td>15.060</td>
<td>2.188</td>
</tr>
<tr>
<td>q=4.07*</td>
<td></td>
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</tr>
<tr>
<td>Stretched skin (30 min), undermined</td>
<td></td>
<td>121 ± 30</td>
<td>9.35**</td>
<td>10.520</td>
<td>15.280</td>
<td>3.748</td>
<td></td>
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<td>q=2.220</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Stretched skin (30 min), not undermined</td>
<td></td>
<td>132 ± 35</td>
<td>5.935**</td>
<td>10.520</td>
<td>15.280</td>
<td>3.748</td>
<td></td>
</tr>
<tr>
<td>Stretched skin (30 min), not undermined</td>
<td></td>
<td>106 ± 11</td>
<td>4.407*</td>
<td>6.601**</td>
<td>8.129***</td>
<td>2.188</td>
<td></td>
</tr>
</tbody>
</table>

When the value of \( q \) is greater than 4.333, the \( p \) value is less than 0.05. The mean, standard deviation (SD) and mean difference (md) of major axes are expressed as length in pixels. One pixel represents an area of 1.6 x 1.5 mm. * : \( p < 0.05 \), ** : \( p < 0.01 \), *** : \( p < 0.001 \)

higher the mean major axis value of the collagen fibers in the plane of the section was, the more elongation and alignment of the fibers had occurred. Thirty minutes of stretching of undermined skin resulted in the highest mean major axis value (132 ± 35). This was statistically significant difference from the control value (Table I). The quantitative data were in agreement with qualitative analysis of the sections (cf. Figs. 5 and 6). After 30 minutes of stretching of undermined skin, collagen fibers ran almost perpendicular to the wound margin. Stretching of not undermined skin for 15 minutes resulted in statistically significantly higher mean major axis value (121 ± 30). There was also a statistically significant difference after 15 minutes of skin stretching between undermined and not undermined skin (Table I). After 30 minutes of skin stretching in not undermined skin, collagen fibers were still clearly stretched (106 ± 11). Although this value had a statistically significant difference from the control value, parallel alignment was less well defined than after 15 minutes stretching. Significant changes were not observed in the muscle layers after 15 and 30 minutes of skin stretching, both in undermined and not undermined skin.
DISCUSSION

Changes in the dermis of skin during stretching with the use of a stretching device for 30 minutes to close a large skin defect have not yet been analyzed. Histological changes have mainly been studied in expanded soft tissues. In these studies, gradual expansion was performed over a period of several weeks and histological changes were usually described on the basis of qualitative microscopical analysis of skin sections. It appeared that the epidermis did not change significantly, whereas a rapid decrease in thickness of the dermis and the panniculus carnosus muscle occurred, especially during the first 2 weeks after implantation of the expander. Larger and more compact bundles of collagen fibers were observed in the dermis of expanded skin. In addition, most of the experiments were performed on laboratory animals such as guinea pigs and rabbits. Both species have skin that has a different composition from that of the human skin. These animals have a “scruff”, which allows the skin to move freely over the deeper layers of tissue and can be picked up in large folds. We have performed our experiments on piglets because their skin closely resembles that of human skin. There are similarities not only in histomorphology but also in vascular anatomy of the skin. However, the muscle layer of pig skin is a noncontinuous coat called panniculus carnosus. This does not exist in humans with the exception of the platysma muscle in the antero-lateral neck region.

Gibson et al. were the first to describe lengthening of skin and realignment of randomly positioned collagen fibers into parallel bundles as a result of a stretching force. Only a few studies have been published on histological changes in rapidly expanded skin, all after using immediate intraoperative tissue expansion. The results of these studies were controversial. One study showed that the more acute the expansion, the more the angle of cross linking diminished, meaning that the fibers run more parallel to the surface. All others reported that significant histological changes in the orientation of the collagen fibers in the dermis of rapidly expanded skin did not occur.

Histomorphological changes as a result of skin stretching are difficult to quantify. To analyze whether significant changes in orientation occur, an objective method is imperative. We used picrosirius red staining in combination with polarization microscopy to objectively determine changes in collagen orientation. The use of polarization microscopy to identify collagen in picrosirius red-stained material is specific. It allows quantitative analyses with sufficient sensitivity and
spatial resolution. This is because even very thin fibrils of collagen, which are undetectable with routine microscopy, are visualized with this method as a source of light against a dark background (Figs. 5 and 6). This method enables image analysis to determine quantitatively the length of the fibers in sections as a parameter of histomorphological changes of collagen fibers caused by skin stretching. Because of its validity, reproducibility, and the fact that the biopsies were quenched in liquid nitrogen to freeze the structure of the tissues immediately, this appeared to be the method of choice to analyze behavior of collagen during stretching.

Skin stretching for 30 minutes only using a device with maximum stretching force of 2.5 kilograms significantly reduces wound closing tension.\(^{29}\) This decrease in tension results from changes in the dermis of the skin that was visualized at the microscopical level using the picrosirius red polarization method. Epidermis did not change significantly under the influence of stretching forces. The dermis consists of 80 to 90 percent collagen fibers. The fibers changed from a random orientation to a more parallel alignment in the plane of the sections during stretching. The fibers stretched rapidly as a result of the forces applied and became aligned in the direction of the stretching force, perpendicular to the wound margin. After undermining the skin, collagen fibers seemed to align less rapidly as a result of skin stretching than when the skin was not undermined, but after a 30 minutes interval of stretching, alignment was less dependent on undermining. Although maximum alignment was seen after undermining in combination with a 30 minutes interval of skin stretching, this was not statistically significant compared to skin stretching without undermining. These results are comparable with the tension decrease that we measured as a result of skin stretching with or without undermining in an experimental study in piglets.\(^{20}\) In this study, it appeared that undermining of the wound margins before skin stretching resulted in a small additional tension decrease, but also had its well-known complications such as skin edge necrosis and seromas. To our knowledge, this is the first time that these rapid changes in orientation of collagen fibers in dermis caused by mechanical force using a skin-stretching device have been described. This alignment of collagen fibers in the direction of the mechanical force explains the rapid relaxation of the skin as a result of skin stretching.
REFERENCES


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DISCUSSION BY BERNARD HIRSHOWITZ, F.R.C.S.

This article is further confirmation of the changes in the alignment of collagen fibers produced by stretching of the skin, applied in a controlled study in five piglets, with the skin both undermined and not undermined. Using polarization microscopy of picrosirius red-stained sections of stretched skin, the collagen fibers, in contrast to a black background, show up in bright red in a parallel direction to the stretching force, which is perpendicular to the wound margin. These changes were evident after 15 minutes of incremental stretching with the Sure-Closure skin-stretching system, and they were also evident after 30 minutes of stretching.

The mechanical response of the skin to loading, because of the viscous mucopolysaccharide ground substance of the dermis, is time dependent. This viscous fluid acts like a lubricant, permitting the relative movement of separate collagen fibers in contact and thereby reducing dry friction effects, but in addition, it provides a viscous resistance to relative motion. Its behavior, in mechanical terminology, is viscoelastic, showing the typical behavior patterns of creep deformation under constant stress and stress reduction under constant strain (i.e., stress relaxation). The stretching of skin causes the irregular, wavy, and multidirectional collagen fibers to be converted to a compact and parallel configuration, the combined effect of which enables sufficient lengthening of skin to permit closure of a large skin defect. The elastic fibers that are interwoven with collagen fibers are randomly coiled, giving the elastic fibers their ability to be stretched and then to revert to their original state. It is the elastic fibers that restore the stretched and elongated collagen to its previous state, following each episode of deformation of the skin, as the collagen fibers themselves do not have the power of retraction. These fibers require staining by Verhoeff's elastic stain to be seen.
Initially, at low loads the collagen fibers move with ease relative to each other, the implication being that relaxed skin can be readily stretched. The resistance to further extension rapidly increases as more and more fibers become aligned and take up the strain. The collagen fibers impart the considerable strength to skin and also limit the extent of deformation when it is pulled. It was found empirically that skin can be safely stretched up to a load of 3 kilograms, and to prevent possible damage, a built-in tension gauge has been incorporated in the tension knob of the Sure-Closure skin-stretching device. In addition, a safety clutch within the knob disengages whenever the tension applied to the skin is in excess of 3 kilograms. Stretching the skin beyond its natural extensibility by using excessive force can cause tearing of the collagen fibers, leading to striae formation. In chronic wounds, the composition of the mucopolysaccharide matrix of the dermis is altered and may be partly replaced by immature scar tissue so that the natural lubrication of the collagen fibers is impaired. Under these conditions, the time needed to harness the skin’s viscoelastic properties is delayed, and hours to 1 or 2 days may be required to fully mobilize these properties of the skin.

To assess the rapid changes in orientation of the collagen fibers in the dermis caused by mechanical force from a skin-stretching device, the stretched skin was stained by the authors with picrosirius red.

The Sirius red molecules are bound to collagen fibrils in a parallel fashion, which greatly enhances the naturally weak birefringence of the fibrils. Using this principle, the authors have demonstrated in a novel and graphic manner that by employing polarized microscopy to identify even very thin fibrils of collagen in picrosirius red-stained sections, quantitative determination can be made of the length of the fibers in sections as a parameter of histomorphologic changes of collagen fibers caused by skin stretching.

The authors have shown that skin stretching up to 30 minutes results in marked histomorphologic changes of collagen fibers in the dermis of both undermined and not undermined skin, which explains the decreased wound closing tensions, allowing primary closure of large skin defects. There was no significant difference in the results between the undermined and not undermined stretched skin. In their study, the authors demonstrated that the results of using a skin-stretching device-lengthening of the skin of the wound margins and decreasing wound closing tension-are the two features that enable closure of a wound with a large skin defect.
REFERENCE
