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CONTRIBUTIONS OF ELECTRON MICROSCOPY TO THE STUDY OF THE HYPERTROPHIC SCAR AND RELATED LESIONS

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Abstract

Prior to 1969, only one study of the hypertrophic scar had been done using electron microscopy, and that one used electron diffraction. Since that time, studies using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have been integral in establishing not only the characteristics of this lesion but in formulating the reasons why the scar develops and how it resolves. The first SEM studies demonstrated a homogeneous, dense dermal matrix which supported the conclusion that the hypertrophic scar and keloid reflected excess collagen. These same studies were integral in identifying the collagen nodule as the basic anatomical unit of these lesions. SEM and TEM studies have been complimentary. The TEM studies revealed the first evidence of the phenomenon of occluded microvessels; but, their significance was not established until later quantitative studies. A hierarchy of fibroblasts was first demonstrated by TEM. Later, evidence came from several different investigators, through tissue culture and molecular differentiation, that the previously observed different electron microscopic features may reflect different fibroblast types of cells. Finally, the degenerative (or apoptotic) events, involving fibroblast-type cells and microvessels have been revealed by TEM studies, and supported by SEM observations. This phenomenon has been presented as a major factor in formation of the nodules, and, also, in the natural resolution of the hypertrophic scar and keloid.

Key Words: Hypertrophic scar, keloid, collagen, fibroblast, fibrocyte, dermis, microvessels, scanning electron microscopy, transmission electron microscopy.

Introduction

The hypertrophic scar and its related lesion, the keloid, are examples of scar tissue which develop following an injury to the skin of critical depth, which appears to be related to the extent of microvascular injury (Kischer *et al.*, 1990). These lesions are unique to humans. The differences between the hypertrophic scar, keloid and normal scar are clinical as well as anatomical and have been previously discussed (Kischer *et al.*, 1982a; Kischer, 1992). Until such time that certain model systems could be designed, studies were, of necessity, confined to surgical excisions and biopsies.

Prior to 1969, only one article (Holmstrand *et al.*, 1961) was published using electron microscopy as a method of study of these lesions; those authors used electron diffraction as a means to determine the orientation of collagen in the scar. All other studies used light microscopy to characterize the hypertrophic scar and keloid; but, often, the data from these studies were inconsistent and conflicting (Mancini and Quaife, 1962; Montgomery, 1967; Sturim, 1968), because characterizations depended on a blend of variable histology with clinical observations. Too often, stages of lesion development were ignored. Their microanatomy varied according to time post-injury and from individual to individual. But, such variations were rarely considered. Sometimes clinical histories were not available, further complicating analyses. Additionally, some lesions were diagnosed clinically as mature when they were still hypertrophic, or classified as hypertrophic when they were found histologically to be keloidal, or contrarily.

Figures

All of the tissues used by the author and shown here are human tissues with the exception of Figure 11, which was taken from a rat. All human tissues were obtained from surgical excisions. They were immediately cooled for transport to the laboratory and fixed within 30 minutes, or were placed in a transport medium for shipping and fixed immediately upon arrival. All tissues were then cut to appropriate size and sufficiently thin to allow for quick penetration of the fixative. All tissues

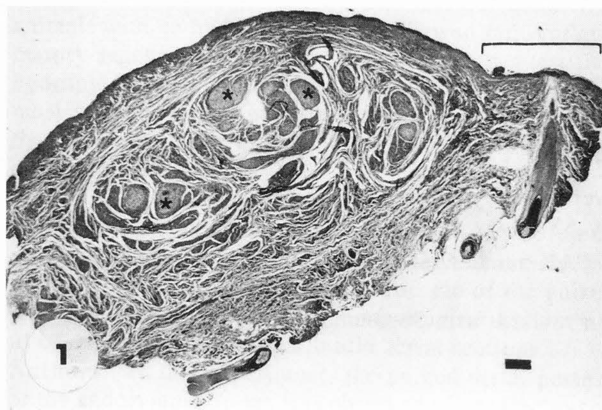
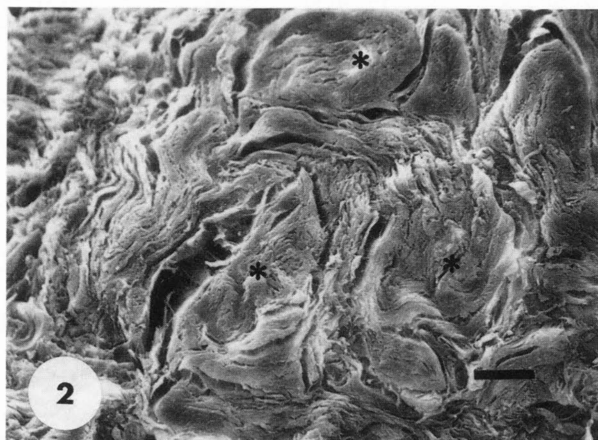
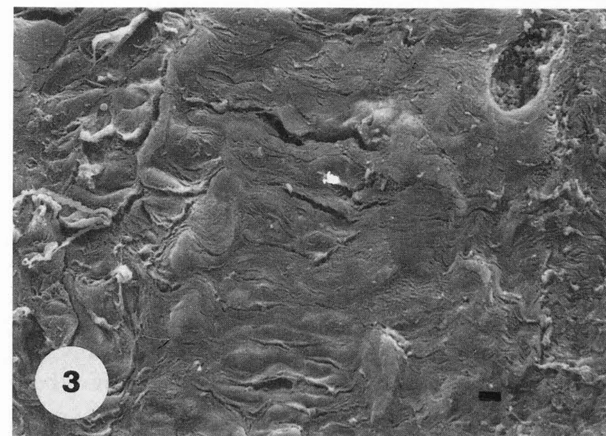


Figure 1. A light microscopic cross-sectional view of a hypertrophic scar with adjacent normal (uninvolved) skin (bracket). Excised from the chest. Thermal burn. Nodules of varying size (*) are seen in cross-section. Masson's trichrome. Bar = 100 μm.





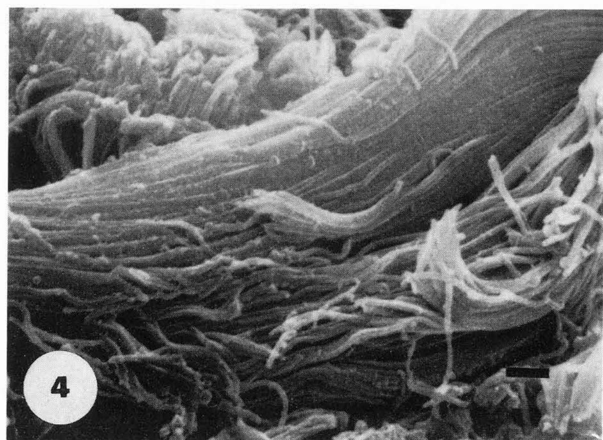


Figure 2. Nodules of HS in cross-section (*). SEM. Bar = 100 μm.

Figure 3. Collagenous matrix of a nodule, note lack of interstitial space. SEM. Bar = 10 μm.

Figure 4. Collagenous matrix of dermis from normal skin, note interstitial space and fibrils. SEM. Bar = 1 μm.

were immersed in Karnovsky's fixative. Those samples for histological study were embedded in paraffin, sectioned and stained by hematoxylin and eosin (H & E) and by Masson's trichrome stain.

Samples for scanning electron microscopy (SEM) study were dehydrated by transfer through graded alcohols, then processed in a Tousimis Sandri-790 critical point dryer. The intermediate fluid was alcohol and the transitional fluid was liquid CO_2. The samples were mounted and coated with approximately 30 nm of gold using a Polaron Sputter Coater, model #5700 with argon gas. Observations were made in an ETEC Autoscan SEM operated at 20 kV.

Transmission electron microscopy (TEM) samples were post-fixed in 2% OsO_4 buffered with sodium cacodylate, dehydrated through graded alcohols, passed through propylene oxide, and embedded in Epox 812 (Ladd Research Co.). Thin sections were cut with a diamond knife, stained with lead citrate and uranyl acetate and examined in a Philips 300 electron microscope.

Collagen

Collagen fibril diameter and distribution patterns are different in skin from various species and during different physiological states of the skin in the same specie (Flint *et al.*, 1984). In human skin, undergoing repair from full thickness injury, it was found that collagen fibril diameters changed over time (Kischer, 1974a).

The microanatomical unit of both the hypertrophic scar (HS) and keloid is the collagen nodule, a rod-like fascicle of uniformly aligned, highly stressed collagen. These units can be readily observed with the naked eye when cut in cross-section and easily observed in tissue section. Figure 1, shows a cross-section of a hypertrophic scar demonstrating the area of scarring clearly and sharply delineated from normal uninvolved skin (bracket). It also shows numerous nodules of varying

size in cross-sectional profile (N). The hypertrophic scar and keloid are defined by the presence of these nodules. In other words, they exist in every sample (Kischer and Brody, 1981), but, too often, they have been ignored (Knapp *et al.*, 1977).

The nodules can be observed by scanning electron microscopy (SEM) (Figure 2) and at high magnification reveal a homogeneous annealed matrix (Figure 3). In the dermis of normal skin, SEM shows a quite different appearance with much interstitial space and variable sizes of collagen fascicles (Figure 4). The appearance of the hypertrophic scar matrix by SEM is complemented by the view through transmission electron microscopy (TEM), in which the interfibrillar matrix demonstrates prominent filamentous material (Figure 5), which appears to link the fibrils together (Inset, Fig. 5). The four-fold increase of one of the glycosaminoglycans (GAGs), chondroitin-4-sulfate (C-4-S), from levels in normal skin, found in hypertrophic scars and keloids, is consistent with the fine structure of these lesions (Shetlar *et al.*, 1972).

The early literature on the hypertrophic scar concentrated on studies of collagen. Although it was determined that the lesions were rich in Collagen Type III, (Bailey *et al.*, 1975; Chaig *et al.*, 1975; Hayakawa *et al.*, 1979) no differences have ever been found in the periodicity of the fibrils.

In a set of experiments we performed, it was found that a marked difference in the interfibrillar material was observed by SEM on fresh post-surgical tissue between the hypertrophic scar and the hypertrophic scar which was mechanically stretched (Figures 6 and 7). We reported that the interfibrillar material was anionic, staining with Ruthenium Red, and became globular upon stretching, which presumably produced rupture (Figure 8 and inset) (Kischer and Shetlar, 1974). The significance of the prominence of this material and its probable relationship with C-4-S is probably rooted in the size and shape of collagen filaments and changes thereof over time of healing (Shetlar *et al.*, 1981). The globular nature upon rupture suggests a recoil or elastic nature of the interfibrillar material and prior to rupture, complements the rigid, hard quality of the clinical lesion.

In the course of our studies we wondered what happened to the severed ends (or burned ends) of collagen fibrils in a wound. SEM observations on Karnovsky's-fixed and critical point dried samples repeatedly showed those ends to be rounded (Figure 9). Again, does this have something to do with the relationships collagen may have with the glycosaminoglycans (C-4-S)? By TEM, profiles of cross-sectioned collagen in normal dermis show a fuzzy peripheral coating (Figure 10), while in the hypertrophic scar the edges are sharper with prominence of the interfibrillar material (Figure 5) (Kischer, 1974a). In case of severance, would this material migrate over the end, cover the fibril, and act as an annealing agent?

We looked at the healing of full thickness wounds in rats by SEM and found that, indeed, the area of wound closure suggested collagen fibrils appearing to be mechanically bound to one another, which could have been mediated through a matrix proteoglycan (Figure 11) (Kischer and Shetlar, 1974).

Further evidence for the involvement of GAGS in the healing (or maturation) process was found from three sources: 1) a reduction of C-4-S in the mature scar (Shetlar *et al.*, 1972); 2) examination of the mature scar by SEM (Figures 12 and 13), which shows a change from the homogeneous annealed dermal matrix to resolving collagen fibrils; and 3) examination of the hypertrophic scar after pressure therapy (Figure 14), which has been proven to accelerate maturation (Kischer *et al.*, 1975; Berry *et al.*, 1985; Clark *et al.*, 1987).

Cells

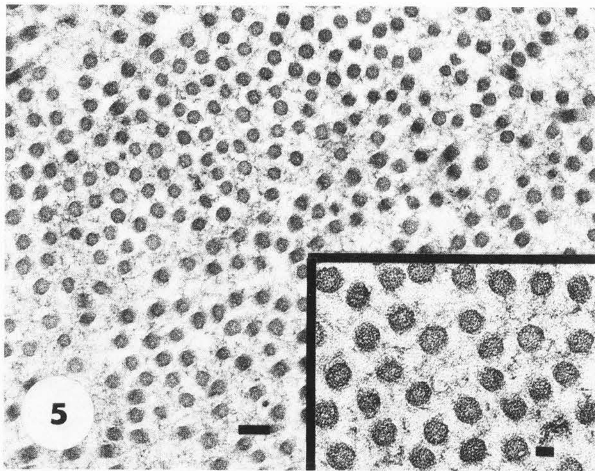
It is clear that the number of interstitial cells in the presumptive dermis (granulation tissue) in a wound are substantially increased (Kischer *et al.*, 1990). In the hypertrophic scar or keloid the fibroblasts form an intricate network with long interlacing and anastomosing filopodia (Figure 15). Among the fibroblasts, morphologic heterogeneity was first suggested by Kischer (1974b) and subsequently, functional heterogeneity was established by tissue culture of cell lines derived from lesions (Kischer *et al.*, 1989; Kischer and Pindur, 1990).

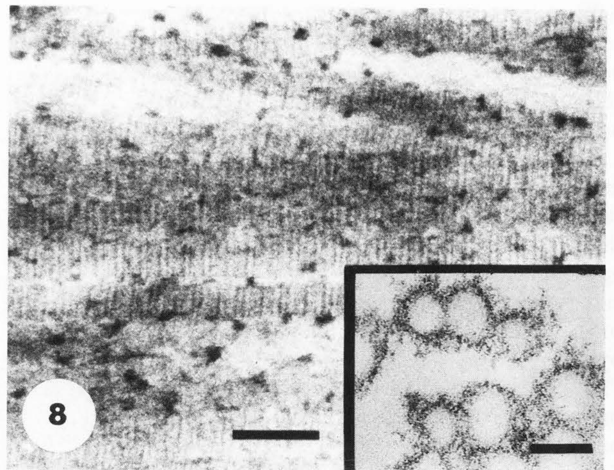
By SEM, it was difficult to identify fibroblast-type cells in hypertrophic scars, probably because of the extreme compactness of cells and collagen in the nodule. In some lesions treated with mechanical pressure, however, the myofibroblast type of cell could be inferred (Figure 16). The SEM view conformed to that observed by TEM in which vast amounts of dilated rough endoplasmic reticulum and microfilament bundles were present (Figure 17).

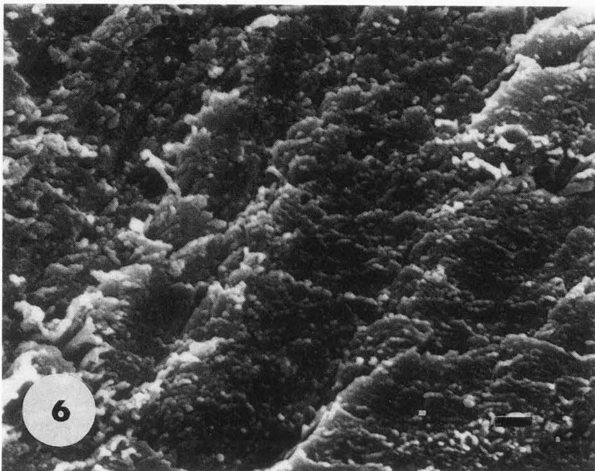
By SEM, fibroblast-type cells are difficult to find in mature scars, as is also the case in normal dermis. This complements the histological view that the increased number of these types of cells in granulation tissues, hypertrophic scars and keloids disappears during the course of maturation. Such, in fact, is the case. Maturing hypertrophic scars demonstrate fewer cells, but many degenerative forms of fibroblasts (Figure 18). These forms could be found by SEM (Figure 19). They also were found in granulation tissues (Kischer *et al.*, 1990) and in a study by Darby *et al.*, (1990).

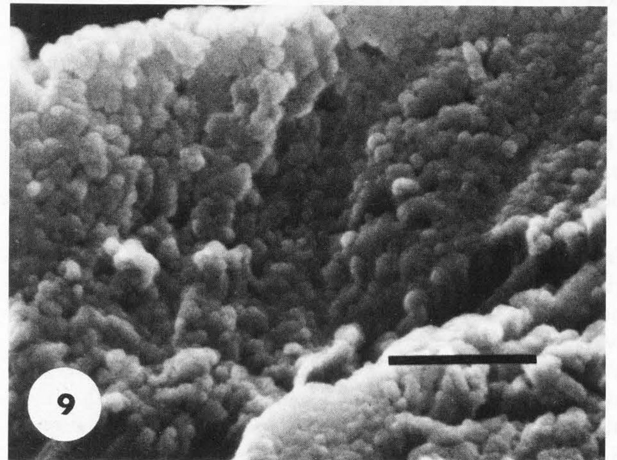
Microvessels

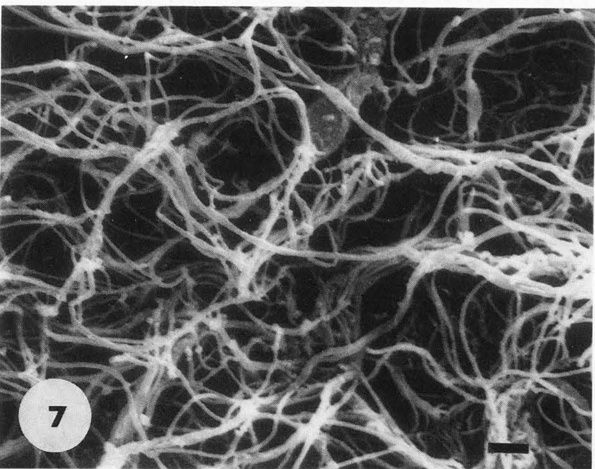
No other finding in the past 25 years of studies of the hypertrophic scar has been more significant than that of occluded microvessels. The occlusion of the lumen is produced by an increased number of endothelial cells. The frequency, incidence and significance of this finding has been reported several times (Kischer *et al.*, 1982a; Kischer, 1987, 1992). It was first observed by TEM (Kischer *et al.*, 1971) (Figure 20). Occlusion is not that easily observed in routine hematoxylin and eosin











sections, the reason being that the effect of formalin fixative produces enough artifacts on the tissues that the occlusion aspect is often distorted (Figure 21). Far less distortion is produced by the aldehyde fixatives used for TEM and SEM. Occluded microvessels are observable by SEM (Figure 22), and are clearly distinguished from those which are patent Figure 23).

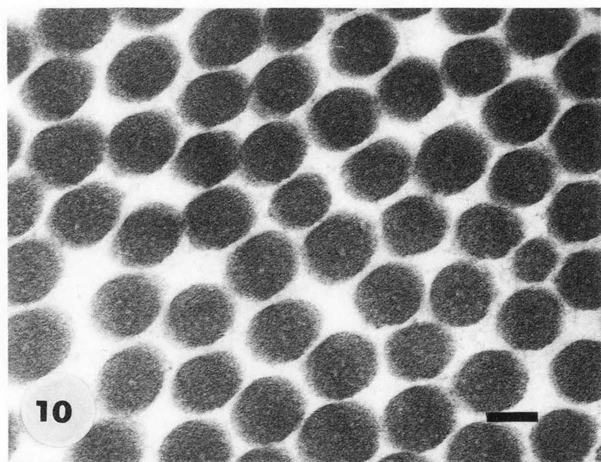
Figure 5. Nodular matrix; cross-sectioned fibrils. TEM. Bar = 0.1 μm. **Inset**: Note prominent interfibrillar material appearing to link fibrils. Bar = 0.1 μm.

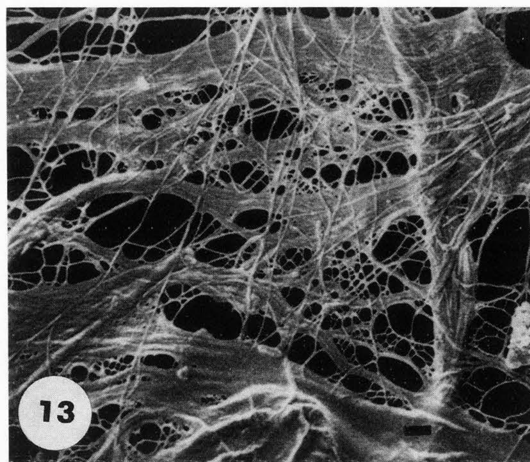
Figure 6. Hypertrophic scar (HS) matrix; note congealed appearance of collagen. Compare with Figure 7. SEM. Bar = 1 μm.

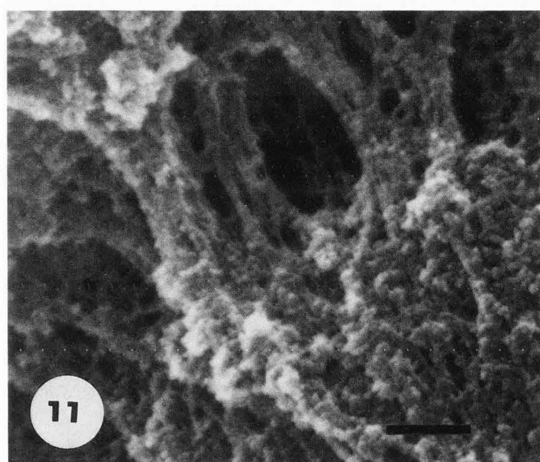
Figure 7. HS matrix under stretch force; collagen fibrils resolved. Compare with Figure 6. SEM. Bar = 1 μm.

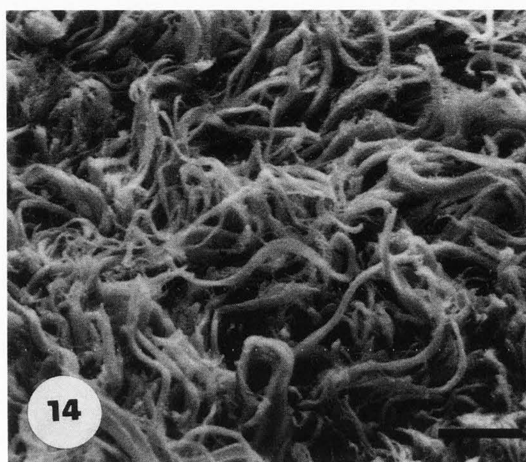
Figure 8. Collagen fibrils from nodule of HS placed under mechanical stretch. Stained with ruthenium red. Interstitial filamentous material has now become globular. TEM. Bar = 0.1 μm. **Inset**: Cross-sectioned collagen fibrils from same tissue and stained with ruthenium red (compare with Figure 5). Bar = 0.1 μm.

Figure 9. Ends of collagen fibrils in nodule of HS; the ends may have been burned, torn, or cut, but they all appear round. SEM. Bar = 1 μm.









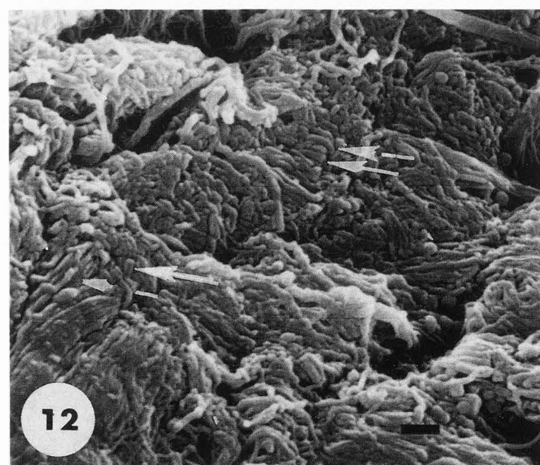


Figure 10. Collagen fibrils in cross-section from normal dermis; note that the edges of the fibrils are not sharp but appear to have a fuzzy coat. Compare with Figure 5. TEM. Bar = 0.1 μm.

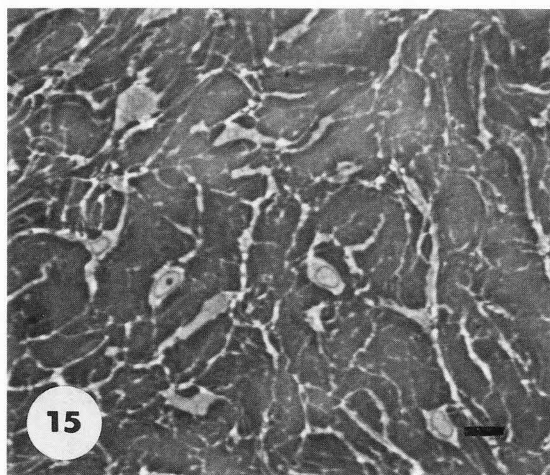
Figure 11. Healing area in wound from back skin of the rat, biopsy taken 10 days after full thickness incision and closure. Annealed ends of fibrils is suggested by the many globular forms observed. Taken in direct midline of healing wound. SEM. Bar = 1 μm.

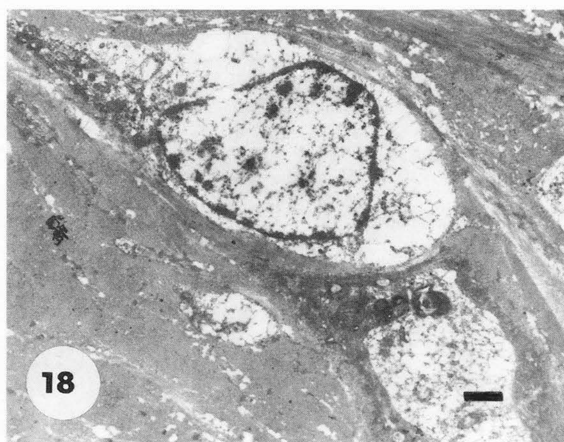
Figure 12. Nodular matrix of HS in early mature stage; note beginning resolution of fibrils. Compare with Figure 6. SEM. Bar = 1 μm.

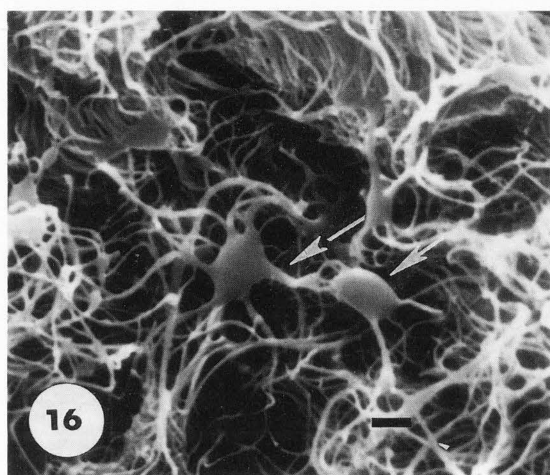
Figure 13. Mature scar in late stage of maturation; note matrix showing interstitial space and resolving individual collagen fibrils. SEM. Bar = 1 μm.

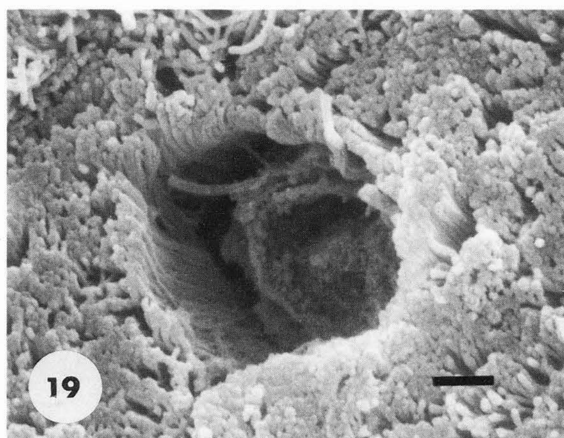
Figure 14. HS under therapeutic mechanical pressure for one year; note nearly total resolution of matrix into individual fibrils. SEM. Bar = 10 μm.

Occluded microvessels are integral to the theory of how the hypertrophic scar develops (Kischer *et al.*, 1982b). This theory states that subsequent to an injury of critical depth to the skin, new microvessels regenerate within a milieu of inflammatory cells. The source of new fibroblasts is unknown but may be from the uninjured peripheral dermis or from the growing tips of the









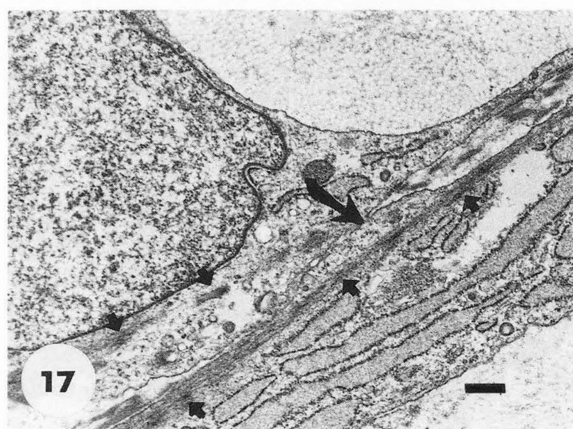


Figure 15. Phase microscopy of thick plastic section from nodule of HS; note long interlacing and anastomosing network of fibroblast-type cells. Toluidine Blue O stain. Bar = 10 μm.

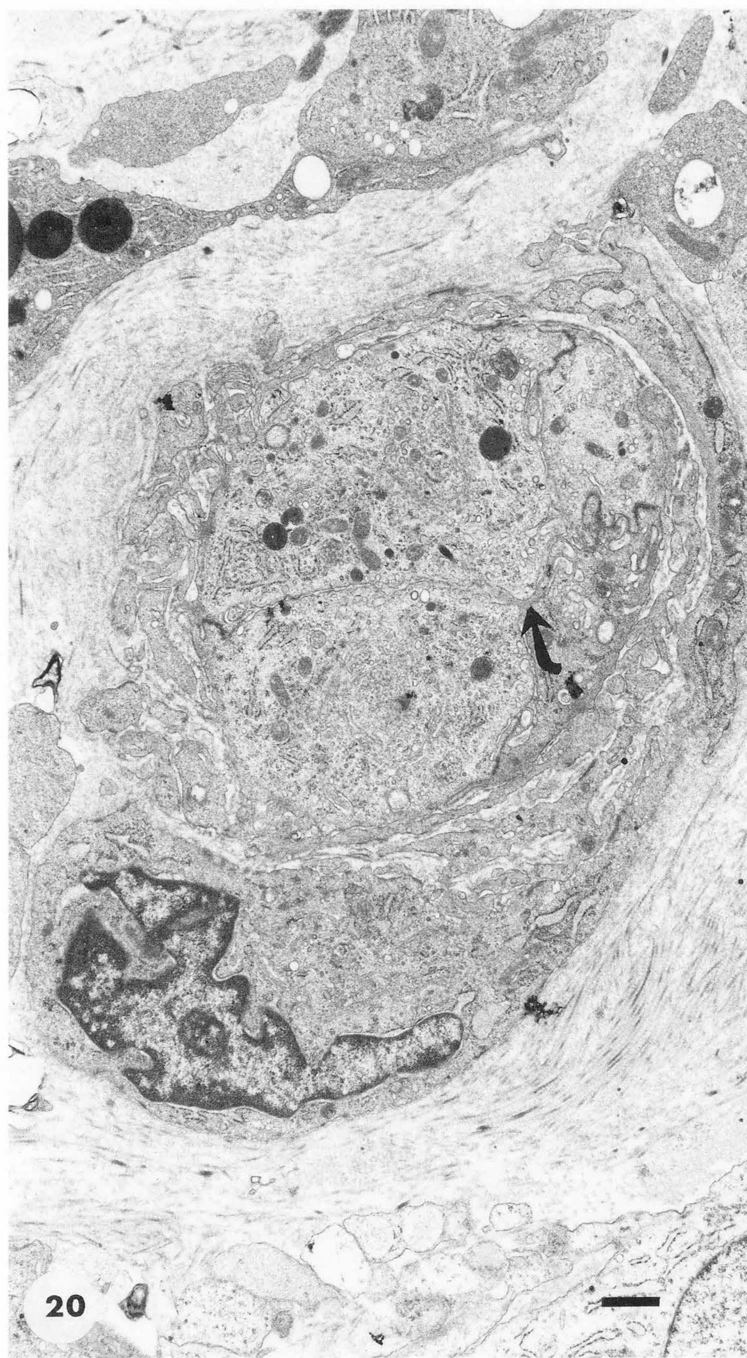
Figure 16. Cells (arrows) in nodular matrix of HS under mechanical therapeutic pressure for three months. SEM. Bar = 1 μm.

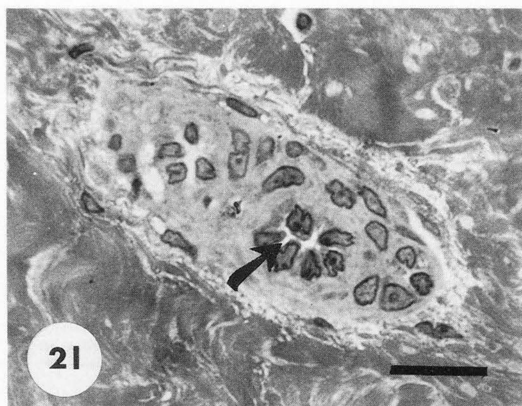
Figure 17. Myofibroblast cells from nodule in HS; note anastomosed area of two cells (large arrow) and bundles of microfilaments in both cells (small arrows). TEM. Bar = 1 μm.

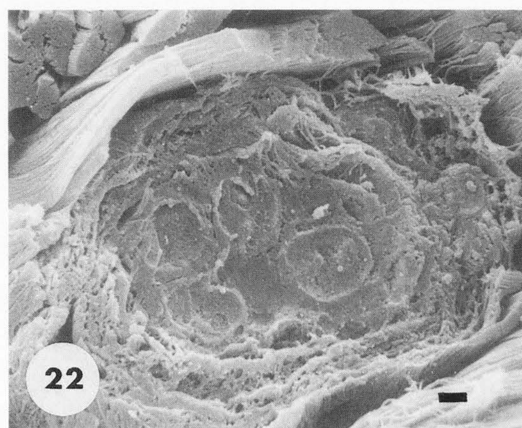
Figure 18. Degenerative forms of fibroblast-type cells in nodule of old HS. TEM. Bar = 1 μm.

Figure 19. Degenerative form of fibroblast-type cell in nodule of old HS. SEM. Bar = 1 μm.

new microvessels (Beranek, *et al.*, 1986; Beranek, 1989). Stimulating factors, perhaps originating from the inflammatory cells, prompt proliferation of fibroblasts and endothelial cells. An excess of endothelial cells occlude many of the microvessels, partially or completely. Granulation tissue is oxygen-poor (Hunt *et al.*, 1978). In the hypertrophic scar, which develops from granulation tissue, occluded microvessels would sustain a condition of hypoxia, predicted by Kischer *et al.* (1975) and subsequently confirmed by direct tissue measurement (Sloan *et al.*, 1978; Berry *et al.*, 1985), and which could stimulate a fibroblast-type cell to produce an excess of collagen (Chvapil, 1974), accounting for the bulk of the lesion. The production of excessive collagen, and, thus, the hypertrophic scar, is sustained as long as the stimulus (presumably the hypoxia) is present. The stimulus would be sustained as long as the occluded microvessels are present.







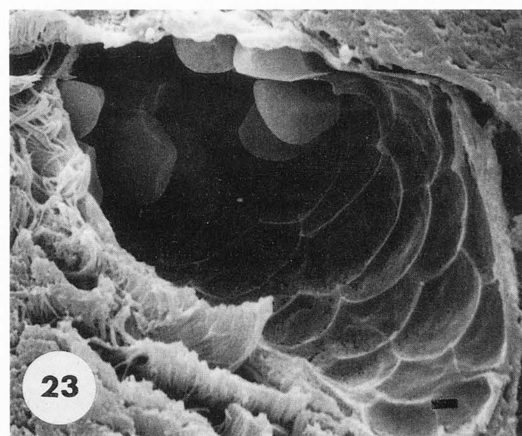


Figure 20. Microvessel in HS; lumen is occluded (arrow). TEM. Bar = 1 μm.

Figure 21. Light microscopic view of microvessel in HS. Although lumen seems to be somewhat patent (arrow), note number of endothelial cells and pericytes. Formalin fixation; H & E stain. Bar = 10 μm.

Figure 22. Occluded microvessel in HS; lumen is not observable. SEM. Bar = 1 μm.

Figure 23. Patent microvessel in normal skin. Compare with Figure 22. Erythrocytes in lumen. SEM. Bar = 1 μm.

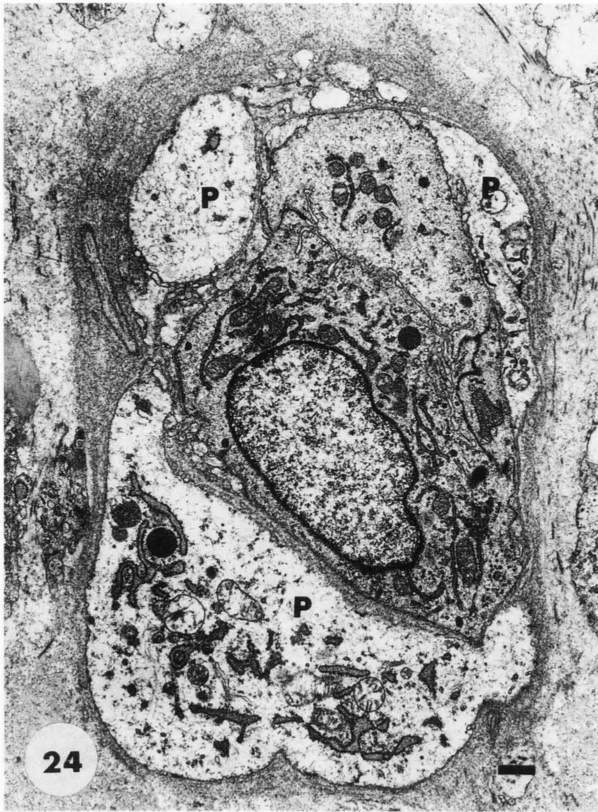


Figure 24. Occluded microvessel in granulation tissue from full thickness wound; note degenerative condition of pericytes (P). TEM. Bar = 1 μm.

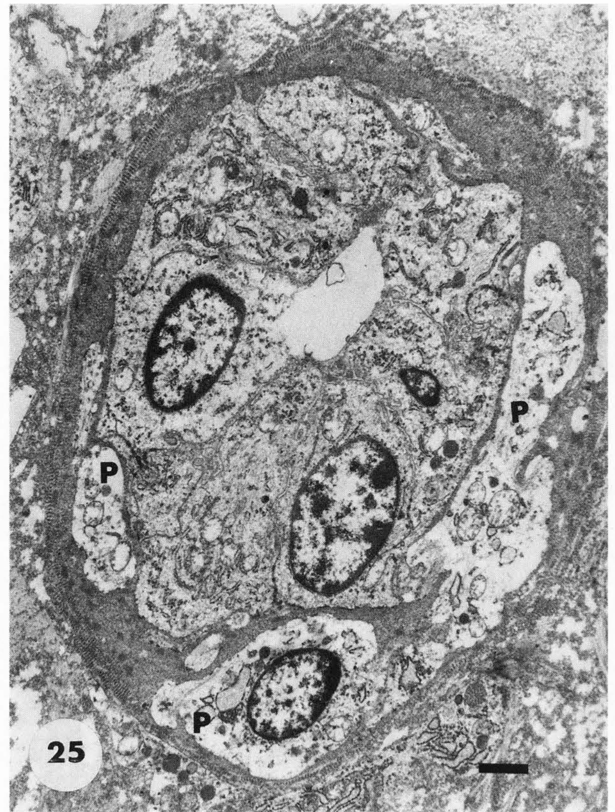


Figure 25. Microvessel in HS; pericytes (P) are degenerative, but endothelial cells may also be degenerative (arrows). TEM. Bar = 1 μm.

TABLE 1. Comparative characteristics of normal dermis, granulation tissue (GRAN), hypertrophic scar or keloid (hs/k), and mature scar (D = diameter).

	NORMAL	GRAN	HS/K	MATURE
Collagen	Variable size of fascicles; Fibril D = 110 nm; Lattice or interlocking network	Few fascicles, Whorl formations; Fibril D = 44 nm; Little organization	Annealed Nodules Fibril D = 60 nm	Small fascicles Uniform and parallel Fibril D = 100 nm
Cells	Minimal number of fibrocytes	Inflammatory cells Increasing number of active fibroblasts	Increased number of active fibroblasts	Few fibroblasts or fibrocytes
Micro-vessels	Few observed in dermis Moderate number of profiles in papillary plexus	Highest number of profiles Many occluded Some degenerative forms	Increased early in lesion Decreased in older lesion Degenerative forms Most are occluded	Few in number No occlusion Few or no degenerative forms

It is known that hypertrophic scars are self-limiting (Peacock and van Winkle, 1976). Thus, most, if not all, would eventually mature and flatten. When this would occur is quite variable and unpredictable. However, one of the phenomena discovered by electron microscopy has been crucial in completing the theory as to the origin and resolution of the hypertrophic scar. That has been the observation of microvascular degeneration (Kischer, *et al.*, 1990). It is known that the granulation tissue and the early developing scar contain excessive numbers of microvessels. The question was: since the maturing hypertrophic scar reveals few microvessels, where did they all go? Observations of the degenerative forms appears to answer that question.

We observed degenerating endothelial cells, pericytes, and fibroblasts in the granulation tissue, as well as in older hypertrophic scars (Figures 24 and 25) and concluded that the microvessels progressively degenerate and are absorbed from early in the granulation stage to late hypertrophy. In granulation tissue and the developing hypertrophic scar, the degeneration is related to the formation and enlargement of nodules.

Degeneration of the microvessels in the hypertrophic scar would eventually alter the hypoxic state to one of virtual anoxia causing the death of many fibroblasts, and allow for release of lysosomes, and therefore, hydrolases, which would act on the excess collagen, thus resulting eventually in flattening of the hypertrophic scar. The maturation would be permanent because the degenerated microvessels are not replaced.

Understandably, maturation, in most cases, constitutes a relatively long period of time. But, as has been pointed out, obviating this extended time can be accomplished by pressure therapy, which is known to accelerate maturation. The possible explanation of the efficacy of pressure treatment has been presented by Kischer (1992). Future clinical therapies may be dependent upon determination of which fibroblast type is producing the excess collagen, which accounts for the clinical appearance of the hypertrophic scar.

Table 1 summarizes the observations made by electron microscopy on the fine structural characteristics of normal dermis, granulation tissue, hypertrophic scar or keloid, and mature scar.

Conclusions

Thus, electron microscopy, both TEM and SEM, have been integral in discovering the specific patterns of collagen during development of the hypertrophic scar, identifying factors which have largely been responsible for developing and sustaining the patterns, and most importantly, accounted for factors which lead to resolution of the lesion.

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Discussion with Reviewers

B. Forslind: Can you comment on factors such as the volume or mass relations between collagen and GAGs in the matrix of the normal dermis as opposed to corresponding findings in the hypertrophic scar and keloids?

Author: Shetlar and his group [(Shetlar MR, Dobrkovsky M, Linares H, Villarante R, Shetlar CL, Larson DL (1972) The Hypertrophic scar. Glycoprotein and collagen components of burn scars. Proc. Soc. Exp. Biol. Med. **138**: 298-300; Shetlar *et al.*, 1972) did the early work on this and found that salt soluble collagen, dermatan sulphate and chondroitin-4-sulphate were elevated in the hypertrophic scar over levels in normal skin. Further, they found that C-4-S was more significantly elevated than any other collagen or GAG component. In addition, all of the morphology done over the past 25 years has complemented and supported this data in demonstrating that the hypertrophic scar and keloid reflect an excess of collagen and proteoglycans.

B. Forslind: The difference in collagen fibril diameter in the papillary versus the reticular dermis is conspicuous in normal skin. Have you any data on the fibrillar diameters in hypertrophic scars and keloids, respectively? Have you any indications of changes in the collagen repetition periods along the fibre axis in the hypertrophic scars and the keloids as compared to normal dermis?

Author: Yes. The work on differing diameters of the collagen fibrils has been done (Kischer, 1974a; Kischer and Brody, 1981). In the hypertrophic scar and keloid, the papillary dermis is virtually obliterated; so, there is only the reticular dermis involved in the lesion. Over time, from the early granulation to mature scar, that is, one which has proceeded through the stage of hypertrophy, the diameter of the collagen fibril increases and its shape changes from angular to ovate to round. The interfibrillar material, most likely proteoglycans, is most prominent in the hypertrophic scar and decreases with maturation, approaching normalcy.

C.J. Doillon: Do you think that microvessels can be mechanically compressed secondary to the increase in collagen nodule size?

Author: We have never seen any evidence for this. In fact, microvascular occlusion occurs initially in granulation tissue. The statistical studies we have done confirm the fact that the occlusion is due to a real increase in the endothelial cells.

C.J. Doillon: Do you think these degenerative fibroblasts could be compressed by the collagen nodules increasing in size?
Author: No. At all stages of healing from granulation tissue, through hypertrophy and maturation, degenerative microvessels and fibroblasts are often seen in round profiles. Some of this degeneration may be through apoptosis, while some also may be due to anoxia through enlarging nodules simply placing a greater diffusion barrier of collagen and matrix between the oxygen and nutrient source and fibroblast-type cells.

W.H. Wilborn: As noted by the author, nodules of lesions are sectioned for viewing by SEM. Could some of the features in Figures 2, 3, 6 and 9 result from sectioning the tissue prior to fixation, or perhaps making the sections with a dull razor blade?
Author: I do not believe so. Virtually all of the tissues handled and processed in this laboratory were dissected after fixation. Please compare Figure 4 with Figure 6 to see the dramatic difference in dermal matrix, both tissues were cut and processed in exactly the same way. Also, we never used dull razor blades.

W.H. Wilborn: Does the preparation in Figure 13 differ from that in other figures? The collagen appears to have been dissected.
Author: No. This is the peculiar character of maturing hypertrophic scar, as often seen by SEM.

W.H. Wilborn: Are you certain that Figure 15 depicts myofibroblasts in a plastic section, and that Figure 16 shows the same cells by SEM? According to the magnification bars, the cells in Figure 16 are only 1 or 2 μm in diameter while those in Figure 15 are much larger. The cells in Figure 16, therefore, would be too small for myofibroblasts. Furthermore, their surfaces appear too smooth. Do you think the cells in Figure 16 are portions of red blood cells?
Author: Certainty is relative. What I can say is that the majority of cells within the nodules of hypertrophic scars, as seen by TEM, show all the known morphological characteristics of myofibroblasts. Figure 15 is a thick plastic section. This tissue has less shrinkage artifact than those tissues processed for SEM through critical point drying. This may account for some of the disparity in diameter of the cells. Please also keep in mind that if you measure diameter of the nucleus, only, in Figure 15, that measurement more closely compares with the measurements of the diameters of the "cells" in Figure 16. During processing for SEM, the body of the cell is likely to collapse about the nucleus; so, what one would effectively be measuring would be the nuclear diameter, not the true cellular diameter. By TEM, the surfaces of the myofibroblasts usually are smooth and do not show spikes or many microvilli. I could not say with certainty that the cells depicted in Figure 16 are not red blood cells. The latter can be very pleomorphic as Bessis [Bessis M (1973) Living Blood Cells and Their Ultrastructure. Springer-Verlag] has elegantly shown. However, the same is now known to be true of fibroblasts, especially the myofibroblast type of fibroblast.

W.H. Wilborn: Figures 18, 23 and 24 are transmission electron micrographs of dermal cells that show various degrees of degeneration. Could the degeneration be due to poor fixation, rather than to the theory you propose?
Author: Let us look at Figure 23. The endothelial cells are wrapped by pericytes and theoretically would be hit last by penetrating fixative. Yet, the endothelial cells appear viable while the pericytes are clearly degenerative. Additionally, we often observe microvessels in which adjacent endothelial cells appear both viable and degenerative. All I can say is that all of our tissues were fixed in the same way. Further, Gabbiani has confirmed apoptotic fibroblasts in his studies (see, Darby *et al.*, 1990). It is an interesting and most important question, because we are now learning how to get beyond the old established dogmas of tissue studies and understanding how the artifacts of formalin fixation for pathological studies obscure vital data.

W.H. Wilborn: Please elaborate on the term "stressed" collagen.
Author: Non-undulating, straight, as if the collagen were under load conditions.

H.P. Ehrlich: Clinically, keloids invade normal dermis around them, while hypertrophic scars do not. Hypertrophic scars resolve, but keloids do not! In general, most figures are related to hypertrophic scars. Would one expect to see the same structures and morphology in keloids?
Author: The author agrees with Peacock [Peacock EE Jr, Madden JW, Trier WC (1970) Biological basis for treatment of keloids and hypertrophic scars. Southern Med. J. **63**: 755-759] in that hypertrophic scars tend to clinically stay within the boundaries of the original injury while keloids tend to overgrow those boundaries. Therefore, it would seem that the keloid and hypertrophic scar are qualitatively the same, but quantitatively different. There is a morphological difference between the hypertrophic scar and keloid, which has been published (Kischer and Brody, 1981). This difference may be seen both histologically and by electron microscopy.

H.P. Ehrlich: Are occluded microvessels a feature of keloids or just hypertrophic scar?
Author: They are a feature of keloids, hypertrophic scar and just about any fibrosis (see Kischer, 1992).