Pilot Ultrastructural Evaluation of Human Preauricular Skin Before and After High-Energy Pulsed Carbon Dioxide Laser Treatment

Désirée Ratner, MD; Annie Viron; Francine Puvion-Dutilleul, PhD; Edmond Puvion, PhD

Background: Carbon dioxide laser resurfacing has recently come into favor for the treatment of photodamaged skin. While the clinical and histologic effects of high-energy short-pulse carbon dioxide lasers on human skin have been investigated, the ultrastructural effects of these lasers have not been documented. Our objective was to study the ultrastructural effects of a high-energy pulsed carbon dioxide laser on photodamaged human skin.

Observations: Before laser surgery, the ultrastructural changes characteristic of photodamaged skin were evident. Immediately after treatment, there was extensive coagulation necrosis of the epidermis and papillary dermis. Thirty days after treatment, there was no evidence of intercellular or intracellular edema, and ordered differentiation of the epidermal keratinocytes, with a loss of keratinocyte dysplasia, was seen. Increased numbers of desmosomes and tonofibrils were noted. New deposition of collagen was present in the papillary dermis. The ultrastructural findings seen at 90 days after treatment were similar to those seen at 30 days, apart from increased organization of collagen fibers in the papillary dermis.

Conclusions: Treatment with the high-energy pulsed carbon dioxide laser appears to reverse the epidermal and dermal changes of photoaging on an ultrastructural level. These changes appear morphologically to be consistent with previously described clinical and histologic changes following laser resurfacing.

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LONG-TERM exposure to UV radiation produces characteristic clinical and histologic changes in the skin.1-8 Actinically damaged skin tends to develop fine and coarse wrinkling, mottled pigmentation, telangiectases, loss of elasticity, and premalignant and malignant lesions. Histologically, epidermal atrophy, keratinocyte dysplasia, solar elastosis, and increased melanocyte activity are generally evident. The ultrastructural changes seen in photodamaged skin, including epidermal spongiosis, degeneration of basal and suprabasal keratinocytes, vacuolization of the dermoepidermal junction, disorganization of collagen bundles and collagen and elastic fiber degradation in the papillary dermis, and inactive fibroblasts with scant cytoplasm and few organelles, have also been described.8-14

Treatment with α-hydroxy acids and topical tretinoin has been shown to improve the clinical and histologic changes associated with photoaging.15-24 Reversal of the ultrastructural changes seen in photodamaged skin with the use of these topical agents has also been demonstrated.8,15,20,23,24 α-Hydroxy acid–treated keratinocytes appear to be connected by fewer desmosomes and exhibit less clumping of tonofibrils. Both α-hydroxy acids and topical tretinoin seem to increase the numbers of anchoring fibrils at the dermoepidermal junction of treated photoaged skin.13,20,23 Normalization of the structure and organization of papillary dermal collagen, reduction in the amount of degenerated microfibrillar material, and increased activity and numbers of fibroblasts have also been described after treatment with topical tretinoin.20,24,25

Dramatic clinical and histologic improvement in photoaged skin may also be produced with medium-depth and deep chemical peeling agents and dermabrasion.26-37 When properly performed, both procedures result in architectural and cytologic normalization of the epidermis, as well as an expanded papillary and reticular dermis composed of dense, parallel arrays of collagen bundles, otherwise known as the dermal repair zone. Nelson et al34 were the first to document the ultra-
tural changes produced by medium-depth chemical peels on photodamaged facial skin. Three months after a single 35% trichloroacetic acid peel, markedly decreased intracytoplasmic vacuoles within and between keratinocytes were seen, as were activated fibroblasts with increased cytoplasm and organelles and abundant deposition of new collagen with clearly defined cross striations arranged in an orderly parallel fashion. Decreased solar elastosis was also noted within the papillary dermis.

Carbon dioxide laser resurfacing has recently come into favor as a means of treating photodamaged human skin safely and effectively. \cite{38-53} Clinical improvement in facial rhytides and photodamage with the new generation of high-energy pulsed carbon dioxide lasers has been well documented. \cite{38-43,51,52} The precise control over the extent of tissue vaporization results in minimization of thermal damage to the skin, thereby reducing the potential risks of scarring and hyperpigmentation, while maximizing therapeutic efficacy. \cite{38-46,51,52}

The histologic changes seen after pulsed laser resurfacing have recently been detailed. \cite{39,51,53} Extensive epidermal necrosis and coagulative changes in the superficial papillary dermis are seen 24 hours after laser administration. Partial or complete reepithelialization is usually seen by day 3, although evidence of papillary dermal collagen damage is still seen. By 90 days after laser treatment, the epidermis is completely intact and dysplasia is absent. A papillary dermal repair zone composed of dense compact collagen bundles aligned in a parallel fashion with the epidermal surface is seen. Decreased numbers of thin elastic fibers oriented perpendicular to the dermoepidermal junction are noted in the superficial papillary dermis, with thicker, more haphazardly arranged fibers in the upper reticular dermis. To our knowledge, the ultrastructural changes seen after treatment with the pulsed carbon dioxide lasers have not yet been described. We sought to determine what changes could be seen not only in the epidermis and dermis, but also at the dermoepidermal junction, using a recent study by Cotton et al\cite{50} as a model for our work.

Three patients (all men) completed the study. One patient (a woman) did not complete the study, as she did not undergo a 90-day biopsy; however, her pretreatment, posttreatment, and 30-day biopsy results were included in our study.

Prior to laser surgery, ultrastructural findings characteristic of photodamaged skin were noted. There was marked epidermal disorganization, as well as prominent intercellular edema with loss of desmosomal connections and intracellular vacuolization of epidermal keratinocytes (Figure 1). On higher magnification, epidermal atypia was clearly evident (Figure 2). Promi-
nent nucleoli were seen, surrounded by a nucleoplasmic area containing highly dispersed chromatin, reflective of heightened nuclear synthetic activity (Figure 2). Condensed chromatin clumps were rare. Sparse tonofibrils were found within the keratinocytes of the stratum spinosum, and the cytoplasm had a cytolytic appearance, reflective of organellar degradation (Figure 2). The dermoepidermal junction exhibited a flattened contour, as relatively few “foolike” processes of the basal cells were seen (Figure 3). Melanocytes were rarely identified. In the superficial dermis, amorphous material consistent with degraded collagen and elastic tissue was seen (Figure 3). Disorganization of the papillary dermal collagen was present (Figure 3). Inactive fibroblasts with relatively few organelles were occasionally seen.

Immediately after laser treatment, there was extensive epidermal coagulation necrosis as well as coagulative change in the superficial papillary dermis (Figure 4). The reticular dermis did not exhibit coagulative change. A single fibroblast (F) is seen in the dermis (uranyl acetate–lead citrate, original magnification x 7500).

Wide intercellular spaces were no longer evident (Figure 5). On higher magnification, innumerable desmosomes filled the intercellular spaces between keratinocytes (Figure 5, inset). Numerous, tightly organized bundles of tonofibrils were uniformly distributed throughout the cytoplasm of all keratinocytes (Figure 6). Atypical keratinocytes were no longer evident, and keratinocyte nuclei exhibited abundant condensed chromatin and less prominent nucleoli. Intracytoplasmic vacuoles were no longer seen. The basal cells were precisely aligned along the dermoepidermal junction, with convolution of the junction due to the increased numbers of foolike processes of the basal cells (Figure 7). Scattered normal-appearing melanocytes were identified in the suprabasal and basal layers. The basement membrane zone exhibited newly prominent anchoring fibrils (Figure 7, inset). New deposition of collagen fibers organized in parallel arrays was present in the pap-
illary dermis (Figure 7). A decreased amount of amorphous degraded material was seen in the papillary dermis. Fibroblasts exhibited increased numbers of organelles, most notably rough endoplasmic reticulum and mitochondria.

By 90 days after treatment, the epidermal keratinocytes were still arrayed in an organized fashion (Figure 8). Their intercellular spaces were not as tightly packed with desmosomes, although bundles of tonofilbrils were uniformly distributed within their cytoplasm. Intracytoplasmic vacuolization of the keratinocytes was not seen. The basal cells were precisely aligned at the dermoeidermal junction, and prominent foot-like processes were again noted. Occasional normal-appearing melanocytes were seen. Prominent anchoring fibrils were identified in the basement membrane zone, comparable in quantity to those seen at 30 days. An even more highly organized network of collagen and elastic fibers was present in the papillary dermis, and amorphous degraded material was absent (Figure 8). Fibroblasts with increased numbers of organelles were noted in the dermis.

**COMMENT**

In recent years, high-energy short-pulse carbon dioxide lasers have grown in popularity for the treatment of photoaged facial skin, both for the treatment of actinic damage and rhytides. The clinical and histologic effects of these lasers have been studied, and it has been shown that the histologic effects of laser resurfacing are microscopically similar to those of phenol peeling, such that, at 90 days after laser treatment, epidermal atypia and dysplasia are corrected and epidermal polarity is restored, the epidermis being then “indistinguishable from that of younger, normal skin.” The presence of a subepidermal repair zone consisting of new subepidermal collagen at 3 months after laser treatment, comparable to that...
seen after medium-depth chemical peels or dermabra-
sion, has also been described.\textsuperscript{30,31} The ultrastructural
changes seen in our small group of patients at 30 and 90
days after laser resurfacing appear to correlate with these
histologic findings.

It is known that ablation of photodamaged epider-
mis and upper dermis, whether by chemical or physical
means, allows reepithelialization from deeper, less pho-
todamaged cells, resulting in the restored structural and
functional integrity of epidermal keratinocytes.\textsuperscript{26-38,50} The
increased number and organization of the tonofibrils in
epidermal keratinocytes that we have seen at 30 and 90
days after laser treatment appears therefore to be signifi-
cant, as these findings correlate with the normalization
of keratinocyte differentiation from the basal layer to the
stratum corneum seen histologically after laser resurfac-
ing. Additionally, the loss of intracellular and intercel-
luar epidermal vacuolization and the presence of folded
and tightly apposed intercellular spaces of adjacent ke-
ratinocytes studded at intervals with innumerable des-
mosomes 30 and 90 days after treatment appear to be sig-
nificant, as these findings are characteristically seen in
normal squamous epithelium.\textsuperscript{\textsuperscript{54}}

Clinical studies have revealed a measurable and re-
producible decrease in fine wrinkling and improvement
in skin texture after laser resurfacing, but the mecha-
nism by which these changes occur is not yet clear.\textsuperscript{39-48}
Fitzpatrick et al\textsuperscript{39} have postulated that, when the carbon
dioxide laser interacts with tissue, 3 zones of tissue dam-
age are produced: a vaporized zone, a zone of irrevers-
ible thermal necrosis, and a zone of reversible thermal
damage, in which collagen shrinkage is thought to take
place. It has been documented that thermal damage to
collagen itself results in shrinkage, but it is not clear how
great a role this shrinkage plays in generating clinical im-
provement in wrinkles.\textsuperscript{55-57} It is also thought that repair
of this layer during healing may account for tightening
of sagging skin and improvement of creases.\textsuperscript{30,36,67} The
clinically evident tightening of sagging skin after laser
surfacing may be in part due to the formation of new col-
lagen, the decrease in the amount of amorphous debris
in the papillary dermis, and the increased activity and
number of fibroblasts that have been documented his-
tologically and ultrastructurally after laser and other re-
surfacing procedures.\textsuperscript{26-38,50}

Nelson et al\textsuperscript{31} have in fact found that collagen's
striation periodicity is reduced after medium-depth
chemical peels, resulting in a more compact architecture,
and that the diameter of individual fibrils is more variable,
consistent with recent production of collagen by activated fibroblasts. Precise quantitative studies will be
required to substantiate whether compression of the
collagen bundles occurs immediately after laser resur-
facing and whether the striation periodicity of the col-
lagen is immediately changed after treatment. If, in fact,
the striation periodicity of the collagen in the papillary
dermis is reduced at 30 and 90 days after treatment, this
may partially explain the clinical perception of tighter,
smoother skin in patients treated with resurfacing la-
sers. The new deposition of dermal collagen that we
have seen ultrastructurally after laser resurfacing seems
to correspond to the papillary dermal repair zone de-
scribed by others, but will need to be further character-
ized, both ultrastructurally and biochemically. While it
does appear that the papillary dermal collagen is more
organized after 90 days than after 30 days after laser res-
urfacing, and that individual collagen fibrils may ex-
hibit more variability in diameter at 30 days than at 90
days after laser resurfacing, it is important to realize
that these are subjective impressions and that these
findings must be documented both qualitatively and
quantitatively when future studies of larger numbers of
patients are performed.

It has been postulated that increased numbers of an-
choring fibrils may help to produce increased adherence
of the epidermis to the dermis, resulting in a pulling,
or "tenting," that may decrease wrinkling, as seen in
patients treated with tretinoin and \(a\)-hydroxy acids.\textsuperscript{15,23,25} While increased convolution of the dermoepi-
dermal junction and increased numbers of anchoring fibrils may play a role in the increased smoothness and
tautness of the skin seen clinically in patients treated with
laser resurfacing, further studies will be needed to verify
and quantitate any true increase in the number of an-
choring fibrils. It is possible that such a phenomenon may
somehow also contribute to the clinical improvement in
superficial rhytides after laser resurfacing.

It is impossible to draw any conclusions about the
ultrastructural effects of laser resurfacing on melan-
cytes at this time. Very few melanocytes were seen in our
pretreatment specimens, but it is probable that this pau-
city of melanocytes was a result of sampling error. While
normal-appearing melanocytes were seen in the basal layer
of the epidermis at 30 and 90 days after treatment, it would
be premature to make any generalizations regarding mel-
anoocyte activity or number at this time. This will be an
important area of future study, especially given the re-
cent reports of delayed hypopigmentation occurring af-
after laser resurfacing.\textsuperscript{38}

Despite the small size of our study, it appears that
treatment with the high-energy pulsed carbon dioxide
laser appears to reverse epidermal and dermal photoag-
ning changes on an ultrastructural level. These changes
appear morphologically to be consistent with previ-
ously described clinical and histologic changes follow-
ing laser resurfacing. Further studies will be necessary
to correlate more precisely the clinical, histologic, and
ultrastructural changes that result from laser resurfacing.
Such studies will undoubtedly provide us with a
wealth of useful information.

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Reprints: Desirée Ratner, MD, Department of Derma-
tology, Columbia-Presbyterian Medical Center, 161 Fort
Washington Ave, New York, NY 10032.
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