

# Study on Histological Changes of the Thin Eyelid Skin After CO<sub>2</sub> Laser Resurfacing

Sergio Lessa, MD<sup>1</sup>  
Roberto Sebastião, MD<sup>2</sup>  
Eduardo Flores, MD<sup>3</sup>

- 1] Assistant professor of the post graduation course at Pontifícia Universidade Católica (PUC) do Rio de Janeiro, Brazil. Assistant Professor of Instituto de Pós-graduação Médica Carlos Chagas, 38<sup>a</sup> Enfermaria at Santa Casa de Misericórdia do Rio de Janeiro (Service of Prof. Ivo Pitanguy). Chief of the Ocular Plastic Surgery Department, 1<sup>a</sup> Enfermaria da Santa Casa da Misericórdia do Rio de Janeiro (Service of Prof. Paiva Gonçalves). Titular Member of the Brazilian College of Surgeons. Titular Member of the Brazilian Society of Plastic Surgery.
- 2] Assistant professor of the post graduation course at Pontifícia Universidade Católica (PUC) do Rio de Janeiro, Brazil. Assistant Professor of Instituto de Pós-graduação Médica Carlos Chagas, 38<sup>a</sup> Enfermaria at Santa Casa de Misericórdia do Rio de Janeiro (Service of Prof. Ivo Pitanguy). Staff Member of the Ocular Plastic Surgery Department, 1<sup>a</sup> Enfermaria da Santa Casa da Misericórdia do Rio de Janeiro (Service of Prof. Paiva Gonçalves). Associated professor of the Ocular Plastic Surgery Department of Universidade Federal Fluminense (Service of Prof. Renato Curi). Titular Member of the Brazilian Society of Plastic Surgery.
- 3] Ocular Plastic Surgeon of the Ocular Plastic Surgery Department, 1<sup>a</sup> Enfermaria da Santa Casa da Misericórdia do Rio de Janeiro (Service of Prof. Paiva Gonçalves). Ocular Plastic Surgeon of 38<sup>a</sup> Enfermaria at Santa Casa de Misericórdia do Rio de Janeiro (Service of Prof. Ivo Pitanguy). Titular Member of the Brazilian Society of Plastic Surgery.

## Address for correspondence:

**Sergio Lessa, MD**

Av. Ataulfo de Paiva, 135 Conj. 1101  
22449-900 – Rio de Janeiro – RJ  
Brazil

Phone: (55-21) 259-1245 Fax: (55-21) 259-0099  
e-mail: slessa@iptec.com.br

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## **ABSTRACT**

*Twenty-six patients, 22 females and 4 males were selected in order to evaluate the histological effects of CO<sub>2</sub> LASER. Ages ranged from 43 to 72 years. Nineteen patients were classified as Fitzpatrick III and 7 as Fitzpatrick II.*

*Upper eyelid skin was examined before operation and after selective photothermolysis at 3 months, six months and one year.*

*All post-operative evaluations showed consistent epidermal and dermal histological changes, such as the epidermal regeneration showing normal anatomy, mainly for evaluations around one year. Derma shows a*

*dramatic transformation of neocollagen on medium and superficial derma, as well as the intense change (restructure) of elastic system and decrease of glucosamineglucans.*

*The two laser passing were analyzed, as well as the whole healing process.*

Cutaneous renewal (resurfacing) with CO<sub>2</sub> LASER has been recognized as a modern and efficient technique for the treatment of facial wrinkles and photoaging. Surgery with LASER provides removal of epidermis and derma thin layers with absolute control of hemostasis, which allows for surgical steps to be accurately followed. Resurfacing is a new procedure that should integrate complex concepts related to dermatology, surgery, LASER Physics and healing process.

When evaluating and treating patients with CO<sub>2</sub> LASER, the surgeon should observe the skin nuances and its regeneration ability. Human skin is better viewed as a layered laminar structure vertically perforated by hair and other cutaneous adnexa. Derma may be divided into superficial or papillary and deep or reticular derma. Epidermis is composed by several specialized layers, the most important being the basal layer, that has the ability of regenerating epidermis through mitotic division each six months or eight weeks<sup>(1)</sup>.

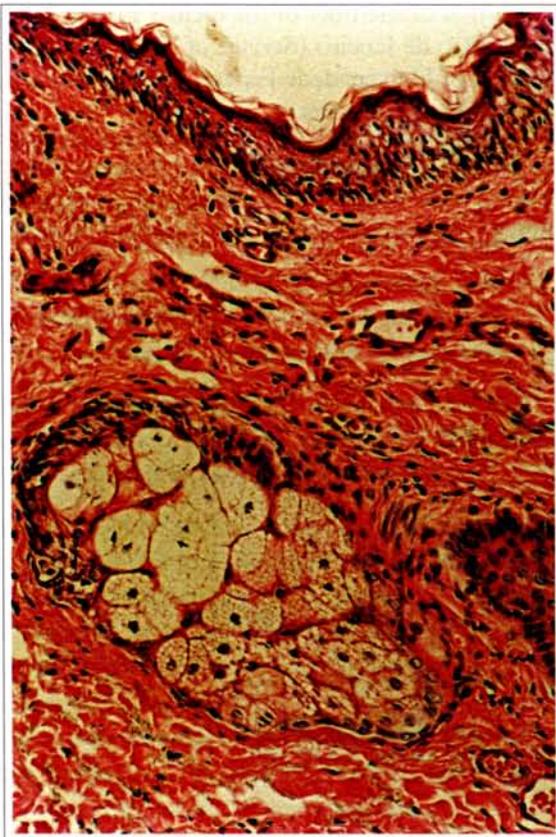


Fig. 1 - Microphotograph showing an advanced process of cutaneous photoaging. Epidermis presents itself even and atrophied with cell changes in its layers. Evident dermal changes with varied elastosis degrees. Collagen fibers present themselves thick with fragmented segments (HE).

*Fig. 1 - Microfotografia mostrando avançado processo de fotoenvelhecimento cutâneo. A epiderme se apresenta plana e atrofiada com alterações celulares em suas camadas. Alterações dérmicas evidentes, com graus variados de elastose. As fibras colágenas se apresentam espessadas com segmentos fragmentados (HE).*

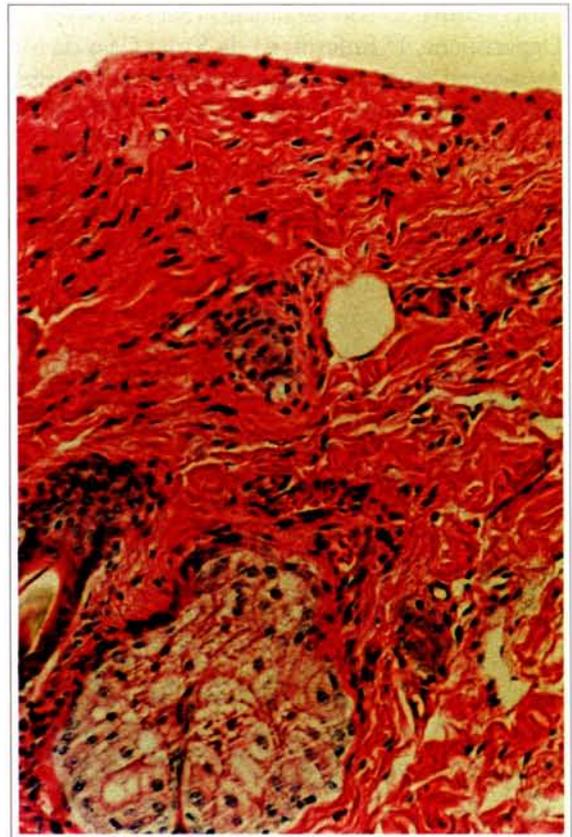


Fig. 2 - Histological analysis after first passing of CO<sub>2</sub> LASER using 300-millijoule energy. Epidermis ablation and basal membrane with the presence of necrosis strip from papillary derma with contraction of more superficial collagen fibers. Dermal adnexa preserved (HE).

*Fig. 2 - Análise histológica após a primeira passagem do LASER de CO<sub>2</sub> usando 300 milijoules de energia. Ablação da epiderme e da membrana basal, com presença de faixa de necrose de coagulação na derme papilar com contração das fibras colágenas mais superficiais. Anexos dérmicos preservados (HE).*

In order to understand LASER cutaneous renewal (resurfacing) we must clarify certain parameters of facial wrinkle ablation and reduction with no residual scars. The state-of-the-art equipment that work with CO<sub>2</sub> LASER are the ultrapulsed ones, that emit enough energy to vaporize tissue with one single pulse and at a lower time interval than that of the thermal relaxation, avoiding heat diffusion and performing the selective photothermolysis of the target tissue. The CO<sub>2</sub> LASER Ultrapulse 5000 C releases energy up to 350 millijoules at less than 1 meter per second, discharging up to 1000 pulses and promoting ablation, i.e., the safe and predictable removal of a given tissue volume<sup>(2)</sup>.

Face is divided into cosmetic zones that vary consid-

erably in thickness, appearance and sebaceous characteristics. Depth and effectiveness of cutaneous renewal (resurfacing) may vary with the change of the number of passing, and energy and density used.

The extremely thin eyelid skin deserves special considerations due to the risk of bad results such as ectropion or apparent scar. When observing the patients operated with Ultrapulse CO<sub>2</sub> LASER with CPG, submitted to upper blepharoplasty, lower transconjunctival and cutaneous renewal (resurfacing) throughout the periorbital cosmetic unit, we noticed a great improvement in the skin quality with the erasure or considerable attenuation of the thin and rough wrinkles.

Immediate or delayed histological changes occurring

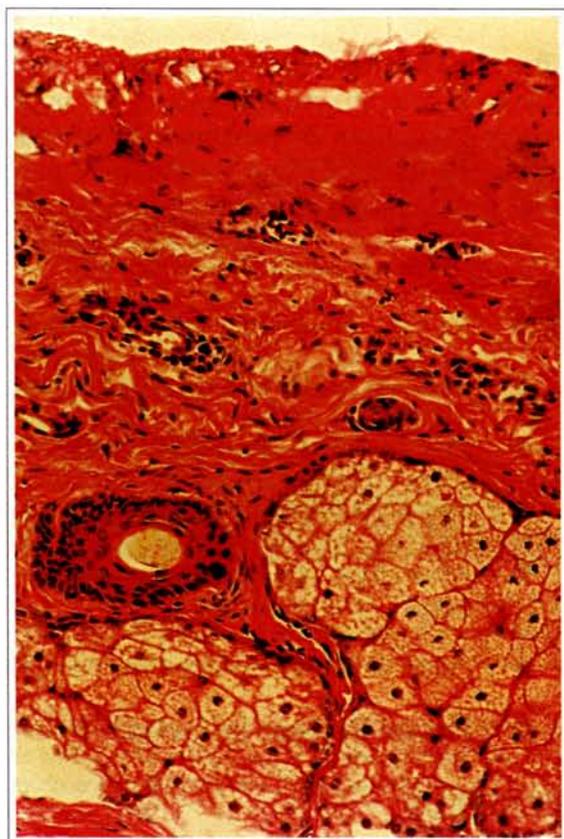


Fig. 3 - Histological analysis after second passing of CO<sub>2</sub> LASER using 200-millijoule energy. Deepening of necrosis from derma papillary coagulation was observed. Basal membrane is integral around preserved dermal adnexa. Contraction of collagen fibers at a deeper plane (HE).

*Fig. 3 - Análise histológica após a segunda passagem do LASER de CO<sub>2</sub> usando 200 milijoules de energia. Observamos um aprofundamento de necrose de coagulação na derme papilar. A membrana basal está íntegra em torno dos anexos dérmicos preservados. Contração das fibras colágenas num plano mais profundo (HE).*

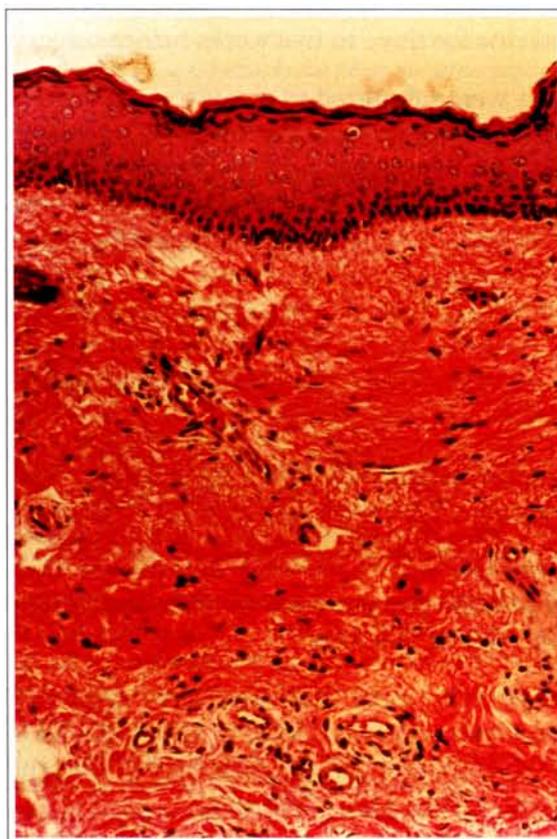


Fig. 4 - Histological evaluation after one year. Completely regenerated thin epidermis with young skin aspect. Great dermal changes with a large band of newly formed collagen at superficial derma were observed. Elastosis elimination from superficial and medium derma. Young skin aspect (HE).

*Fig. 4 - Avaliação histológica após um ano. Epiderme espessa, completamente regenerada, com aspecto de pele jovem. Observamos grandes modificações dérmicas com larga banda de colágeno neoformado na derme superficial. Eliminação de elastose na derme superficial e média. Aspecto de pele jovem (HE).*

at eyelid skin after CO<sub>2</sub> LASER use were little studied and are not much conclusive<sup>(3, 4, 5, 6)</sup>.

The purpose of this work is to accurately evaluate the changes occurring at thin eyelid skin after cutaneous renewal with Ultrapulse CO<sub>2</sub> LASER with CPG, and analyze the penetration depth, the use of proper energy, the accurate number of passing, the ephitelization and the effects at medium and long term.

## MATERIAL AND METHODS

Twenty-six patients were selected for this study. None of them had been previously submitted to blepharoplasty or chemical exfoliation of skin. All patients were submitted to pre-operative treatment according to a protocol that included the use of 0.025% retinoic acid, 4% hydroquinone and photoprotector for three to five weeks before surgery.

The patients were submitted to upper transcutaneous blepharoplasty, lower transconjunctival blepharoplasty and cutaneous renewal (resurfacing) of the periorbital cosmetic unit, except for the upper pretarsal skin, with Ultrapulse CO<sub>2</sub> LASER. Twenty-two patients (84.6%) were females and 4 patients (15.4%) were males. Nineteen patients (75%) were classified as type III and 7 patients (25%) were classified as type II according to Fitzpatrick's<sup>(7)</sup> classification for skin, hair and eye color.

Age of patients ranged from 43 to 72 years old, with 56-year average.

All patients presented varying degrees of skin photoaging with the presence of thin and rough wrinkles in variable amounts.

The equipment used to perform selective photothermolysis was series 5000 C Ultrapulse CO<sub>2</sub> LASER with computer pattern generator (Coherent Medical Group, Palo Alto, California).

Upper blepharoplasty was performed after subcutaneous infiltration of 0.5% lidocaine with adrenaline at concentration of 1:400,000. Skin segments taken out from skin did not exceed 45 millimeters long and 7 millimeters wide.

Selective photothermolysis of periorbital aesthetic unit was performed after upper and lower transconjunctival blepharoplasty sparing upper pretarsal area. Ray release was ultrapulsed with 300-millijoule energy and 60 watt power in the first passing, and 200-millijoule

energy and 40 watt power in the second passing. Pattern used was number 6 with 6 horizontal and vertical points with diameter 2.25 and density number 6. Mechanical removal of reminding devitalized tissue with gauze soaked with 0.9% sodium chloride physiological solution was carried out after each LASER passing followed by drying of all areas.

Following, upper eyelid preseptal skin pieces were removed from saline, dried and divided into pieces of similar area. A passing of CO<sub>2</sub> LASER upon one piece, two passing upon the next piece and no passing upon the last piece were carried out according to the same selective photothermolysis of periorbital aesthetic unit parameters. These eyelid cutaneous pieces were preserved into 0.9% sodium chloride solution and 10% formaldehyde at 3:1 ratio and sent to histopathologic analysis.

After three months, six months and one year after selective photothermolysis, biopsies of the upper lateral preseptal skin from 26 patients were carried out following the same care and for the same purposes of those for the pieces previously examined.

Staining methods used for all pieces were as follows:

- ◆ HE: for architecture overall evaluation
- ◆ Gomori: for architecture overall evaluation
- ◆ PAS: for basal membrane evaluation
- ◆ Resorcina-fuccina: for elastic fiber analysis
- ◆ Alcian blue: for glucosamineglucan evaluation
- ◆ Picrus sirius: for collagen fiber evaluation
- ◆ Masson-Fontana: for melanin evaluation

## RESULTS

The results of histopathologic analysis before and after selective photothermolysis and after cutaneous renewal process are described as follows.

1. Histopathologic analysis of thin eyelid skin before selective photothermolysis (Figs. 1, 5 and 7).  
All pieces showed an advanced photoaging process. Epidermis presents itself flat and atrophic. Keratinocytes present atypia and

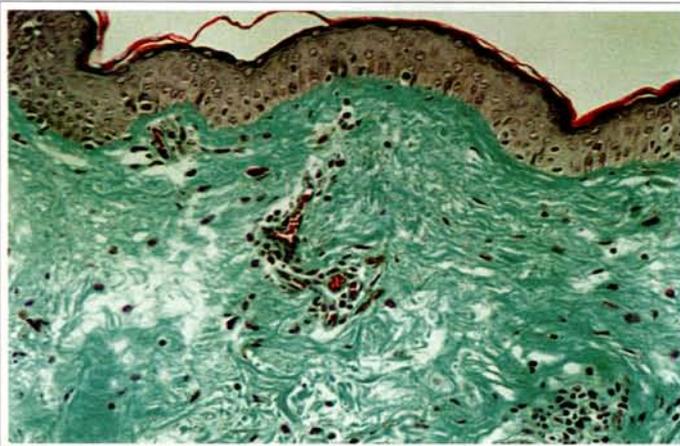


Fig. 5 - Pre-operative histological section showing cutaneous aging aspects and typical epidermal and dermal changes (Gomori).

*Fig. 5 - Corte histológico pré-operatório mostrando os aspectos de envelhecimento cutâneo com alterações típicas epidérmicas e dérmicas (Gomori).*



Fig. 6 - Histological evaluation after sixth month. Regenerated epidermis and great dermal change. Collagen fiber proliferation with redirection and compacting of papillary derma fibers were observed.

*Fig. 6 - Avaliação histológica após o sexto mês. Epiderme regenerada e grande modificação dérmica. Observamos proliferação das fibras colágenas com reorientação e compactação das fibras na derme papilar (Gomori).*

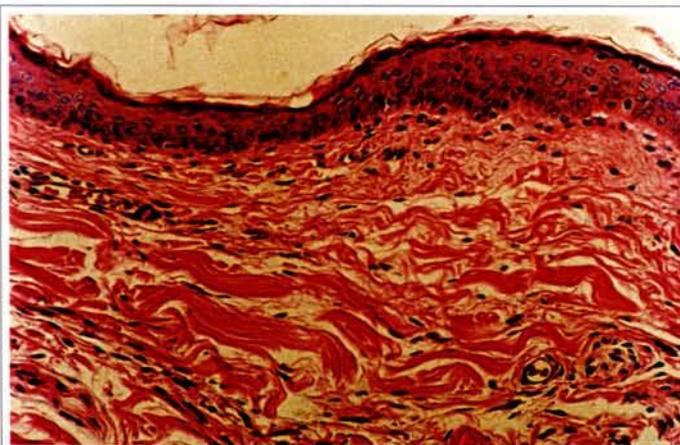


Fig. 7 - Post-operative histological analysis. Thin epidermis with cell polarity loss. Cell atypia and irregularities of the melanin deposit distribution were observed. Derma showing typical aging changes (HE).

*Fig. 7 - Análise histológica pré-operatória. Epiderme fina, com perda de polaridade celular. Observamos atipia celular e irregularidades na distribuição dos depósitos de melanina. Derme mostrando alterações típicas de envelhecimento (HE).*

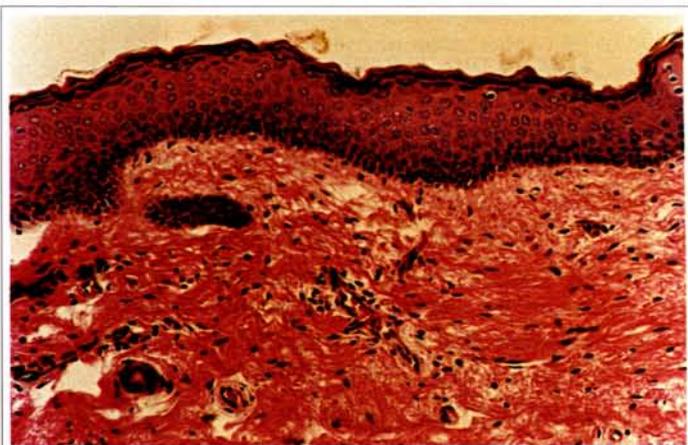


Fig. 8 - Histological analysis after one year. Complete, regenerated and thick epidermis. The epidermal cell layers are quite evident, with cell polarization retaking. Regularization of melanin deposits on basal keratinocytes. Derma showing a large band of normal collagen (HE).

*Fig. 8 - Análise histológica após um ano. Epiderme completa, regenerada e espessa. As camadas das células epidérmicas são bem evidentes, com retomada da polarização celular. Regularização dos depósitos de melanina nos queratinócitos basais. Derme mostrando larga banda de colágeno normal (HE).*

vary in form, size and pigmentation cytoplasmatic characteristics. Melanocytes along basal layer present themselves increased both in size and number. Masson-Fontana staining shows dense irregularly distributed melanin deposits. Cell adhesion is jeopardized, with a clear loss of cell polarity. Dermal changes are evident, with varied degrees

of elastosis. Collagen fibers present themselves thickened with fragmented segments. Glucoamineglucans are found to be highly increased, occupying the spaces where collagenolysis occurred. Dermal elastosis is clear both at superficial derma and at the deeper one.

2. Histopathologic analysis of thin eyelid skin. Selective photothermolysis after the first passing of LASER, using 300-millijoule energy. Epidermis and basal membrane ablation. Presence of necrosis from superficial papillary dermis coagulation. Minimal thermal reaction of papillary derma with contraction of the most superficial collagen fibers. Dermal adnexa preserved (Fig. 2).
3. Histological analysis of eyelid skin. Selective photothermolysis after two passing of LASER using 200-millijoule energy at the first passing and 200-millijoule energy at the second passing. Deepening of necrosis from papillary derma coagulation. Integral basal membrane around cutaneous adnexa. Contraction of collagen fibers at a deeper plane of papillary derma. Elastic degeneration observed at papillary and medium derma is transformed into a coagulated mass.
4. Cutaneous regeneration after selective photothermolysis (Fig. 3). Epidermal regeneration with a reconstituted and completely uniform basal membrane was observed in the third post-operative month. Keratinocytes present themselves normal, atypia was eliminated and polarity presents traces of recovery. Melanocytes show more regular distribution. Structural change at derma is still more important: a strip of normal collagen is evident at the papillary derma. Formation of collagen is observed at horizontal bands parallel to surface. Completely regenerate derma, with a more even distribution of melanocytes was encountered in the sixth month. Derma shows reconstruction and collagen fiber proliferation with redirection and compacting of papillary derma fibers. Collagen is more abundant and organized while glucosamineglucans have concurrently decreased. Elastosis of deeper derma is observed (Fig. 6). More defined histological patterns are observed one year after selective photothermolysis. Epidermis completely regenerated with an aspect of young skin. Cell polarization is more structured, with an increased cell proliferating activity being observed. Epidermal cell layers are quite evident. Return of regularity of the melanin deposits at basal keratinocytes.

A large band of newly formed collagen at superficial derma, with a great and evident decrease of glucosamineglucans at derma are observed. Elimination of elastosis at superficial and medium derma, with re-structure of the superficial dermal elastic system. (Figs. 4 and 8).

All these findings are compatible with the great aesthetic change observed in the cases operated.

## DISCUSSION

Cutaneous regeneration (resurfacing) with CO<sub>2</sub> LASER is an accurate and effective method for the treatment of cutaneous photoaging<sup>(8,9)</sup>. Shoenrick et al<sup>(5)</sup>, Chernoff et al<sup>(10)</sup> Weinstein<sup>(6)</sup> and Apfelberg<sup>(11)</sup> described the use of CO<sub>2</sub> LASER upon the periorbital area. David et al<sup>(12)</sup> described the use of CPG scanner.

Scanner allows for a precise control of overpassing, a parameter called density. Photothermolysis became absolutely accurate with the specification of three parameters: energy, density and number of passing.

When Ultrapulse CO<sub>2</sub> LASER with CPG is used, the first passing produces epidermis ablation and some thermal reaction at superficial papillary derma. The superficial vaporization is equivalent to approximately 50 to 60m in depth<sup>(5,10)</sup>. An additional thermal necrosis of 50 to 70m is also observed. The second passing destroys little tissue as there is less water to absorb LASER energy, however, it produces a thermal injury of approximately 40 to 60m<sup>(3)</sup>. Eyelid skin presents epidermis with depth ranging from 50 to 130m and derma with depth from 215 to 300m. Two passing of CO<sub>2</sub> LASER remove epidermis and superficial derma with extreme precision, eliminating the thin periorbital wrinkles.

We concluded that an important dermal segment is preserved, keeping cutaneous adnexa integral or with the basal membrane preserved at the lower portion. Thus, tissue ablation and subsequent thermal damage do not injure dermal adnexa that are vital for the skin ephitelization process. Burkhardt and Maw<sup>(15)</sup> recently concluded that tissue injury induced by the use of Ultrapulse CO<sub>2</sub> LASER with CPG in multiple passing is confined to reticular derma, sparing adnexal structures responsible for re-epithelization.

The most important cell in healing process after photothermolysis is the specialized keratinocyte from

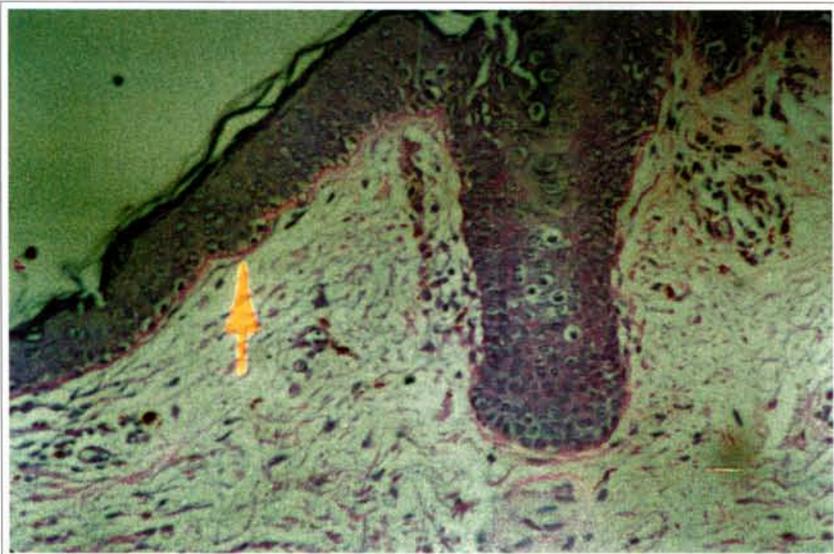


Fig. 9a - Pre-operative histological analysis. Epidermis and basal membrane (arrow) are observed (PAS).

*Fig. 9a - Análise histológica pré-operatória. Observamos a epiderme e a membrana basal (seta) (PAS).*

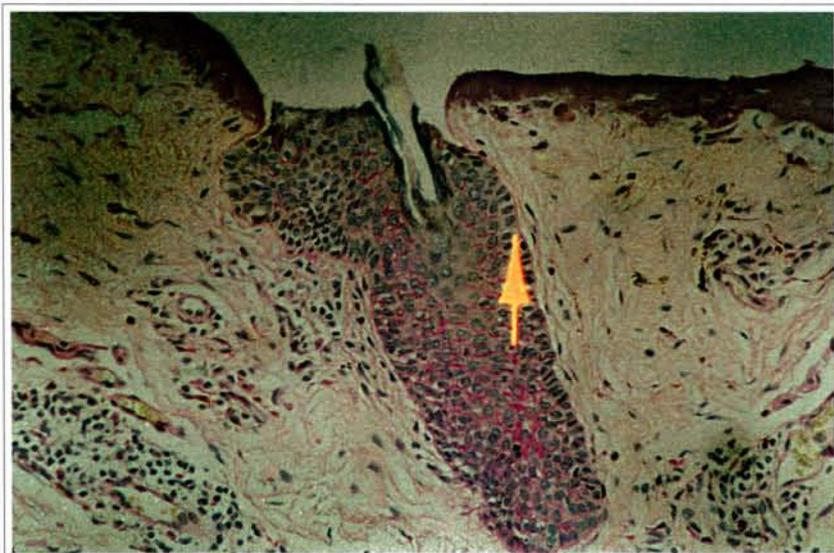


Fig. 9b - Histological analysis after the second passing of CO<sub>2</sub> LASER. A vaporization of superficial cell occurs in the first passing that is equivalent to approximately 50 to 60m in depth. Also an additional thermal necrosis of 60 to 70m occurs. The second passing destroys little tissue, however it produces a thermal injury around 40 to 60m, as showed in figure. Photothermolysis spares dermal adnexa and preserved basal membrane is observed (arrow). Tissue ablation and thermal damage do not injury dermal adnexa vital for skin epithelization process (PAS).

*Fig. 9b - Análise histológica após a segunda passagem do LASER de CO<sub>2</sub>. Na primeira passagem ocorre uma vaporização das células superficiais que equivale a aproximadamente 50 a 60m em profundidade. Ocorre também uma necrose térmica adicional de 50 a 70m. A segunda passagem não destrói muito tecido, porém produz uma injúria térmica de aproximadamente 40 a 60m, como vemos na figura. A fototermólise poupa os anexos dérmicos, e observamos a membrana basal preservada (seta). A ablação tecidual e o dano térmico não lesam os anexos dérmicos vitais no processo de epitelição da pele (PAS).*

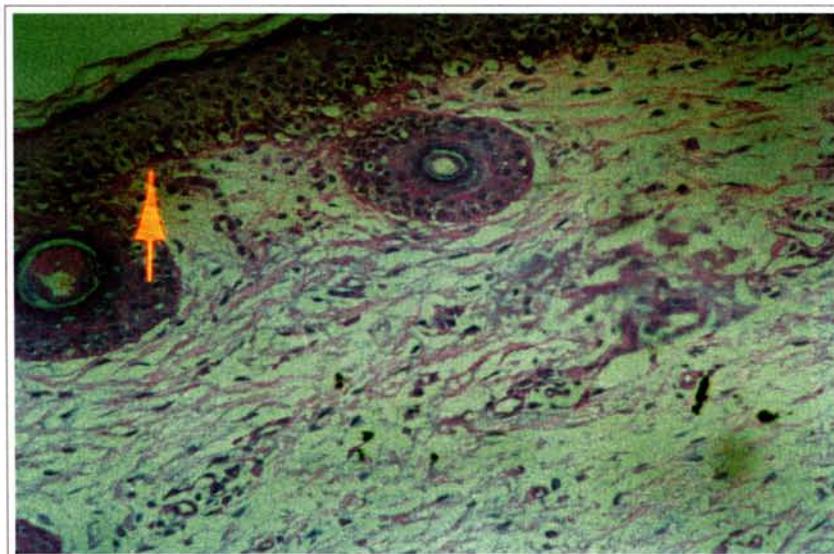


Fig. 9c - Histological analysis after the third month showing regenerated epidermis and basal membrane integrity (PAS).

*Fig. 9c - Análise histológica após o terceiro mês mostrando a epiderme renovada e a integridade da membrana basal (PAS).*



Fig. 10a - Sixty-eight years old patient presenting advanced process of cutaneous aging and eyelid xanthelasma.

*Fig. 10a - Paciente com 68 anos de idade apresentando avançado processo de envelhecimento cutâneo e xantelasma palpebral.*

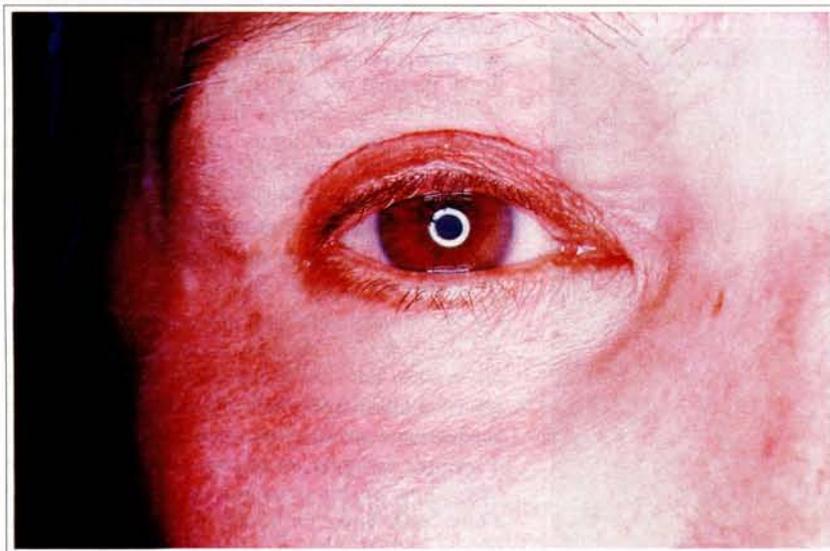


Fig. 10b - Post-operative at eighth month. Upper blepharoplasty, lower transconjunctival, resurfacing and canthexia were carried out. Cutaneous regeneration of the whole periorbital aesthetic unit.

*Fig. 10b - Pós-operatório no oitavo mês. Foram realizados: a blefaroplastia superior, a inferior transconjuntival, o resurfacing e a cantopexia. Renovação cutânea de toda a unidade estética periorbitária.*

pileous follicle sheath. A great number of these cells form the upper portion of pilosebaceous channel where it binds to epidermis. Keratinocytes and pilosebaceous complexes are spared during resurfacing and under special circumstances revert to an embryogenic state and are differentiated into epidermal cells that emerge through pilosebaceous channel and re-epithelize the cutaneous surface<sup>(1)</sup>. (Figs. 9a, 9b and 9c).

We have observed that clinical re-epithelization of aesthetic periorbital unit occurs between six and ten days for most patients.

Our histological findings confirm changes described by Stuzin<sup>(16)</sup> that show complete epidermal regeneration and the intense changes of facial derma.

Periorbital resurfacing using two passing of 300 millijoules and 200 millijoules provides an outstanding formation of neocollagen and the correction of dermal elastosis associated to a perfect and fast healing process. The transformation of the aesthetic pattern is remarkable (Figs. 10a and 10b).

We believe that the ideal model of periorbital resurfacing — with excellent aesthetic results, low rate of complications and proven histological changes being kept for the long term — is obtained with the employment of Ultrapulse CO<sub>2</sub> LASER with CPG.

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