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Histologic and Ultrastructural Analysis of Melasma After Fractional Resurfacing

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Background and Objective: Fractional photothermolysis is a popular treatment option for photodamaged skin and other cutaneous conditions. Recently, successful improvement in melasma has been achieved with this laser system. We undertook this study to evaluate the ultrastructural changes associated with fractional laser treatment of melasma.
Study Design/Materials and Methods: Ten subjects with skin types III and IV and a clinical diagnosis of epidermal melasma were treated with a 1,550-nm erbium:-glass laser delivering light in a fractional manner (Fraxel SR 750, Reliant Technologies, Inc., Mountain View, CA) every 2 weeks for a total of four sessions. Biopsies were obtained from all subjects both before treatment and at 3 months following the final treatment. All biopsies were analyzed by light and electron microscopy for treatment-induced changes. In addition, a secondary endpoint of the study was to assess for clinical improvement in melasma following fractional resurfacing. This assessment was performed by the investigator using pre- and post-treatment photographs.
Results: Light microscopy on post-treatment specimens showed a relative decrease in melanocytes compared to the pre-treatment ones. Post-treatment electron microscopy revealed fewer melanocytes and a relative absence of melanin in the surrounding keratinocytes compared to pre-treatment specimens. In addition, six subjects with skin type III were determined to have good improvement, whereas four subjects with skin type IV had fair improvement, as assessed by the investigator.
Conclusion: Post-treatment ultrastructural changes are consistent with an elimination process and may help to explain clinical improvement following laser treatment. Lasers Surg. Med. 40:134–138, 2008.

Key words: melasma; fractional resurfacing; histology; electron microscopy

INTRODUCTION

Melasma is a common pigmentary disorder that mainly affects females of childbearing age, but may also be seen at any age in women, as well as in men. Clinically, it presents as light to dark brown discrete macules or confluent patches on sun-exposed areas of the face, usually in centrofacial, malar, or mandibular distribution. Based on the location of pigment within the skin, melasma is usually divided into the epidermal, dermal, and mixed subtypes. With the exception of patients with skin types V and VI, these subtypes may be assessed using a Wood's lamp.

Although multiple therapeutic modalities have previously been tried and touted as being successful, truly efficacious treatment options for this condition have been few and quite elusive. Such treatment approaches include numerous topical agents, chemical peels, dermabrasion, and a variety of lasers and light-based devices [1].

Fractional laser technology has recently become popular for the purpose of photorejuvenation. This approach has changed the light-based treatment paradigm from that of layers of photothermolysis to one of columns of thermal damage. Recently, an erbium-doped fiber laser, emitting light at 1,550 nm, has been shown to be effective in the treatment of photodamaged skin, acne scars, post-surgical scarring, poikiloderma of Civatte, pigmented lesions, striae distensae, as well as melasma [2–7].

We undertook this prospective study in an attempt to evaluate the ultrastructural changes associated with, and the mechanism leading to, successful fractional laser treatment of melasma.

MATERIALS AND METHODS

The study protocol and consent form were approved by the Institutional Review Board of Pascack Valley Hospital, Westwood, NJ. Ten female subjects—six with skin type III and four with skin type IV—with a clinical diagnosis of epidermal facial melasma, as determined by Wood's lamp examination, were enrolled in the study. An explanation, including the risks, benefits, and potential complications, was given to the patients, and written informed consent was obtained. All patients had been previously treated with at least topical agents. A significant number of patients also had undergone chemical peels and other laser treatments.

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Anesthesia was achieved with a topical anesthetic cream, consisting of a eutectic mixture of 2.5% lidocaine and 2.5% prilocaine, applied for 1 hour prior to each treatment. A blue dye (FD&C No. 1), which serves as a guide marker for the intelligent optical tracking device of the laser handpiece, was applied to the entire face to demarcate the area of laser treatment. All subjects were treated with a 1,550-nm erbium:glass laser delivering light in a fractional manner (Fraxel SR 750, Reliant Technologies, Inc., Mountain View, CA) every 2 weeks for a total of four sessions. The settings of 2,000–2,500 MTZ/cm^2 were used at energy levels ranging from 6 to 10 mJ per microthermal zone.

All subjects were instructed to avoid the use of bleaching agents during the course of treatment and for 3 months thereafter. They were also instructed on proper sun protection and the use of broad-spectrum sunscreens of their choice.

In order to evaluate for microscopic and ultrastructural treatment-induced changes, biopsies were obtained from all subjects both before and 3 months after the final treatment. Biopsies were taken from the least conspicuous site within clinically involved area of the face. All biopsy specimens were split for processing for light and electron microscopy. For the electron microscopy, sampling bias was minimized by obtaining two to three sections for each specimen and by analyzing two to three areas within each section. All specimens were analyzed by a single blinded dermatopathologist.

As a secondary endpoint, the investigator performed assessment of clinical improvement in melasma by using subject photographs obtained before and at 3 months following treatment. A quartile system was used with a rating of excellent defined as $>75\%$ lightening; good as 51–75% lightening; fair as 26–50% lightening; and poor as 0–25% lightening of the melasma.

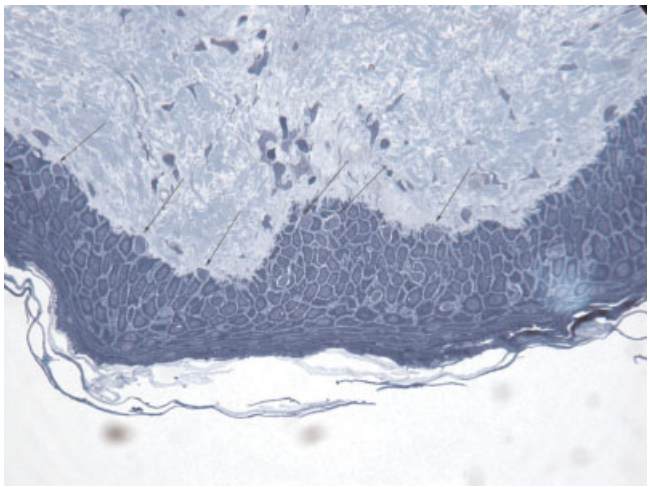

Fig. 1. Pre-treatment biopsy from a patient with skin type III. Note an increased number of melanocytes (arrows) (methylene blue, original magnification 20×). [Figure can be viewed in color online via www.interscience.wiley.com.]

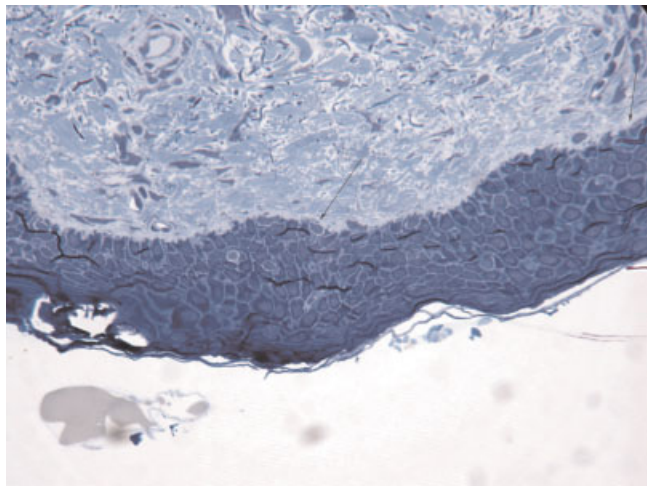

Fig. 2. Post-treatment biopsy from the same patient, showing a relative decrease in the number of melanocytes (arrows) (methylene blue, original magnification 20×). [Figure can be viewed in color online via www.interscience.wiley.com.]

RESULTS

All pre-treatment histologic analyses showed varying degrees of epidermal hyperpigmentation and rare dermal melanophages, changes consistent with epidermal melasma (Fig. 1). Post-treatment biopsies showed a relative decrease in the number of melanocytes (Fig. 2). This decrease appeared to be consistent for all biopsy specimens without notable individual differences.

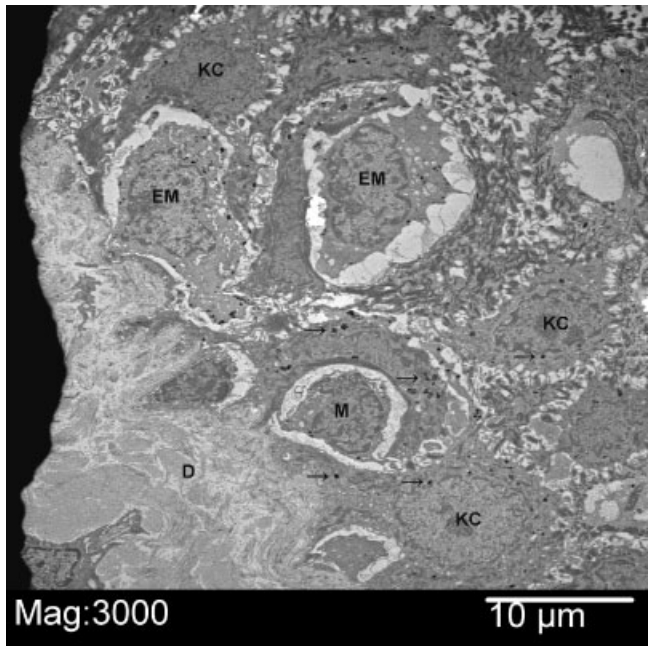

Fig. 3. Pre-treatment electron microscopic analysis, showing the presence of normal (M) and enlarged melanocytes (EM), with an increased number of melanin granules (arrows) within keratinocytes (KC). Dermis (D) is unremarkable.

Subject	Skin Type	Pre-Treatment Melanocyte Count	Post-Treatment Melanocyte Count
1	III	10	5
2	III	10	6
3	III	8	5
4	IV	12	6
5	IV	11	10
6	III	9	6
7	IV	10	7
8	III	10	5
9	III	9	6
10	IV	13	12

Average Number of Pre-treatment Melanocytes = 10.2

Average Number of Post-treatment Melanocytes = 6.8

Fig. 4. Pre- and post treatment melanocyte counts.

Pre-treatment electron-microscopic analyses showed the presence of enlarged melanocytes, with an associated increase in melanin-containing keratinocytes (Fig. 3). Ultrastructural analysis of specimens obtained 3 months following treatment revealed fewer enlarged melanocytes and a decreased total number of melanocytes. The percent decrease in melanocytes from pre- to post-treatment varied greatly from one subject to the next with the mean decrease among all subjects being 33% (Fig. 4). There was no correlation between the degree of clinical improvement and the percentage decrease of melanocytes. Additionally, there was a significant relative decrease or complete absence of melanin granules in the surrounding keratinocytes. The decrease in melanin appeared to be relatively homogeneous throughout the evaluated epidermis. Finally, the basement membrane zone appeared intact (Fig. 5).

Clinically, all subjects tolerated treatments well. No transient or long-term adverse effects, including post-inflammatory hyperpigmentation or scarring, were noted. At 3 months following the last treatment, six subjects with skin type III were rated by the investigator as having good improvement, whereas the remaining four subjects with skin type IV were deemed to have fair improvement in their melasma (Figs. 6 and 7). Since all biopsy specimens showed treatment-induced changes, no direct correlation between the degree of clinical improvement and microscopic or ultrastructural changes could be made.

DISCUSSION

In addition to the indisputable contribution by ultraviolet radiation, etiological factors underlying the development of melasma most commonly include pregnancy—hence the term "the mask of pregnancy"—and oral contraceptive use [8–10]. Additional factors, such as genetic predisposition, hormonal abnormalities, and ingestion of phototoxic medications, have also been implicated in this condition [9,11].

Histological examination of melasma demonstrates enlarged melanocytes with prominent dendritic processes,

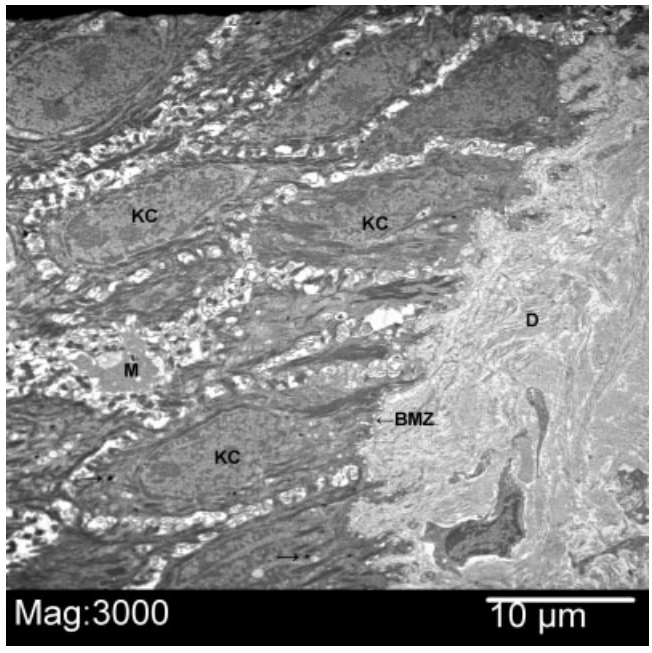

Fig. 5. Post-treatment electron microscopic analysis from the patient in Figure 3, showing fewer, normal-appearing melanocytes (M) and a relative decrease or absence of melanin granules (arrows) in the surrounding keratinocytes (KC). Dermis (D) is unremarkable and the basement membrane zone (BMZ) appears intact.

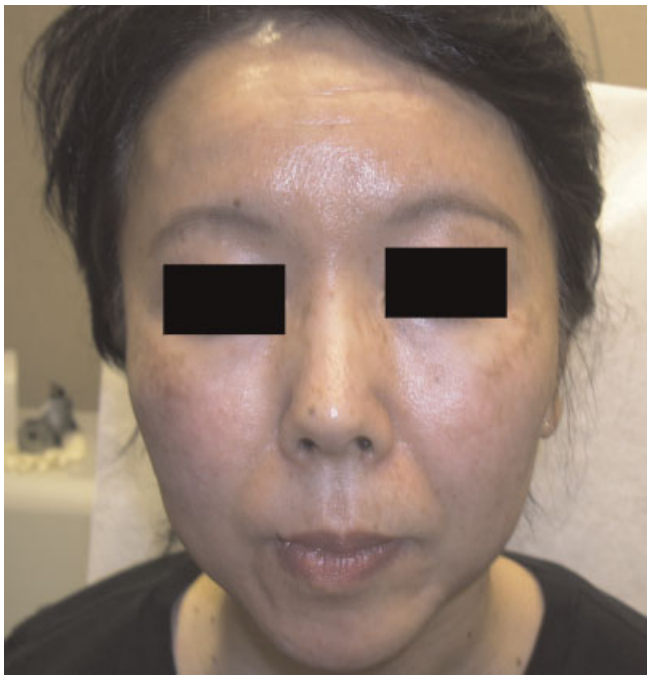

Fig. 6. Clinical appearance of melasma in subject with skin type IV before fractional resurfacing. [Figure can be viewed in color online via www.interscience.wiley.com.]

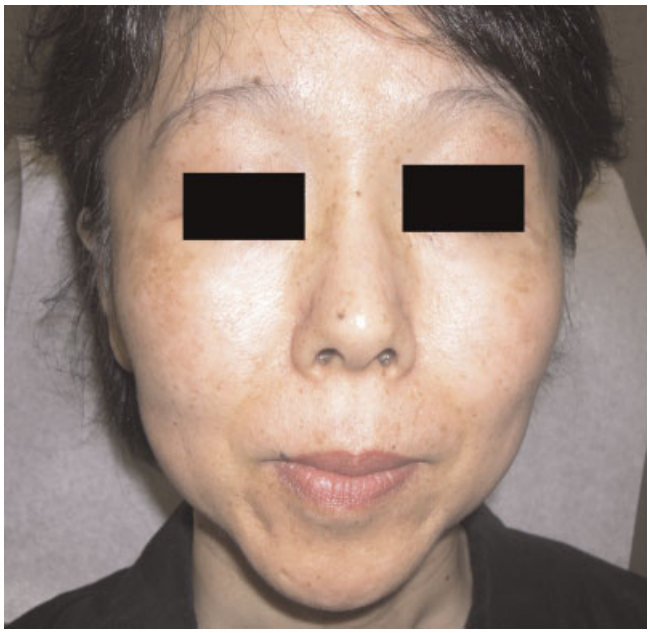

Fig. 7. Clinical appearance of melasma in the same patient 3 months following fractional resurfacing. Improvement was rated as fair. [Figure can be viewed in color online via www.interscience.wiley.com.]

increased epidermal melanin content, slightly increased number of dermal melanophages, and mild perivascular lymphohistiocytic infiltrate [12].

Therapeutic options for this condition have generally been disappointing, with relatively frequent failures designated as refractory melasma. Rigorous sun protection is a critical component of every treatment regimen for melasma. Additionally, therapies that have shown at least some effectiveness in this condition are most commonly directed at either decreased production of melanin or improved degradation and elimination of the pigment. Until recently, these included topical preparations, such as bleaching agents [13], corticosteroids [14], and retinoids [15], as well as physical modalities, most commonly various chemical peels [16–18], dermabrasion [19], intense pulsed light [20], and ablative and non-ablative laser treatments [21–23]. However, the latter methods may result in significant epidermal damage, leading to stimulation of melanocytes at the periphery of the treatment area with subsequent post-inflammatory hyperpigmentation.

Fractional photothermolysis has recently been evaluated in the treatment of melasma and is currently the only FDA-approved laser therapeutic modality for this condition [6,7]. A dermally focused laser beam is absorbed by water, creating columns of thermal damage, also known as microscopic treatment zones (MTZs). The surrounding tissue is relatively unaffected by the laser and serves as a source of cells and inflammatory mediators involved in the wound repair process [24].

Histological studies of photodamaged skin have indicated post-treatment presence of thermally damaged components just below the stratum corneum. The composition of these button-shaped formations was originally thought to be epidermal in nature, leading to the term micro-epidermal necrotic debris (MENDs) [24]. More recent evidence indicates that such designation is a misnomer, as dermal components are readily identified there, as well [25]. Additionally, melanin concentration within the columns of damage was found to be significantly higher than that in the surrounding tissue [26], with clinical correlation of minute brown crusts evident at 3 days post-treatment, just as MENDs are being eliminated through the skin.

Subsequently, it has been proposed that, in addition to epidermal debris, dermal contents, such as melanin and elastotic material of solar elastosis, may also be eliminated—either passively or actively—through the formed channels in the skin that result in a compromised dermo-epidermal junction. This was recently confirmed with the help of light microscopy [25]. These developments have lead to a theory of melanin shuttle following fractional photothermolysis. It would appear that our noted post-treatment ultrastructural changes, showing a decrease in the number of melanocytes and the amount of melanin granules within keratinocytes, are consistent with this elimination process and may help to explain the improvement noted after laser treatment. Alternatively, a decrease in epidermal melanin noted at a follow-up period longer than the epidermal turnover cycle may also imply a delayed repopulation of pigment.

Finally, it should be noted that the previously reported findings of clinical improvement in melasma following fractional resurfacing, in combination with our noted post-treatment histologic and electron microscopic "improvement" should in no way be interpreted as a "cure" for melasma. Future studies should evaluate other even more specific measurements of melanocyte counts. These include MART 1, S100 and/or Fontana Masson stains. Finally, although fractional resurfacing can improve melasma, the etiologic factors of this skin dyschromia are likely still present following treatment. Although all of our patients were improved after treatment, none were totally cleared. Thus, recurrences are to be expected over time.

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