Laser Resurfacing-Induced Hypopigmentation: Histologic Alterations and Repigmentation with Topical Photochemotherapy

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BACKGROUND. Hypopigmentation is a relatively common side effect of CO_2 laser resurfacing. Little is known regarding the histologic features of the areas of pigmentation loss. To date, hypopigmentation is considered a permanent complication of this procedure.

OBJECTIVE. To assess the histologic features of hypopigmentation caused by laser resurfacing and to evaluate the efficacy and safety of topical psoralen photochemotherapy.

METHODS. Ten patients were included in this pilot study. Four had baseline biopsies performed. Histologic parameters assessed included epidermal melanin, dermal melanophages, perivascular inflammation, Mel-5 immunostaining for melanocytes, and dermal fibrosis. Seven patients were treated twice a week with topical photochemotherapy utilizing 0.001% 8-methoxypsoralen.

RESULTS. All of the biopsy specimens demonstrated varying quantities of epidermal melanin and residual epidermal melanocytes. Mild perivascular inflammation was evident in two specimens. Superficial dermal fibrosis was noted in all biopsy specimens. Topical photochemotherapy induced moderate to excellent repigmentation in 71% of the treated patients. Adverse effects were minimal.

CONCLUSION. The results of this investigation suggest that hypopigmentation induced by laser resurfacing may result from a suppression of melanogenesis rather than destruction of area melanocytes. The preliminary data further suggest that hypopigmentation caused by laser resurfacing can be effectively treated by topical photochemotherapy.

LASER RESURFACING has enjoyed immense popularity for treatment of actinic damage, rhytides, and acne scars since its introduction in 1993.[1,2] The historical gold standards for resurfacing are high-energy pulsed and scanning CO_2 lasers. The Er:YAG laser has increased in popularity in an attempt to decrease thermal damage and clinical side effects associated with CO_2 laser resurfacing.[3] The efficacy and complications of laser resurfacing procedures are well documented.[4,5] However, long-term complications continue to emerge. Enthusiasm for resurfacing procedures has been tempered by the development of long-term pigmentary problems as well as the postoperative wounding that occurs in all patients. Pigmentary disorders including hyperpigmentation and hypopigmentation are relatively common side effects of laser resurfacing.[5–8] The frequency of hyperpigmentation varies from 2 to 37%. It is primarily observed in darker skin types. Laser-induced hyperpigmentation is usually temporary and is often amendable to topical bleaching agents. In contrast, hypopigmentation is more often a late sequalae, usually occurring after 6 or 7 months. Published studies report frequencies ranging from 1 to 20%.[3,5–8] To date, hypopigmentation is considered a permanent complication of laser resurfacing. Furthermore, there is a virtual dearth of data regarding the histologic features of hypopigmentation following this procedure.[9]

Since 1947 psoralens have been used as repigmenting agents for vitiligo, clinically characterized by depigmentation.[10] Myriad clinical studies have documented the efficacy of 8-methoxypsoralen (8-MOP) applied topically or taken orally in combination with sunlight or artificial high-intensity long-wave ultraviolet (UVA) light sources in patients with vitiligo.[11–17] The acronym PUVA was introduced in 1974 to describe the use of oral psoralen and high-intensity long-wave photochemotherapy units.

Topical photochemotherapy protocols for vitiligo utilize varying concentrations of 8-MOP in combination with artificial UVA light sources or sunlight. Grimes et al.[15] reported the efficacy and safety of various concentrations of 8-MOP for repigmentation of vitiligo. Low-dose (0.1%) 8-MOP was as effective as high-dose (1%) 8-MOP, while causing substantially fewer blistering reactions. Subsequent studies by Grimes[16] demonstrated substantial efficacy with minimal side effects using a concentration of 0.001% 8-MOP ointment in

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combination with sunlight exposure. We are aware of no published studies involving repigmentation of laser resurfacing-induced hypopigmentation.

In light of our vast pigmentation experience utilizing topical PUVA, the purpose of the present pilot investigation was to assess the efficacy and safety of topical psoralen photochemotherapy for treatment of hypopigmentation induced by laser resurfacing. In addition, this pilot study assessed the histologic and immunohistochemical features of laser-induced hypopigmentation.

Patients and Methods

Patients

Ten patients with laser-induced hypopigmentation were included in this investigation. All patients were referred to the Vitiligo and Pigmentation Institute or the UCLA Dermatology Clinic because of hypopigmentation following laser resurfacing. Eight were women and two were men. Seven patients were resurfaced for rhytides and three were treated for acne scarring. Nine were Caucasian and one was African American. All patients noted the onset of pigment loss within 2–8 months following laser resurfacing. Nine were resurfaced with a CO_2 laser. One patient with acne scars was resurfaced with an Er:YAG laser. Data regarding scanners, fluencies, and energy densities received by each patient was not known.

Methods

Light Microscopic and Immunohistochemical Studies. After informed consent was obtained, 2 mm punch biopsies were taken from the hypopigmented resurfaced skin of four patients. Biopsy specimens were placed in 10% neutral-buffered formalin fixative and immediately mailed to the Skin Pathology Laboratory at Boston University School of Medicine, Boston, Massachusetts. From paraffin-embedded specimens, 4 μm sections were cut and stained with hematoxylin and eosin. Fontana–Masson staining was used for determination of epidermal melanin, melanocytes, and dermal melanophages. Hematoxylin and eosin and Fontana–Masson stained sections were compared to normal historic controls.

Mel-5 Staining for Melanocytes. Four-micron sections were cut, floated onto positively charged slides, and air-dried overnight without heat. Sections were stained with a 1:5 dilution of Mel-5 antibody (Signet Laboratories, Dedham, MA) for 32 minutes after predigestion of 4 minutes with protease using the Ventana 320 automatic immunostainer (Ventana Medical Systems, Inc., Tucson, AZ) and their alkaline phosphatase detection kit.[18]

Topical Photochemotherapy Protocol. Seven of the 10 patients included in the study were treated with topical photochemotherapy. Methoxsalen lotion 1% (8-methoxysalen, ICN Pharmaceuticals, Costa Mesa, CA) was diluted to concentrations of 0.001% and 0.01% in aquaphor. A thin coat of 0.001% was applied to hypopigmented areas of skin 30 minutes prior to UVA exposure. The initial UVA dose was 0.20 J/cm^2. The dose was increased by 0.20 J to 0.50 J/cm^2 weekly according to the patient's skin type and sensitivity. After mild to moderate asymptomatic erythema was achieved, the UVA dose was maintained at a level sufficient to retain mild to moderate asymptomatic erythema. The treated areas were washed with cetaphil liquid cleanser and water immediately after UVA exposure. A broad-spectrum sunscreen was applied by patients prior to leaving the treatment center. Patients were treated twice a week. After 8–10 treatments, the concentration of methoxsalen ointment was increased to 0.01% if therapeutically indicated.

The UVA light source was a Daavlin Spectra 311/350 high-intensity combination UVA/narrow-band UVB phototherapy unit. The unit consisted of 24 F72T12BL-HO UVA lamps and 24 TL-01 Philips UVB lamps. The UVA irradiance was 23 mW/cm^2. Patients were treated with only high-intensity UVA light and topical methoxsalen ointment. None received narrow-band UVB.

Repigmentation and erythema were evaluated by phototherapy technicians and two of the investigators (PEG and JK). Repigmentation was graded as follows: 0%, no improvement; 1–25%, minimal improvement; 25–50%, mild improvement; 50–75%, moderate improvement; and 75–100%, excellent improvement. Wood's light examination performed at baseline in all of the patients was positive. Erythema grading was 0, none; 1+, mild asymptomatic; 2+, moderate asymptomatic; 3+, moderate symptomatic; and 4+, blistering. Serial photographs were taken with a Yashica Dental Eye camera and Ektachrome 100 film.

Results

The histologic features of laser-induced hypopigmentation were evaluated in four patients. Melanin appeared to be markedly decreased in two of four specimens (Figure 1A–D). Immunostaining with Mel-5 identified basal epidermal melanocytes in all patients (Figure 1E–H) which appeared to be present in normal numbers except in one patient who showed a slight decrease (Figure 1E). Mild perivascular inflammation was evident in two of the four patients (Figure 1I–L). Melanophages were seen in three of four biopsies (Figure 1A–D, I–L). Superficial dermal fibrosis was noted in all four biopsies, which was quite prominent in two patients (Figure 1I–L).

Table 1 summarizes the clinical features, cumulative UVA dose, number of treatments, and repigmentation responses for each patient treated with topical photochemotherapy. Of the seven patients, all experienced some degree of improvement. Moderate to excellent repigmentation occurred in 5 of 7 patients (71%) (Figures 2–4) . Complete repigmentation occurred in one patient with localized hypopigmentation of the malar areas. The number of treatments ranged from 13 to 38

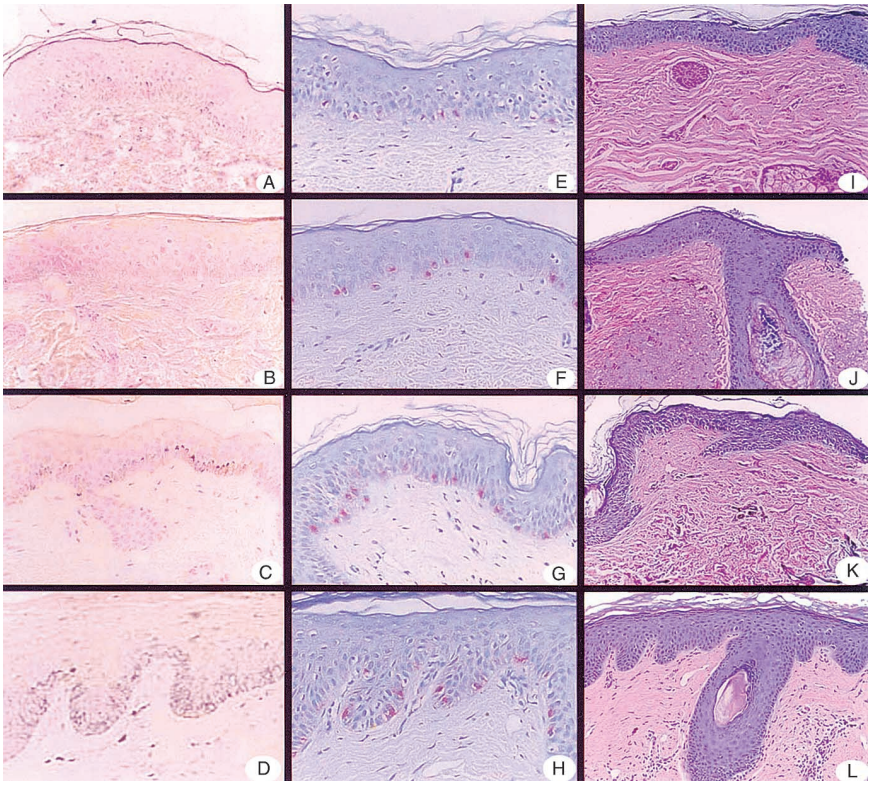

Figure 1. Composite photomicrographs showing representative areas of four patients (A–D). Sparse (A, B) to moderate (C, D) epidermal melanin. Note melanophages in upper dermis (C, D) (Fontana–Masson; magnification 40×). Basal melanocytes on immunostaining with Mel-5. Note slightly reduced numbers in (E) but normally appearing in (F, G) (H, magnification 40×). Upper dermal fibrosis is seen in all patients (I–L). Note mild to moderate perivascular lymphoid cell infiltrate and melanophages in K and L. (Hematoxylin and eosin; magnification 20×).

(mean 25 ± 10 SD). Cumulative joules ranged from 15 to 95 (mean 48 ± 28 SD). The predominant pattern of repigmentation observed in the treated patients was diffuse gradual darkening of areas of hypopigmentation.

In general, topical photochemotherapy was well tolerated by all of the patients. None experienced blistering reactions. Treatments were temporarily discontinued for 1 week in two patients with moderate symptomatic erythema characterized by pruritus and slight edema. Mild perilesional border hyperpigmentation occurred in 3 of 9 patients (43%). The hyperpigmentation either significantly improved or completely disappeared during the course of treatment.

Patient follow-up periods after topical photochemotherapy ranged from 2 to 12 months (mean 5 months). Two patients experienced 25–50% loss of pigmentation within 6–8 weeks after discontinuing photochemotherapy. Both experienced a rapid improvement upon re-treatment.

Discussion

CO_2 and Er:YAG resurfacing are popular procedures for improving actinic damage, acne scars, and rhytides. However, pigmentary issues, specifically hypopigmentation, impact the long-term safety of resurfacing. Hypopigmentation has been considered a permanent complication. Nanni and Alster[5] reported a frequency of 1%. In contrast, other studies have reported higher frequencies of hypopigmentation. Bernstein et al.[7] re-

Table 1. Therapeutic Responses in Patients Treated with Topical Photochemotherapy for Laser-Induced Hypopigmentation

Patient	*Age*	*Gender*	*Resurfacing indication*	*Areas resurfaced*	*Number of treatments*[a]	*Cumulative Joules*[b]	*Percent repigmentation*
1	35	Male	Acne scars	Malar areas	38	63	100%
2	59	Female	Rhytides	Full face	25	50	50–75%
3	62	Female	Rhytides	Full face	28	55	50–75%
4	49	Female	Rhytides	Full face	37	95	75–100%
5	28	Female	Acne scars	Perioral	16	15	25–50%
6	49	Female	Rhytides	Full face, neck	21	28	25–50%
7	31	Male	Acne scars	Full face	13	19	50–75%

[a] Mean treatments = 25 ± 10 SD.
[b] Mean cumulative joules = 48 ± 28 SD.

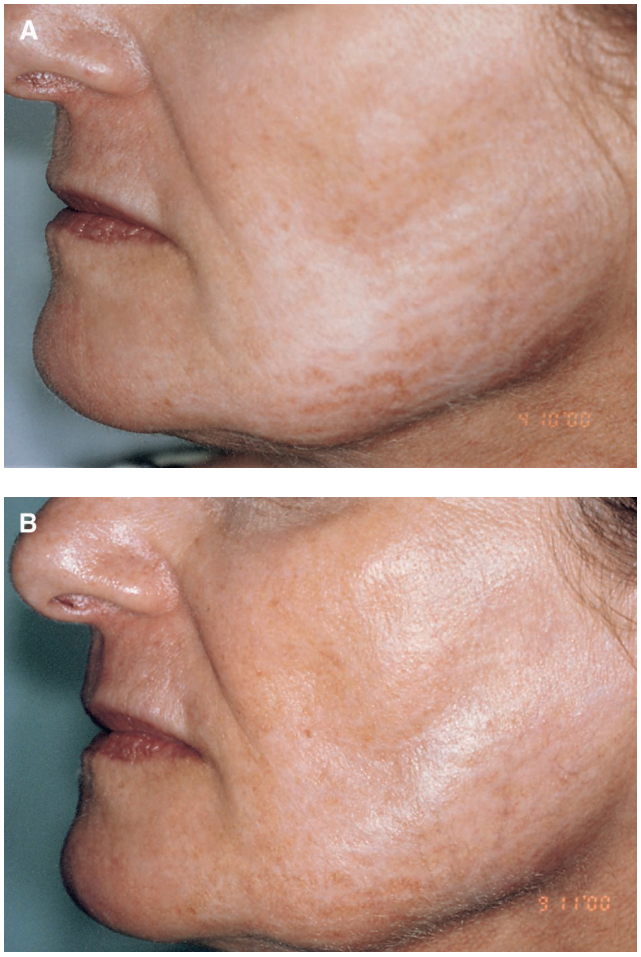

Figure 2. (A) Before and (B) after 28 treatments. Repigmentation 50–75%.

ported hypopigmentation in 16% of 104 patients followed retrospectively for an average of 8.2 months. Schwartz et al.,[19] in a retrospective series of 211 patients, reported hypopigmentation in 8%. Manuskiatti et al.[8] described long-term complications of CO_2 resurfacing in 104 patients resurfaced between 1993 and 1996. The frequency of hypopigmentation was 19.2%, of which 84% were considered mild and 16% moderate. In the aforementioned series, hypopigmentation was often not evident until 6 months after surgery.

The histologic features of laser-induced hypopigmentation are not well delineated. Liew et al.[20] described the histologic changes of hypopigmentation in nine patients treated for hair removal using the ruby laser. S-100-positive melanocytes remained constant, whereas DOPA oxidase activity appeared to decrease. These findings suggest that the ruby laser causes hypopigmentation by blocking melanin synthesis rather than destroying melanocytes.

Laws et al.[9] assessed the histologic features of hypopigmentation after CO_2 resurfacing in a 62-year-old woman.

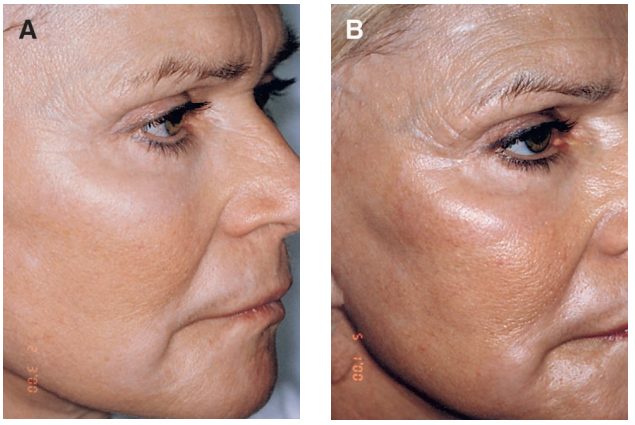

Figure 3. (A) Before and (B) after 25 treatments. Repigmentation 50–75%.

No decrease in the number of melanocytes was noted compared with a pretreatment biopsy. There was, however, a decrease in the amount of epidermal melanin visible with Fontana–Masson staining.

The present investigation described the histologic alterations and topical photochemotherapy-induced repigmentation of laser resurfacing-induced hypopigmentation. All of our patients demonstrated positive staining for epidermal melanin. In addition, melanocytes were present as evidenced by positive Mel-5 immunostaining. Mel-5 antibodies remain one of the most reliable and accurate immunostains for melanocytes.[18] It detects a 75 kDa glycoprotein of melanosomes. Other widely known and available antibodies such as S-100 and HMB-45 are often unreliable for identification of normal melanocytes. In addition, S-100 also stains nerves, Schwann cells, and Langerhans cells.

Wood's light examination in all of our patients was positive. This observation suggests the presence of residual pigment as confirmed by our histologic studies.

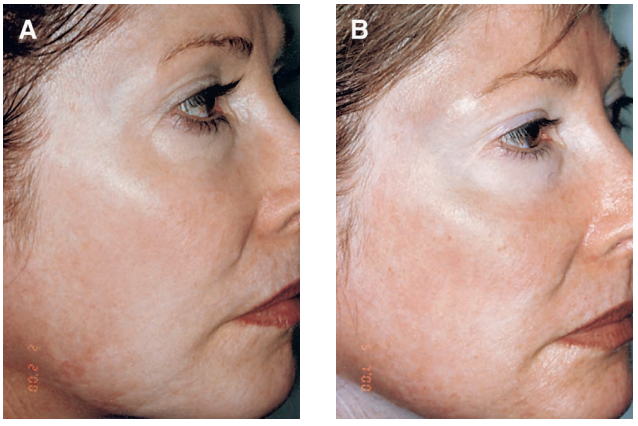

Figure 4. (A) Before and (B) after 37 treatments. Repigmentation 75–100%.

Previous studies by Jarrett and Szabo[21] and Pitts and Grimes[22] have suggested that the presence of residual melanocytes in patients with vitiligo portends a better prognosis for repigmentation. The rapidity of the overall repigmentation response observed in our patients suggests that residual melanocytes and melanin probably facilitated the expediency of repigmentation.

In light of the presence of melanocytes and reduced melanin in our patients, we postulate that melanogenesis is impaired as a consequence of laser resurfacing. Our data support Liew et al.'s [19] finding of residual epidermal melanocytes after ruby laser treatment for hair removal. All of our biopsied patients also demonstrated superficial dermal fibrosis. Resurfacing may have altered the complex microenvironment of melanocytes, basement membrane, and collagen. Morelli et al.[23] suggested that PUVA therapy can alter type IV collagen secretion by changing the profile of melanocyte integrin receptors, thereby potentially facilitating the process of repigmentation. PUVA therapy may also inactivate inhibitors of melanogenesis and stimulate melanocyte proliferation and hypertrophy.[13,16,23–26]

In 1993 Grimes et al.[15] began utilizing lower concentrations of topical 8-MOP for treatment of vitiligo in combination with sunlight. The diluted concentration of 0.001% proved to have significant efficacy while substantially decreasing the frequency of blistering reactions often observed with higher concentrations of ≥0.1% 8-MOP. Preliminary promising data from the Vitiligo and Pigmentation Institute using this approach in patients with hypopigmentation induced by chemical peels and dermabrasion suggested the potential for similar beneficial effects with laser resurfacing.

Concerns remain regarding the acute and long-term complications of PUVA therapy. Minimal acute complications were observed in our patients. None experienced blistering reactions. Perilesional hyperpigmentation was temporary and faded during treatment. Hence our low-concentration topical psoralen photochemotherapy protocol was well tolerated. However, the use of higher concentrations of 8-MOP are contraindicated in light of the likelihood of causing phototoxic blistering reactions in laser-resurfaced skin. Unlike oral photochemotherapy, the number of treatments and cumulative UVA exposure were relatively low.[12] Hence this should minimize any long-term risks of further actinic damage and cancer risks. In contrast to psoriasis,[27] no studies have documented an increased risk of skin cancer in vitiligo patients treated with topical or systemic photochemotherapy.[26–28]

The results of this investigation suggest that topical photochemotherapy utilizing 0.001% methoxsalen may offer substantial improvement in patients with laser-induced hypopigmentation. Our preliminary data suggest that laser-induced hypopigmentation is amenable to treatment. However, long-term larger studies are necessary to further document the sustained efficacy and permanency of repigmentation.

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Commentary

Since its introduction in the early 1990s, ablative laser resurfacing for skin rejuvenation has passed through the three stages of all technological development: early adoption, general evaluation, and acceptance. First, there was initial enthusiasm and exuberance for the procedure, then a leveling off or assessment period. Finally, the technique found its home in the therapeutic array for facial rejuvenation. Many side effects or sequelae of ablative laser resurfacing are temporary and easily correctable. The most dreaded complication is the late onset of hypopigmentation or depigmentation.

This side effect has been reported to occur as frequently as 20% and often does not appear until 6 to 12 months after laser resurfacing has been completed. The reported pigmentary alterations include a full spectrum of severity ranging from lines of demarcation between the treated and non-treated site, true hypopigmentation, and frank depigmentation. Until recently, the physician had no significant treatment to offer to the patient other than instructions for strict sun avoidance, application of camouflage makeup, and diligent use of sunscreens.

Dr. Pearl Grimes and her associates have addressed this problem in a scientific and methodical manner. First, they demonstrated that there are residual melanocytes in the areas of dyspigmentation. Then they bridged medical and surgical dermatology by taking our knowledge of PUVA treatments for treating pigmentary abnormalities such as vitiligo and applying it to the treatment of postoperative laser hypo or de-pigmentation.

The main advantage of this study is that it now gives physicians a therapeutic option for their patients. However, topical PUVA can be an arduous process requiring as many as 38 treatments. Only one patient in this study had complete repigmentation but 71% showed that the topical photochemotherapy induced moderate to excellent results. The last desire of the physician trying to clear or improve one complication is to cause another one and topical PUVA is not without potential risks of phototoxic reactions, hyperpigmentation of surrounding normal skin, erythema, pruritus, and edema.

This topical photochemotherapy regimen is the first in a series of efforts of dermatologic surgeons to address this complication. Dr. Roy Geronemus presented preliminary work at the recent American Academy of Dermatology meeting in Washington, DC, March, 2001, which showed the use of the excimer laser (308 nm) also to be beneficial in treating these pigmentary disturbances. The advantage of this technique is that it requires fewer treatments and preliminary results suggest excellent outcomes. The availability of this new laser equipment will influence its adoption as a treatment.

Dermatologic surgeons have led the way in ablative laser resurfacing by developing the technique and reporting results and complications. Once again, they step forward to address the complications and develop new treatments. This reflects the strength of the dermatologic surgery community's background in skin biology. The result is one of hope for our patients with laser resurfacing-induced hypopigmentation.

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