High-Intensity Flashlamp Photoepilation

A Clinical, Histological, and Mechanistic Study in Human Skin

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Objective: To examine the clinical, histological, and immunohistological effects of flashlamp photoepilation.

Design: Nonrandomized control trial with blinded histological study and follow-up of 1 to 20 months.

Setting: Private academic practice.

Subjects: Sixty-seven subjects (10 males and 57 females) with areas of excess body hair.

Interventions: Single (9 subjects) or multiple (58 subjects) treatments (noncoherent, 590-1200 nm, 2.9-3.0 milliseconds, 40-42 J/cm²) to hairy skin. From subjects given a single treatment, biopsy samples were taken immediately after treatment and at different intervals for up to 20 months.

Mean Outcome Measures: Clinical measures include hair counts and morphologic features before and after treatment. Histological measures include terminal-vellus and anagen-other ratios, hair shaft diameter, and morphologic features (routine and immunohistochemical detection of bcl-2, bax, p53, Ki67, cyclin D1, and hsp70) before and after treatment.

Results: Mean hair loss after photoepilation was 49%, 57%, and 54% for a single treatment and 47%, 56%, and 64% for multiple treatments at follow-up of less than 3 months, 3 to less than 6 months, and 6 months or longer (P<.05 for all comparisons). Transient erythema was seen in all subjects; no scarring occurred. Histologically, treatment caused morphologic damage confined to hair follicles and shafts. Terminal-vellus and anagen-telogen ratios, mean hair shaft diameter, and immunohistochemical profiles were not significantly modified by treatment. Treatment did not alter other skin adnexa, epidermis, or vessels.

Conclusions: Flashlamp treatment leads to significant, long-lasting epilation. The predominant mechanism seems to be via selective photothermal damage to large, pigmented hair follicles rather than induction of a programmed state of follicular cycle arrest or follicular miniaturization.

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Several devices for removing undesired hair by the delivery of radiant energy to the skin (photoepilation) are currently in clinical use or under investigation. With all these photoepilation systems, it is hypothesized that melanin in the follicular epithelium or hair shaft absorbs radiant energy, thereby mediating follicular damage and resultant cessation of hair growth. However, the precise mechanisms responsible remain unclear. Photoepilation could induce a prolonged, telogen-type quiescent state, possibly involving a program of apoptosis or dysregulation of the cell cycle; according to that hypothesis, reversion to an active anagen state might then account for the late recurrence of hair growth observed with the use of some photoepilation devices. Alternatively, the absorption of energy might induce destructive photothermal or (less likely in the case of long-pulse duration) photomechanical effects, causing follicles to undergo nonspecific cell death or oncosis and eventually in generalized follicular necrosis and resorption. Some authors propose that photoepilation may involve short-term growth delay and long-term clinical hair loss. The latter may be caused by miniaturization of hairs to a vellus-like state. These various mechanisms may not be mutually exclusive. By optimizing exposure factors such as pulse duration and wavelength, it may be possible to vary these effects to achieve a better therapeutic index.
SUBJECTS, MATERIALS, AND METHODS

SUBJECTS

The study group consisted of 67 subjects aged 15 to 67 years (mean, 36 years), including 10 males and 57 females, with areas of excess body hair. Subjects were of Fitzpatrick skin phototype I (2 subjects), II (29 subjects), III (34 subjects), or IV (2 subjects). All subjects had black or brown terminal hair at body sites to be treated and were screened by physician interview and physical examination to exclude endocrine dysfunction, immunosuppression, recent oral retinoid drug use, drug-induced hypertrichosis, sensitivity to infrared radiation or visible light, collagen-vascular disease, or androgen-producing tumors. Previous modalities used at the flashlamp irradiation sites included waxing (12 subjects), depilation (22 subjects), and shaving (25 subjects); any such treatments were discontinued at least 4 weeks before flashlamp irradiation. Informed consent was obtained from all subjects.

PHOTOEPILATION PROTOCOL

The EpiLight hair removal system, an intense, broad spectrum, pulsed-light source, was used for all treatments. Hairs were trimmed to 1 mm, and a cooling gel (1°C) transparent to the irradiation wavelengths was applied to the surface. Nine subjects received a single flashlamp treatment and 58 received multiple flashlamp treatments with a nonoverlapping technique to the hairy skin of the inguinal region, chest, abdomen, face, back, or arms. Flashlamp exposure parameters were as follows: filter (long pass), 590 nm (for subjects with Fitzpatrick skin type I), 615 nm (skin type II), 645 nm (skin type III), or 693 nm (skin type IV); fluence, 40-42 J/cm²; exposure area (spot size), 10 × 45 mm; pulse duration, 2.9 to 3.0 milliseconds; and pulse delay, 30 milliseconds. No anesthesia or postoperative care was required in any subject.

CLINICAL ASSESSMENT

A 1-cm² grid was used to count hairs at baseline and at various subsequent times, including 1, 2, 3, 4, 6, 8, 9, 12, 14, 16, 18, and 20 months after flashlamp treatment for different subjects. Subjects were entered in the study at different times between February 1997 and January 1998, accounting for different final follow-up periods (range, 0.25-20 months; mean ± SD, 4 ± 4 months) (Figure 1). The treated area was determined by measuring the distance from anatomic landmarks (umbilicus, iliac crest, nipple, mouth, chin, or shoulder) and was confirmed by reference to standardized photographs. Photographic documentation was performed with the Yashica Medical Eye II (Tokyo, Japan), equipped with a macro lens, a shooting area of 24 × 360 mm, and a shooting distance of 15.5 cm, using identical lighting and patient positioning with respect to anatomic landmarks. The hairs were counted separately by 2 investigators (N.S.S. and a physician assistant) with the aid of a hand lens at each time; later, the average value was calculated. The same 2 individuals performed the hair counts in an unblinded manner. The hair counts were then tabulated as a function of the number of hairs counted immediately before treatment. To determine any possible differences regarding short or long follow-up, hair counts were tabulated in 3 separate groups with cutoff times at 3 and 6 months: less than 3 months, 3 to less than 6 months, and 6 months or more. In a group of 19 subjects, the percentage of grossly altered hairs (ie, kinked, tapered, or hypopigmented) also was recorded on the skin (a trichogram was not performed because that would affect the hair count data).

RESULTS

CLINICAL FINDINGS

Groups of subjects receiving 1 or multiple treatments demonstrated significant reductions in hair counts at all times compared with unirradiated controls (Figure 1 and Figure 2). In subjects who received a single treatment there was a mean hair loss of 61%, 58%, and 48% at less than 3 months, 3 to less than 6 months, and 6 months or more of follow-up, respectively (P = .002, P = .002, P = .03, respectively). In subjects who received multiple treatments there was a mean hair loss of 47%, 56%, and 64% at less than 3 months, 3 to less than 6 months, and 6 months or more of follow-up, respectively (P < .001 for all) (Figure 1). Overall, no significant differences were detected in subjects treated once vs multiple times (P = .82). However, at less than 3 months of follow-up, there was more pronounced hair loss in subjects treated once (61%) than in those who received multiple treatments (47%) (P = .04).

No relationship of hair loss to age, sex, or anatomic site was apparent (follow-up ≥ 3 months; P = .53, .53, and .84, respectively). No significant differences were found between single and multiple treatments in relation to age or sex (P = .68 and .13, respectively); multiple treatments were more frequently delivered to face areas (P < .001). No significant differences were identified when comparing the 4 different wavebands used in the study (P = .41).

The treatments were well tolerated and without serious adverse effects. Immediate, mild follicular ery-
HISTOLOGICAL ANALYSIS

All histological and immunohistochemical preparations were studied jointly by 2 dermatopathologists (C.R.S. and V.G.P.) in a blinded manner. Histological study was performed only in the 9 subjects who received a single treatment (5 females and 4 males). Contralateral, unirradiated sites also underwent biopsy for comparison. Three- or 4-mm punch biopsy samples were taken from control and treated regions immediately after treatment sessions. Follow-up biopsy samples were taken at different periods during the study (Table 1). Biopsy samples of treated sites were obtained from the areas of maximal gross epilation. In the case of control sites, biopsy samples were taken from regions symmetrical to the treated areas; no clear variation of apparent hair density was evident. Specimens were bisected or trisected transversely by hand, routinely fixed in 10% buffered formalin, transversely paraffin-embedded (in 3 specimens, an additional tissue fragment was also embedded in conventional, vertical orientation), and stained with hematoxylin-eosin. The ratios of terminal-vellus (Te/V) and anagen/catagen-telogen (A/other [nonanagen]) hairs were calculated in all specimens; small hairs showing features of telogen or early anagen phases were classified as undetermined. The diameter of all available hair shafts was measured in all specimens.

A standard 3-step immunohistochemical method was performed with primary antibodies against the following antigens: Ki67 (a proliferation marker); hsp70 (a heat shock protein); and p53, cyclin D1, bcl-2, and bax (proteins involved in the regulation of the cell cycle) (Table 2). Five-micrometer sections were air-dried and deparaffinized with xylene and absolute alcohol. Antigen retrieval was performed to detect Ki67, hsp70, p53, bcl-2, and bax expression (Antigen Retrieval Solution; Dako, Carpinteria, Calif), with overnight incubation at 72.5°C. After protein block with normal serum (JRH Biosciences, Lenexa, Kan), the appropriate dilutions of the primary antibody, biotinylated IgG (Vector Laboratories, Burlingame, Calif), ABC Elite tertiary complex (Vector Laboratories), and 3, 3’ diaminobenzidine (Sigma-Aldrich Corporation, St Louis, Mo) solution were added in sequence with intervening phosphate-buffered saline solution washes. The diaminobenzidine reaction was enhanced with 1% cupric sulfate, and slides were lightly counterstained with Harris hematoxylin. All hair follicles identifiable in the specimens were studied, and counts were performed in a single section per antibody. For all markers, a qualitative measurement of the degree of expression was determined for the follicular epithelium. Because discrete numbers of labeled cells were observed with anti-Ki67, a quantitative measurement was performed for Ki67 expression. Most labeled cells were located in the hair bulb and in the most peripheral layer of the follicular epithelium. In the case of hair bulbs, a semiquantitative measurement of percentage of labeled cells was performed. Numbers were tabulated as an index of labeled cells divided by the total number of cells in the basal layer of follicles.

Skin biopsy samples from the areas contralateral to the treated areas were studied as controls for the routine histological and immunohistochemical studies.

STATISTICAL ANALYSIS

Student t and χ² tests were used to determine whether the groups receiving 1 or multiple treatments had significant differences regarding age, sex, or anatomic location. The Student t test was used to compare any significant differences between single vs multiple treatments regarding hair counts. The Student t test was used to compare the pretreatment and posttreatment counts, different wavebands, anagen-other and terminal-vellus ratios, and diameters of hair shafts. A software package (JMP; SAS Institute, Cary, NC) was used for statistical analysis. P=.05 was considered significant.

thema occurred in all subjects but was transient in most of them; a single subject had mild, localized erythema that persisted for 7 days after flashlamp exposure and then resolved spontaneously. No pigmentary or scarring complications developed in any subject, but most developed gross kinking of some hair shafts immediately after treatment (range, 0%–22%; mean ± SD, 10.2% ± 6.5%). Hairs displayed several morphologic features at the various observation points, ranging from normal terminal hairs to dystrophic shapes, including apparently reduced diameters, abnormal tapering, and distorted contours.

HISTOLOGICAL FINDINGS

Biopsy samples taken immediately after treatment showed clumping of melanin, distortion of the hair shaft and hair canal, and homogenization of the keratin layer predominantly in hair follicles with large, pigmented hair shafts; coagulative necrosis of the hair shaft was easily detected (Figure 3). Also observed was thinning of the follicular epithelium of large hair follicles. At 48 hours, more than 50% of follicles (those of larger size) contained apoptotic keratinocytes, thick basement membrane, and perifollicular edema with a sparse lymphocytic infiltrate, features similar to those described in the catagen phase of normal follicles. Some hair bulbs had many vacuolated and apoptotic keratinocytes; hemorrhage was observed perifollicularly and also in rare hair papillae (Figure 4). At 1 week, morphologic features were similar to those described at 48 hours. At 2 weeks and 1, 2, 6, 8, 12, 18, and 20 months, many follicles had apoptotic keratinocytes, perifollicular fibrosis, and perifollicular melanophages (Figure 5). Rare hair follicles in those late biopsy samples showed marked alteration of the follicular epithelium, with separation between epithelium and dermis (Figure 5). Some hair follicles, mainly those with small, hypopigmented shafts, did not demonstrate any morphologic change. In no cases were any morphologic alterations identified in other skin adnexa, overlying epidermis, vessels, or other dermal structures. Unirradiated control skin showed normal-appearing hair follicles.

Hair counts were performed in all the available biopsy samples (Table 3). In these biopsy samples, after a single treatment, terminal-vellus and anagen-other ratios were not significantly different at the various observation points (P = .94 and .84, respectively). The diameter of the hair shafts did not vary significantly between control (untreated) and treated areas either im-
IMMUNOHISTOCHEMICAL FINDINGS

All markers were assessed in all available hair follicles from all biopsy samples. For all markers, there were no significant differences between nontreated and treated areas at any time studied.

Anti-Ki67 Expression

In hair follicles from biopsy samples taken immediately after treatment, there was a decreased number of cells expressing this proliferation marker compared with normal, unirradiated skin (mean ± SD, 17.2 ± 7.3 vs 15.5 ± 7.1, control vs immediately after treatment, respectively). However, the differences were not statistically significant (P = .65). In the few available hair bulbs showing marked morphologic damage after treatment, more than 80% of the cells in the damaged hair bulbs expressed this proliferation marker; in contrast, in normal, unirradiated hair follicles, fewer than 50% of keratinocytes in the hair bulbs expressed Ki67 (Figure 6).

bcl-2 Expression

In treated and unirradiated skin, this anti-apoptotic regulatory protein was expressed in melanocytes located in the epidermis and hair follicles, dendritic cells in the hair papilla, keratinocytes in the bulb, blood vessels, and, weakly, in the basal layers of epidermis and hair follicles.

bax Expression

In treated and unirradiated skin, this proapoptotic regulatory protein was consistently expressed in the inner root sheath and hair shaft of follicles, sebaceous glands, clear cells in eccrine glands, nerves, and blood vessels. Most hair papillae were not labeled, and only rare cells were labeled in damaged follicles of treated areas.

Cyclin D1 Expression

In treated and unirradiated skin, this cyclin involved in control of the phosphorylation of the retinoblastoma protein was weakly expressed in the cytoplasm of follicular and epidermal keratinocytes; scattered keratinocyte nuclei were strongly labeled in some hair follicles.

p53 Expression

In treated and unirradiated skin, expression of p53 was seen as nuclear labeling of rare keratinocytes in the epidermis and hair follicles.

hsp70 Expression

In treated and unirradiated skin, hsp70 expression was patchy in epidermis and hair follicles.

COMMENT

Several pulsed lasers with various wavelengths, pulse durations, spot sizes, and energy densities recently have been used for photoepilation. Q-switched 1064-nm Nd:YAG laser irradiation has been used to remove hair from mucosae or transplanted skin11,12 and subsequently was combined with topical application of carbon suspension in an attempt to increase absorption of radiant energy by follicles; however, use of the latter modality has been associated with variable results and high recurrence rates.13-15 Use of free-running pulsed ruby (694-nm) and alexandrite (755-nm) lasers reportedly yields better epilation;8,10,16-19 transient hyperpigmentation in some darker-skinned subjects has been noted. Most long-pulse devices are theoretically based on the principle of thermokinetic selectivity20: The absorbed energy destructively heats larger targets such as hair follicles, whereas smaller structures containing the same chromophore are merely warmed below the threshold for destruction. Use of the ruby laser reportedly induces initial follicular growth delay, originally postulated to be associated with a prolonged telogen phase.8 In a later article,10 the same authors reported that long-term clinical hair loss correlated with miniaturized hairs having a velluslike consistency. The 800-nm diode laser is also in use for photoepilation; in principle, its long, penetrating wavelength may facilitate targeting of deeper follicular structures.9

The flashlamp instrument used in the present investigation differs from the previously described laser sys-
tems in that it is a noncoherent, noncollimated, and poly-
chromatic visible-infrared source. Some authors refer
to it as “nonselective” because they theorize that “the
broadband light increases the likelihood of non-target ab-
sorption and also the risk of adverse effects.” However,
our data do not support these concerns. Only 1 of 67 sub-
jects in our investigation developed significant ery-
thema, which persisted for 7 days and later completely
subsided; no scarring occurred in any of them. Also, our
data demonstrate a great degree of tissue selectivity, with
histological effects exclusively confined to hair follicles.
From our total group of 67 subjects, histological analy-
ysis was performed on all 9 in whom a single treatment
was used. This is a larger number than in other pub-
lished photoepilation literature and includes bi-
opsy results from a wide range of times after treatment
(immediately until 20 months), with multiple biopsies
performed in between. Five specimens were obtained 6
months or more after treatment. Thus, this study offers
comprehensive data on the clinical and histological
changes induced by flashlamp photoepilation. A fol-
low-up study is ongoing to determine whether there are
any histological differences in subjects who undergo single
vs multiple treatments.

In the present investigation, the percentage of hairs
present was relatively stable by 3 months after treat-
ment, with no clear relationship to age, sex, or anatomic
site. The variable efficacy of photoepilation among sub-
jects may be caused by differential melanization of hair
structures as an individual pigmentary phenotype or by
differences in the anagen-telogen ratio among the par-
ticular sites treated.

### Table 1. Hair Counts on Histological Slides From 9 Patients Who Received a Single Treatment

<table>
<thead>
<tr>
<th>Region</th>
<th>Immediately</th>
<th>2-3 d</th>
<th>10 d</th>
<th>1-1.5 mo</th>
<th>2-2.5 mo</th>
<th>6-8 mo</th>
<th>12 mo</th>
<th>18-20 mo</th>
</tr>
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<tbody>
<tr>
<td>Chest</td>
<td>2Te (1A/1C), 1V (A)</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td>2Te (2A), 0V</td>
<td>2Te (1U), 1V (A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctl</td>
<td>2Te (2A), 0V</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td>1V (U)</td>
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</tr>
<tr>
<td>Chest</td>
<td>1Te (A), 1V (A)</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td>2Te (2A), 0V</td>
<td>2Te (1U), 1V (A)</td>
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<td></td>
</tr>
<tr>
<td>Ctl</td>
<td>1Te (A), 0V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td></td>
</tr>
<tr>
<td>Lower abdomen</td>
<td>2Te (2U), 0V</td>
<td>1V (A)</td>
<td>1V (A)</td>
<td>1V (A)</td>
<td>1V (A)</td>
<td>1V (A)</td>
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</tr>
<tr>
<td>Ctl</td>
<td>1Te (U), 0V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td></td>
</tr>
<tr>
<td>Groin</td>
<td>0 Hairs</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td>2Te (2C), 0V</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td></td>
</tr>
<tr>
<td>Ctl</td>
<td>1Te (U), 1V (U)</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td></td>
</tr>
<tr>
<td>Groin</td>
<td>1Te (C), 2V (2C)</td>
<td>3Te (2C/1U), 3V (3A)</td>
<td>3Te (2C/1U), 3V (3A)</td>
<td>3Te (2C/1U), 3V (3A)</td>
<td>3Te (2C/1U), 3V (3A)</td>
<td>3Te (2C/1U), 3V (3A)</td>
<td>3Te (2C/1U), 3V (3A)</td>
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<tr>
<td>Ctl</td>
<td>1Te (C), 2V (2C)</td>
<td>3Te (2C/1U), 3V (3A)</td>
<td>3Te (2C/1U), 3V (3A)</td>
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<td>3Te (2C/1U), 3V (3A)</td>
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<tr>
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<tr>
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<td>3V (2A/1U)</td>
<td>3V (2A/1U)</td>
<td>3V (2A/1U)</td>
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<tr>
<td>Groin</td>
<td>1Te (1A), 1V (U)</td>
<td>1Te (1C), 0V</td>
<td>1Te (1C), 0V</td>
<td>1Te (1C), 0V</td>
<td>1Te (1C), 0V</td>
<td>1Te (1C), 0V</td>
<td>1Te (1C), 0V</td>
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<tr>
<td>Ctl</td>
<td>1Te (A), 0V</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td>1V (U)</td>
<td>1V (U)</td>
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</tr>
<tr>
<td>Groin</td>
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<td>1Te (A), 0V</td>
<td>1Te (A), 0V</td>
<td>1Te (A), 0V</td>
<td>1Te (A), 0V</td>
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<td>1Te (A), 0V</td>
<td></td>
</tr>
<tr>
<td>Ctl</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td>0 Hairs</td>
<td></td>
</tr>
<tr>
<td>Suprapubic†</td>
<td>2Te (2C), 1V (A)</td>
<td>4 Te (3C/1A), 0V</td>
<td>4Te (1A/3C), 0V</td>
<td>4Te (1A/3C), 0V</td>
<td>4Te (1A/3C), 0V</td>
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<tr>
<td>Ctl</td>
<td>1Te (C), 0V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td>0 V</td>
<td></td>
</tr>
</tbody>
</table>

* Tx indicates treated area; Ctl, control area; Te, terminal; V, vellus; A, anagen; C, catagen; Tl, telogen; and U, undetermined.
† An extra set of biopsy samples was taken immediately after treatment and at 2 to 3 d.

### Table 2. Antibodies Used in the Study

<table>
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<th>Antibody</th>
<th>Antigen</th>
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<td>Anti-bax</td>
<td>bax</td>
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<td>Biogenex, San Ramon, Calif</td>
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<tr>
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<td>Ki67</td>
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<td>Zymed, Burlingame, Calif</td>
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<td>DCS6</td>
<td>cyclin D1</td>
<td>1:100</td>
<td>Zymed</td>
</tr>
<tr>
<td>Anti-bcl-2</td>
<td>bcl-2</td>
<td>1:40</td>
<td>Dako, Carpinteria, Calif</td>
</tr>
<tr>
<td>Anti-hsp70</td>
<td>hsp70</td>
<td>1:300</td>
<td>Dako</td>
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<tr>
<td>Do-7</td>
<td>p53</td>
<td>1:50</td>
<td>Dako</td>
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</table>
the functional melanocytes of the anagen hair matrix region, they may be intrinsically more resistant to melanin-mediated photoepilation. This hypothesis is supported by the finding that the morphologic changes seen in this study were most prominent in the large, melanized hairs, whereas most of the small, hypopigmented hairs appeared unchanged. In mice, it has been shown that pigmented anagen hair follicles are more sensitive to ruby laser exposure than are catagen or telogen follicles. Some studies in humans reported that nonpigmented hairs show little or no response to ruby laser exposure.

Because of the apparently different responses of hair follicles, some authors proposed that multiple treatments, performed after a delay of several weeks to months, may be more efficacious than a single exposure. In the present flashlamp study, in which subjects were not randomly assigned to 1 or more treatments, overall there were no statistically significant differences in hair counts after a single vs multiple treatments. However, additional randomized prospective trials will be necessary to resolve this issue.

A notable finding of this study is the persistence of epilation, which endures more than 6 months after a single or multiple treatments. However, the sampling error intrinsic to the small size of biopsy samples customary in this type of study precludes direct, quantitative comparison between clinical and histological hair counts. It has been speculated, based on the study of a single patient, that long-term ruby laser photoepilation involves inversion in the terminal-vellus ratio. Our data do not support this hypothesis for the flashlamp device because there was a relative stability in the ratio before treatment and at last follow-up and there was no significant reduction in the diameter of hair shafts. However, the same sampling error mentioned previously also represents a potential pitfall when calculating the terminal-vellus ratio. Even so, from our data, miniaturization of hairs does not seem to be a major mechanism of photoepilation with use of this particular device; instead, damage to hair follicles is probably more important.
Trimming, not waxing, was used to prepare the selected area for flashlamp treatment. Waxing produces transient alopecia (in the sense that there is a lag time before any regenerating hairs reach the epidermal surface and become available to be counted). This phenomenon is not present in the case of trimming, which eliminates only the distal, external portion of the hairs and does not interfere with the assessment of hair regrowth. This fact is also supported by results of a recent study in which no significant difference was found between baseline and follow-up counts in sites that were shaved but not exposed to ruby laser irradiation. Therefore, the present study compares pretreatment and posttreatment counts without requiring comparison with a nontreated site. Results of the study of contralateral, normal areas show that no morphologic changes could be directly attributed to any of the hair removal treatments (waxing, depilatory, etc) previously used by some subjects. However, it is theoretically possible that previous wax epilation might modify the efficacy of subsequent flashlamp treatment by inducing a new phase of anagen growth in the few subjects (n = 12) who had used wax epilation. Also, any previous treatment was discontinued at least 4 weeks before irradiation. Other possible factors that might conceivably affect the results under this experimental design are hormonal and seasonal effects. However, it is unlikely that these factors were significant in the present study because subjects entered the study at various times during a 10-month period and no significant regrowth was seen up to 20 months after treatment.

From the few histological studies published on photoepilation with the ruby laser, analysis immediately after treatment revealed cytoplasmic eosinophilia and elongation of nuclei in follicles; fragmentation of hair shafts; and basophilic, homogenized dermal collagen. No histological information is available from follow-up biopsy samples. No previous histological data have been reported with this EpiLight device. In our study, biopsy samples taken immediately after treatment show findings qualitatively similar to those described in laser-treated skin and are probably related to thermal coagulation (cellular hypereosinophilia and clumping of melanin). In those early biopsy samples, no definite fibrosis, but rather perifollicular edema, is identified. In the follow-up biopsy samples in our study, there is an increased number of apoptotic cells in many follicles. Although these apoptotic bodies indicate that use of the high-intensity flashlamp induces individual cell death, when comparing control and treated areas, no significant differences were detected for the expression of some of the key proteins known to be involved in regulation of apoptosis (bax/bcl-2 pathway and p53/cyclin D1). Also, we did not detect any change in a representative molecule of the heat shock response. It is possible

Figure 4. Top, Horizontal section of a control biopsy specimen showing a hair follicle, including the hair bulb and hair papilla. Bottom, Biopsy specimen 48 hours after treatment. Notice the vacuolization of the cytoplasm of keratinocytes, distortion of the follicular lumen, and perifollicular hemorrhage (hematoxylin-eosin, original magnification ×100).

Figure 5. Top, Horizontal section of a biopsy specimen 26 weeks after treatment. Notice the vacuolization of keratinocytes and marked perifollicular fibrosis in 1 follicle. Bottom, Horizontal section of a biopsy specimen 52 weeks after treatment. Notice the granular melanin in the hair shaft and the perifollicular fibrosis with melanophages (hematoxylin-eosin, original magnification ×200).
that the changes induced in these proteins may not be detectable at the immunohistochemical level or that other molecular pathways may be involved. Thus, the molecular mechanisms underlying these histological findings require further elucidation. Regarding the expression of proliferation markers (Ki67), we did not detect significant differences at any time during follow-up, but there was a trend for hair follicles from late follow-up biopsy samples to have fewer proliferating cells than those in pretreatment biopsy samples.

At the tissue level, the mechanism by which this flashlight device induces hair loss seems to be different from that reported with other devices. Use of the ruby laser has been reported to induce marked and rapid hair loss followed by partial regrowth. According to published data using the pulsed ruby laser at 40 J/cm² in shaved skin, at 1-month follow-up the hair count was reduced to 9% of the pretreatment count (Figure 5). However, this dramatic extent of epilation was short-lived. According to the same data, at 3- and 6-month follow-up, approximately 55% and 70% of hairs, respectively, compared with pretreatment control counts were present, representing partial regrowth. A comparable regrowth phenomenon was not observed in a published study of the flashlamp system in 31 subjects treated with a range of 34 to 55 J/cm². In that study, approximately 50% of treated sites showed good response (defined arbitrarily by us as hair counts <50% of pretreatment counts) immediately after treatment, and this trend increased to 70% good response by 3 months (Figure 1 through Figure 5). Our results in subjects receiving a single treatment show a good response (again arbitrarily defined as hair counts <50% of pretreatment counts) in 100%, 89%, 89%, and 78% of them at 1, 2, 3, and 6 months or more of follow-up, respectively. Our results demonstrate a slight rebound in hair counts 1 to 3 months after receiving a single treatment (P = .10). It seems unlikely that follicles after flashlamp treatment enter into a prolonged resting phase because the hair counts obtained at long follow-up periods (18 and 20 months) showed no clinical regrowth and still showed histological changes. Moreover, the Ki67 data at no time showed evidence of quiescence of the hair follicles with regard to cell proliferation.

Reasons for the apparent differences in the extent of hair regrowth induced by use of the ruby laser and flashlamp require further investigation. One likely explanation relates to differences in pulse duration. The ruby laser used in previously published studies emitted 270-microsecond pulses, an order of magnitude briefer than the 3-millisecond pulse used in the present flashlamp study. Thus, the ruby laser delivered 10× greater power density, which may have facilitated the early fragmentation and shedding of hair shafts. As noted by Dierickx et al, a 3-millisecond pulse may permit more heat conduction and thermal damage to nonpigmented regions of the follicle.

In summary, our data demonstrate that use of the high-intensity flashlamp induces long-lasting epilation by causing selective thermal damage of large, pigmented hair follicles. A shift in treated follicles to a telogen phase is not apparent. It is likely that the energy delivered by this photodevice is absorbed by melanin, with subsequent damage to melanin-containing structures—predominantly the hair bulb and the hair shaft—and possibly thermal conduction to the remainder of the follicle. The relatively mild inflammatory reaction observed histologically and clinically probably explains the absence of clinical scarring. Multicenter studies are in progress to determine the optimal treatment interval to achieve maximal efficiency of hair removal.

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