Temporary Hair Removal by Low Fluence Photoepilation: Histological Study on Biopsies and Cultured Human Hair Follicles

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Background and Objectives: We have recently shown that repeated low fluence photoepilation (LFP) with intense pulsed light (IPL) leads to effective hair removal, which is fully reversible. Contrary to permanent hair removal treatments, LFP does not induce severe damage to the hair follicle. The purpose of the current study is to investigate the impact of LFP on the structure and the physiology of the hair follicle.

Study Design/Materials and Methods: Single pulses of IPL with a fluence of 9 J/cm² and duration of 15 milliseconds were applied to one lower leg of 12 female subjects, followed by taking a single biopsy per person, either immediately, or after 3 or 7 days. Additionally, we present a novel approach to examine the effects of LFP, in which ex vivo hairy human scalp skin was exposed to IPL pulses with the same parameters as above, followed by isolation and culturing of the hair follicles over several days. Samples were examined histologically and morphologically.

Results: The majority of the cultured follicles that had been exposed to LFP treatment showed a marked treatment effect. The melanin containing part of the hair follicle bulb was the target and a catagen-like transformation was observed demonstrating that hair formation had ceased. The other follicles that had been exposed to LFP showed a less strong or no response. The skin biopsies also revealed that the melanin-rich region of the hair follicle bulb matrix was targeted; other parts of the follicle and the skin remained unaffected. Catagen/telogen hair follicles were visible with unusual melanin clumping, indicating this cycle phase was induced by the IPL treatment.

Conclusions: Low fluence photoepilation targets the pigmented matrix area of the anagen hair follicle bulb, causing a highly localized but mild trauma that interrupts the hair cycle, induces a catagen-like state and eventually leads to temporary loss of the hair. Lasers Surg. Med. 40: 520–528, 2008.

Key words: temporary hair removal; low fluence; photoepilation; biopsies; histology; human hair follicle; intense pulsed light (IPL)

INTRODUCTION

Clinical results of photoepilation treatments reported in the literature in general show variability in hair reduction effectiveness, both in rate and duration of clearance. Based on “selective photothermolysis” as the proposed mechanism of action [1], this variability can partly be explained by the broad range of applied parameters such as fluence, pulse width and spectrum of the light. Similarly, variability between subjects such as skin type, hair color, and hair follicle (HF) geometry also contributes to these differences [2–4]. In general, however, clinical results observed for photoepilation can be classified into temporary effects and permanent effects.

Permanent hair reduction as defined by the FDA is “the long-term, stable reduction in the number of hairs re-growing after a treatment regime, which may include several sessions. The number of hairs re-growing must be stable over a time period greater than the duration of the complete growth cycle of HF’s, which varies from 4 to 12 months according to body location”.1 The hair cycle has four stages, active growth (anagen), regression (catagen), resting with an anchored club hair (telogen) and exogen (when the hair is shed) [5]. Anagen is deemed to be the stage of the cycle most susceptible to photoepilation treatment [6].

Histological studies show that permanent effects are often the result of extensive immediate post-treatment damage in the HF’s leading to miniaturization of coarse terminal hairs to fine vellus-like hairs or even complete degeneration of the follicles [2,7]. The reported types of


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immediate damage include severe damage to the hair shafts, the follicle inner root sheath (IRS) and outer root sheath (ORS), including thermal coagulation and vaporization injury [8–13]. It has also been shown that effective hair reduction can be obtained even though the hair shaft remains in place for several days before being shed [14]. It is hypothesized that permanent HF damage is obtained if the stem cells of the follicle in the “bulge” area are damaged, disabling the processes of hair follicle re-growth [1,2,15,16].

However, in various so-called permanent photoepilation studies also a temporary effect is reported [7,17–20]. This effect is often independent of the degree of permanent hair reduction (as determined at least four months after the treatment). This temporary effect consists of a strong hair clearance 2–3 weeks after the treatment which is maintained for approximately 12 weeks, followed by partial or complete re-growth [18,21]. Apparently, most of the affected HFs go through a non-hair producing period, without losing the ability to develop a hair. Histological studies reported in the literature suggest this temporary effect is a treatment-induced disturbance of the hair cycle, consistent with a catagen-like transition of the HF [8,18,21] without destruction of the stem cell region. For this catagen-inducing effect, it has been proposed that it is sufficient to damage the matrix cells of anagen HFs, coagulate blood vessels of the follicle papilla or partially destroy the ORS [22].

**Low Fluence Photoepilation**

In the search for a reversible treatment regime, we showed that low fluence photoepilation (LFP) can result in effective temporary hair removal [23]. Our method consists of a light treatment using intense pulsed light (IPL) using fluences that are significantly lower than those generally used for permanent hair reduction. The fluencies we applied for LFP are in the 10 J/cm² range, while permanent hair reduction is usually achieved with fluences in the range of 20–60 J/cm² [20]. Our clinical studies [23] show that LFP results in extremely effective hair removal with up to 95% hair reduction after two treatments in 4 weeks. Subsequent treatment every 2 weeks for a period of 12 weeks maintained the site hair free. However, complete hair re-growth was observed 12 weeks after the last treatment. Low fluence photoepilation, therefore, presents an option to achieve high clearance rates without permanent damage to the HF.

The literature includes only a few reports of studies in which low fluencies were applied in a clinical setting. Drosner et al. [24] report effective hair reduction in the 10 J/cm² range using an alexandrite laser and suggest this approach could lead to long lasting results. McCoy et al. [16] applied fluencies from 10 to 40 J/cm² using a 3 milliseconds ruby laser. At a fluence of 10 J/cm² they found damage to the hair shaft consisting of homogenization (disruption of the cellular structure by thermal damage) and after 4 weeks follicles appeared to have moved to late catagen and telogen phases. Liew et al. [25] used a single ruby laser treatment on ex vivo scalp skin at 14 and 20 J/cm² and did find damage at the bulge region, but not to the hair bulb, although they only investigated the acute effects. In a white paper, Manstein et al. present research on ex vivo HFs from scalp skin showing that exclusive bulb damage to the HFs is possible without damaging other parts of the follicle.² Despite the relatively low fluencies applied in these studies, the reported damage still extends over a large part of the HF, similar to that reported for higher fluencies [26], but without necrotic damage. Trelles et al. [27] showed by skin histology that the use of a filterless flashlamp-based system delivering 7.5 J/cm² (SpaTouch) leads to partial degeneration of the HFs and coagulation changes in the adjacent tissue. Also clinically this device leads to relative strong treatment effects including side effects like edema, crusting and even blistering [12]. These strong effects are most probably the result of the “Light and Heat Energy” technology³ by which also a substantial amount of heat energy is delivered to the skin.

Based on our clinical results [23] and the above-mentioned types of damage to hair follicles, we hypothesize that our parameters lead to an effective induction of catagen and telogen with no permanent damage to the hair follicle. The aim of this study was to validate this hypothesis. The study approach was twofold. In the first place we tracked treatment responses in individual HFs over time as a result of LFP treatment. We designed a novel approach of illuminating ex vivo human skin followed by isolation of HFs through micro-dissection and maintenance of the hair follicles in vitro for at least five days during which a detailed morphological analysis was conducted. Secondly, to investigate clinical effects in skin and to compare in vitro with in vivo data, biopsies were taken from subjects over a time-course following a single LFP treatment.

**MATERIALS AND METHODS**

**IPL Systems**

In this study, excised human scalp skin from cosmetic operations was exposed to IPL treatment. To illuminate these ex vivo skin samples, a bespoke non-commercial IPL device was built in our workshop that delivered 9 J/cm² during a pulse of 15 milliseconds with exponential decay. The spectrum ranged from 600 to 1,100 nm and is displayed in Figure 1. The optical coupling was accomplished by a sapphire window sized 10 × 50 mm² and an optical gel. Each footprint area received a single pulse.

Treatment of subjects was performed with an Ellipse Flex IPL system (Danish Dermatologic Development). A hair bearing area on the lower leg was chosen as suitable location for a biopsy. After shaving and application of an optical gel, this area was exposed to a single pulse of 9 J/cm² and 15 milliseconds.

In Vitro Model

Human scalp skin was obtained from Caucasian females aged 35–60 years undergoing facelift surgery. After gently washing the skin samples using phosphate buffered saline (PBS), hair bearing parts were trimmed and were warmed up to 37°C in a humidified CO₂ incubator. A layer of optical coupling gel was applied to the surface of the skin samples which were divided randomly into a control and a treatment group; the latter group was given a single treatment following steps:

- Each HF was placed free-floating in 1 ml serum-free culture medium in a well of a 24-well plate. Medium used was Williams E medium supplemented with Glutamine (2 mM), Insulin (10 μg/ml), hydrocortisone (100 ng/ml), antibiotics (Pen/Strep). Glutamine, Insulin and hydrocortisone were previously defined as the only supplements required for serum free maintenance of human hair follicles in vitro [30]. HFs were incubated in a humidified atmosphere of 5% CO₂/95% air.

During the days of culturing, gross morphology analysis was carried out on all individual HFs at 24–48 hour intervals using an inverted phase contrast microscope. Following 24 hours in vitro maintenance a set of two treated and two control HFs were stained with Propidium iodide (PI) and were analyzed using a fluorescence microscope. PI is not absorbed by viable cells but can penetrate cell membranes of dying or dead cells, thus acting as a marker for cell death.

Biopsies

Approval for the study was obtained through the medical ethics committee (Catharina Hospital Eindhoven). A total of 10 Caucasian females consented to having one 3 mm punch biopsy taken. The skin of the calf of the subjects was shaved and the LFP treatment was applied (9 J/cm², 15 milliseconds). The treatment was well tolerated by all subjects. Skin types ranged from Fitzpatrick type 1 to VI, age varied from 19 to 50 years, (mean 37 years); hair color ranged from mid-blonde to black and leg hair density was variable ranging from 2 to 20 hairs/cm² with the mean being 9 hairs per cm².

Biopsies were taken from the exposed area either immediately after treatment (within 1–2 hr, n = 4), 3 days after treatment (n = 4) or 7 days after treatment (n = 2). Each subject had only one biopsy taken and only at one point in time. For each biopsy a location was selected accommodating at least one HF. The area to be biopsied was anesthetized using chloroethyl spray and then the biopsy tool was punched into skin in the direction of hair growth, to also include the lower part of the HFs. A total of 10 biopsies were obtained during the study. They were fixed in paraformaldehyde (4%, phosphate buffer) and processed into wax and sectioned, either in the transverse direction (perpendicular to the long axis of the follicle) or in the longitudinal direction, (parallel to the long axis of the hair follicle). All sections were de-waxed and stained with hematoxylin–eosin for light microscopy.

RESULTS

In Vitro Maintenance of Hair Follicles Isolated From LFP Treated and Control Scalp Skin

In total 89 HFs were cultured, originating from three different patients undergoing facelift surgery. Of these follicles, 44 were harvested from skin samples exposed to IPL treatment, the other 45 were dissected from the skin samples that were not treated with light. All HFs were tracked individually each 24–48 hour via light microscopy.
The advantage of this method over other previously reported methods is that the behavior of the follicles can be monitored in vitro and specific treatment effects can be studied.

**Control (sham treated) samples.** Of the 45 control HF, 37 (82%) showed normal hair growth for at least 5 days. The remaining control HF showed atypical or no hair growth. A typical example of normal hair growth is shown in Figure 2A. The HF is shown after 1, 2, 4, and 5 days of culture and the growth of the hair fiber is clearly visible with the follicle elongating each day, as described previously [30]. In Figure 2B an enlarged picture of the bulb of the same HF is shown during the second day of culture. The dermal papilla (DP) remains visible but is half covered with melanocytes. The bulb is intact and the border of the inner root sheath (IRS) and outer root sheath (ORS) of the follicle can be easily distinguished. These are all signs of healthy hair growth [31].

**Treated samples.** Cultured hair follicles from the skin samples exposed to IPL treatment in general showed atypical hair growth. The effects observed in the 44 HF from treated skin can be classified into four types of responses, which will be referred to as type I to IV, described as follows:

I. Marked changes were observed in the lower part of the HF within 1 day of treatment (Fig. 3). As can be observed in Figure 3A, after 1 day in culture, the base of the hair fiber is rounded and the melanocytes have retracted upwards away from the DP and form a characteristic melanin condensate in comparison with the control. Figure 3B shows that the base of the hair shaft, apparently containing trapped melanocytes and melanin, has retracted further during the subsequent 3 days leaving an epithelial strand below. The clear decrease of length of the hair fiber is visible. The DP itself is reduced in size and is relatively dark, possibly indicating loss of viability of the cells. This Type I effect was seen in 27/44 follicles examined (61%).

II. The characteristic feature observed in this group is a thickened epithelial column formed between the retracted fiber base and the melanin zone, which can be seen after approximately 4 days (Fig. 4). The
overall response is similar to type I response, that is, rounding of the base of the fiber, rounding of the DP and upward retraction of the hair fiber. The thickening effect was seen in 6/44 HFs examined (14%).

III. Type III HFs are characterized by visible melanin clumping in the area of the matrix and after 4–5 days these melanin clumps are visible in the hair shaft (Fig. 5). The fiber, however, remains close to the dermal papilla. This effect was seen in 5/44 follicles examined (11%).

IV. In 3/44 HFs (7%), hardly any treatment effect was seen (type IV). There is a slight retraction of the melanin and appearance of isolated melanocytes around the DP. There is, however, no evidence of melanin clumping and the HFs continue to form pigmented hair (Fig. 6).

An overview of the treatment responses and their occurrence is given in Tables 1 and 2, respectively. In 2/44 HFs normal hair growth was observed and 1/44 HF appeared to be not viable (referred to as “other” in Table 2).

Propidium iodide was used to assess follicle viability following treatment. Figure 7A,B shows a control and a treated HF, respectively, after 1 day in culture. In contrast to the control HF, the treated hair follicle shows an upward retracted hair fiber leaving the dermal papilla uncovered. A clear concentration of bright matrix/DP cells can be seen at the level of the white arrow in the treated hair follicle, meaning these cells has lost their viability. The location of these cells coincides with the area of high melanin concentration of normal anagen hair follicle anatomy. These cells probably have been subjected to a relatively high temperature increase during the IPL treatment.

**Histological Analysis of Biopsies**

**Skin tissue.** Immediately after treatment no signs of damage to the epidermis were observed, even in individuals having skin type IV. The biopsies taken 3 days after treatment did not reveal gross changes in morphology of the skin. One week after treatment, the epidermis also appeared normal with good differentiation and normal melanin distribution. The dermis contained undamaged normal fibroblasts, collagen and micro-vessels at all times.

**Hair follicles.** In one of the four biopsies taken 3 days after the treatment no HF was visible, the other three biopsies contained, respectively 1, 2, and 3 hair follicles. Analysis of these biopsies shows that the anagen HFs have been targeted in a very specific way. The upper bulb shows no signs of any damage; the ORS and IRS and fiber appear normal (Fig. 8A). Also no damage is observed in the sebaceous gland. However, lower down the HF, signs of a trauma induced catagen-like state are evident. These signs include separation of the fiber base from the dermal papilla, clumping of the cells in the matrix area and trapping of melanin/melanocyte remnants (Fig. 8B). These features are uncommon for natural catagen HFs, indicating that this catagen-like state is induced by the light exposure. Melanin clumps are trapped in the keratinized clump of cells. As is normal for a catagen HF, the bulb size appears to be reduced and the DP is rounded as compared to the anatomy of anagen hair follicles (Fig. 8C). In the matrix cells nuclear fragmentation is observed which is a sign of apoptosis. The connective tissue sheath shows signs of a thickening hyaline membrane and is highly cellular. In
another HF taken after 3 days, melanin clumping in a disrupted lower hair matrix is observed (Fig. 9).

From the \( n = 2 \) biopsies taken seven days post treatment the first biopsy contained three HFs, the second contained one hair follicle. Again, the upper follicles showed no apparent damage and the hair fibers looked normal. Catagen hair follicles were observed but it could not be determined whether these are treatment induced catagen transitions. Of all HFs from the biopsies taken 7 days after treatment only one hair follicle bulb was visible. This bulb again shows melanin clumping and trapping of melanin in the hair fiber (Fig. 10).

Due to the small size of the biopsies and the low density of leg hair, only two of the \( n = 4 \) biopsies taken directly after treatment contained HFs. The upper hair follicle regions looked normal and there was no damage to the hair shaft. Only one hair bulb was identified and it looked normal.

**DISCUSSION**

In this study, the mode of action of a single low-fluence photoepilation (LFP) treatment has been investigated using an in vitro model and compared with histological analysis of biopsies taken from human leg skin following identical treatment regimes. The in vitro model enabled a detailed analysis of HF morphology and hair growth dynamics during the first few days after exposure of the follicles to LFP. A limitation of this model is that only anagen HFs can be investigated. Telogen hairs cannot be obtained by the procedure used due to their strong anchorage in the upper dermis, in contrast to anagen hair follicle bulbs, which are loosely anchored in the subcutaneous fat. However, we assumed this to be a relatively unimportant limitation, as only anagen hairs are particularly sensitive to trauma from external insults, including photoepilation [32]. Another assumption we made was that the treatment response of anagen HFs from human scalp skin can be extrapolated to anagen hairs of other body locations. This is more than likely, as hair follicle structure and growth of anagen hairs are largely comparable for HFs over different body locations and experience with photoepilation has shown treatment response on various anatomical areas, despite differences in hair follicle geometry over these areas [5]. We used scalp skin because of its relatively good availability from cosmetic face procedures, and the proven suitability of HFs originating from this tissue for the culture procedure [31,33,34].

Models for hair follicle responses to photoepilation have been used by other groups but have certain drawbacks. For instance, Poureshagh [35] and Liew et al. [25] have investigated the acute impact of photoepilation treatment on hair follicles following illumination of ex vivo skin samples without maintaining them in vitro. This approach, however, does not allow determination of whether an actual catagen-transition would have taken place, as there was no in vitro follow up. A published abstract of Shander et al. [36] does report application of in vitro maintenance of HFs for investigating photoepilation. However, in this study HFs are exposed to light after isolation, such that optical properties of the skin and their effect on the light propagation are not incorporated in the model.

**TABLE 1. Morphology Descriptions of Treatment Effects as Observed in Hair Follicles of the In Vitro Model as a Result of the Low Fluence Photoepilation Treatment**

<table>
<thead>
<tr>
<th>Treatment effect</th>
<th>Morphology description days 1 and 2</th>
<th>Morphology description days 4 and 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Rounding of the base of the fiber. Melanin trapped. Upward retraction of the hair fiber away from the DP. DP rounded</td>
<td>Continued upward retraction of the hair fiber away from the DP</td>
</tr>
<tr>
<td>Type II</td>
<td>Rounding of the base of the fiber. Melanin trapped. Upward retraction of the hair fiber away from the DP. DP rounded</td>
<td>Within the epithelial column, a “keratinised” structure appeared attached to the base of the hair shaft. Continued upward retraction of the hair fiber away from the DP</td>
</tr>
<tr>
<td>Type III</td>
<td>Some melanin clumping but no obvious retraction of the shaft</td>
<td>The hair matrix remained connected to the DP with evidence of continued fiber formation. Might see melanin clump in the shaft</td>
</tr>
<tr>
<td>Type IV</td>
<td>Very little effect. Slight retraction of the melanin and appearance of isolated melanocytes around the DP</td>
<td>HFs continued to form pigmented hair. No evidence of melanin clumping</td>
</tr>
</tbody>
</table>

**TABLE 2. Occurrence of Treatment Effects in the Treated HFs and Normal Hair Growth in the Control Hair Follicles of the In Vitro Model**

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
<th>Normal</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 45 HFs</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>37 (82%)</td>
<td>8 (18%)</td>
</tr>
<tr>
<td>Treated 44 HFs</td>
<td>27 (61%)</td>
<td>6 (14%)</td>
<td>5 (11%)</td>
<td>3 (7%)</td>
<td></td>
<td>3 (7%)</td>
</tr>
</tbody>
</table>
To our knowledge this is the first report of a model in which in vitro maintenance of hair follicles (HFs) follows a light treatment of ex vivo whole skin. The ability to study hair growth dynamics during the first 5 days following exposure to LFP is of particular importance. This is because the actual course of the hair follicle changes induced by the exposure is crucial for this specific photoepilation treatment, rather than the extent of initial damage only.

Westgate et al. [30] demonstrated in detail that in vitro hair growth resembles hair growth in vivo very well. The results we observed in the control HFs of the in vitro model also showed normal maintenance of hair growth and hair follicle architecture. This indicates that the isolated HFs are viable and confirms that the conditions for normal hair growth are not perturbed when applying the sham LFP procedure. Furthermore, as the light exposure procedure closely resembled the in vivo situation, including the presence of whole skin during the light treatment, as well as correct initial tissue temperature, we expect the model to be an excellent means by which to explore the mechanisms of LFP.

The majority of HFs in both ex vivo and in vivo treatment regimes showed a similar treatment effect, this being a catagen-like response in the subsequent days. Some HFs from the in vitro model showed a less pronounced effect which may be due to variation in exposure, for example, as a result of the position of the HF in the skin sample (deep versus shallow). However, in this study we have not investigated exact causes of the different responses. The observed catagen-like response is in line with the proposed mechanism for the temporary effect seen in the so-called permanent photoepilation treatments [8,18,21].

The localized nature of the initial thermal damage that we observed is striking when compared to that which is...
generally reported for HF morphology after treatments aimed at permanent hair reduction, even those pertaining to fluencies in the 10 J/cm² range. We found that the fully keratinized pigmented hair shaft, the IRS and ORS were not damaged, as judged from morphology and histology. Within one to three days after the treatment, however, a melanized epithelial cell condensate was observed just above the dermal papilla and this feature was observed in both in vivo and ex vivo treated skin samples. The propidium iodide stained HFs confirmed that thermal cell damage and death is induced by the LFP treatment and that this is focused in the matrix area and region of the DP. The observation that thermal damage is highly localized can be explained by the significantly higher density of melanin in this area. Exposure of skin to IPL with our parameters (a fluence of 9 J/cm² in a pulse of 15 milliseconds) and subsequent generation of heat apparently suffices to interrupt the hair growth cycle, without causing damage to structures outside the matrix and DP area.

The clinical implications of our observations indicate that even a single LFP treatment is sufficient to cause temporary hair removal. Without significant hair follicle damage, the follicle is likely to recover to produce a new hair some weeks after treatment. The clinical benefits of LFP treatment have also been examined by our group and will be reported separately [23].

In conclusion, we have developed a unique model for studying the treatment effect of LFP on hair follicle behavior and our studies strongly suggest that LFP induces an immediate catagen-like transition in anagen hair follicles, with minimal damage to the majority of the hair follicle structures. This finding is further strengthened by the analysis of the clinical biopsies, where catagen-like hair follicles were found having similar characteristics. The absence of damage in structures of the hair follicle other than the matrix area confirms the notion that LFP targets the melanin rich bulb matrix area causing local damage sufficient to precipitate hair loss.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 10. Clumping and trapping of melanin is seen in section of a follicle taken 7 days after treatment.


