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Effect of intense pulsed light treatment on human skin *in vitro*: analysis of immediate effects on dermal papillae and hair follicle stem cells

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Summary

Background Hair follicles house a permanent pool of epithelial stem cells. Intense pulsed light (IPL) sources have been successfully used for hair removal, but long-term hair reduction may require several treatments. Many questions remain regarding the impact of IPL treatment on the structure of the hair follicle, more specifically on hair follicular stem cells and dermal papilla cells, a group of specialized cells that orchestrate hair growth.

Objectives To characterize the destruction of human hair follicles and surrounding tissues following IPL treatment, with more attention paid to the bulge and the bulb regions.

Methods Human scalp specimens of Fitzpatrick skin phototype II were exposed *ex vivo* to IPL pulses and were then processed for histological analysis, immunodetection of stem cell-associated keratin 19, and revelation of the endogenous alkaline phosphatase activity expressed in dermal papilla cells.

Results Histological analysis confirmed that pigmented structures, such as the melanin-rich matrix cells of the bulb in anagen follicles and the hair shaft, are principally targeted by IPL treatment, while white hairs and epidermis remained unaffected. Damage caused by heat sometimes extended over the dermal papilla cells, while stem cells were mostly spared.

Conclusions IPL epilation principally targets pigmented structures. Our results suggest that, under the tested conditions, collateral damage does not deplete stem cells. Damage at the dermal papilla was observed only with high-energy treatment modalities. Extrapolated to frequently treated hairs, these observations explain why some hairs grow back after a single IPL treatment.

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Conflicts of interest

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What's already known about this topic?

- Clinical observations have been made following intense pulsed light (IPL) treatment.
- Few studies analysing the overall histology of treated tissues exist, and no link has been made with stem cells or the effectiveness of the destruction of the dermal papilla.

What does this study add?

- This study characterizes the destruction of human hair follicles following IPL treatment, with more attention paid to epithelial stem cells and dermal papilla cells.
- Epithelial stem cells are preserved after IPL treatment, and these cells play important roles in epidermal renewal and during wound healing.

Laser and intense pulsed light (IPL) are classified as performing 'permanent hair reduction', with limitations on thin vellus, white and blond hair, and contraindication on dark skin. The IPL technique relies on the selective absorption of a brief radiation pulse to generate and confine heat at certain pigment targets.[1] The targets must have a greater optical absorption than their surrounding tissues at some wavelength, as is the case for dark hairs contrasting with pale skin. Each hair follicle undergoes repeated cycles consisting of an active growth period (anagen) followed by a regression phase (catagen) and a resting phase (telogen). The melanogenically active anagen stage of the hair cycle is the mostly likely to be affected by IPL because hair is intensively pigmented during this stage only.[2] Thus, a favourable treatment outcome depends on various factors, including proper patient selection, setting adjustments, the IPL system used, the hair cycle and operator experience. Often, repeated treatments are required to obtain satisfactory results.[3–7] Although the clinical efficacy of these treatments has been reported, their effects on surrounding tissues are not well known.

Hair follicle regeneration and cycling are dependent on mesenchymal–epithelial interactions between a population of fibroblast-like cells situated in the bulb of the hair follicle, known as dermal papilla cells, and surrounding epithelial cells, called matrix cells (for a review see Yang and Cotsarelis).[8] Dermal papilla cells orchestrate hair follicle development and growth.[8–12] They produce and secrete several growth factors and signalling molecules that instruct matrix cells to proliferate, move upward and differentiate into the internal layers of the hair follicle.[12,13] Thus, close association between the dermal and epidermal cells of the lower follicle bulb is essential to the production of hair fibre. Matrix cells derive from epithelial stem cells located in the bulge region of the hair follicle.[14–17] Bulge epithelial stem cells are also paramount for the renewal of the epidermis and its repair after wounding.[16] Unlike the hair bulb, which undergoes cyclic degeneration during the hair cycle, the bulge area marks the bottom of the permanent portion of the hair follicles. Bulge stem cells are thus preserved and can ensure the production of cells to regenerate new hair at the beginning of each hair cycle. Keratin 19 (K19) is a marker for bulge stem cells, and K19 labelling can be observed in hair follicles of all stages.[14,18]

Bulge epithelial stem cells and dermal papillae are two key elements responsible for hair regrowth. Therefore, the dermal papilla-specific population represents an attractive target for permanent hair removal strategies. The bulge epithelial stem cells should be preserved so as not to affect epidermal regeneration. The present study aimed to analyse the immediate histological changes following IPL treatments performed *ex vivo* on human scalp skin specimens in order to identify the main cell populations targeted by the treatment. The results obtained provide helpful clues to understand clinical outcomes better.

Materials and methods

The study was approved by the Comité d'Éthique de la Recherche du CHU de Québec for the protection of human subjects. All skin samples were obtained after informed consent was given.

Sampling and intense pulsed light systems

Experiments were conducted on adult scalp specimens, from skin phototype II of the Fitzpatrick scale,[19] removed during reductive facelift surgery from five subjects aged between 55 and 66 years, mean age 59 years. After phosphate-buffered saline washings, hair-bearing parts were shaved to prevent light obstruction and were cut into approximately 1 × 1·5-cm strips. A contact gel was applied to the skin strips and the IPL device handpiece was gently pressed against the skin. The cooling option (Peltier effect) available on the handpiece was not selected, to prevent temperature differences between samples. IPL treatment was applied to all samples except the control specimen, where the gel and device handpiece were applied, but no pulse was generated. Treatment of specimens was performed with devices of the Apilux series: Apilux 1G, Apilux 2G and Apilux Smart Pro (Dectro International, Québec, QC, Canada). The adjustable output fluence setting varies from 10 to 50 J cm^{-2}. All three devices share the technology of IPL, and the properties of the light sources (xenon) are identical to each other. The wavelength range is 420–1200 nm. To target pigmented hairs, the 620 nm cut-off filter was used. Settings commonly used on phototype II skin were selected. For each device, the different settings tested are detailed in Table 1. To avoid misinterpretation of results due to differences in device performance, for a given fluence parameter the output energy was measured using the FLASH Handheld IPL Probe Power Detector (Model FLASH-500-55-W-IPL; Gentec Electro Optics, Inc., Québec, QC, Canada). For each setting, a minimum of five readings were taken and the average calculated. The measured values were then divided by the surface area of the crystal to provide the J cm^{-2} value. The error in measurement was estimated to be 20%. After treatment, each strip was cut into 4–6 smaller pieces, which were processed for further histological analysis and labelling.

Histological analysis and endogenous phosphatase activity

For histological analysis, the biopsies were fixed with Histo-Choice MB fixative (Amresco, Solon, OH, U.S.A.) and embedded in paraffin. Sections 6-μm thick were stained with haematoxylin–phloxine–saffron dyes. The endogenous alkaline phosphatase activity was detected by the Handjiski method[20] followed by Harris's haematoxylin counterstaining.

Immunolabelling

Direct immunofluorescence staining was performed on 5-μm-thick acetone-fixed (10 min at −20 °C) frozen sections of the tissues as described previously.[14] The mouse monoclonal antihuman K19 clone A53-B/A2[21] (a generous gift from U.

Table 1 Rating of the effects observed for each tested parameter

Device model	Fluence setting (J cm^{-2})	Power output[a] (J cm^{-2})	N	T1 (ms)	D1 (ms)	T2 (ms)	n	Rating of effects observed[b]	Representative picture
Apilux 1G	0	0	0	0	0	0	4	None	
Apilux 1G	15	16	5	9	27	8	3	Low	
Apilux 1G	25	21	5	9	27	8	3	Low	
Apilux 1G	35	26	5	9	27	8	3	Moderate	Figure 5
Apilux 1G	35	25	5	7	27	7	3	Moderate	
Apilux 1G	35	25	3	9	27	8	3	Moderate	Figure 2
Apilux 1G	35	30	5	5	27	8	3	Moderate	Figure 5
Apilux 1G	35	29	5	15	27	8	3	Moderate	
Apilux 1G	35	26	5	9	27	4	3	Moderate	
Apilux 1G	35	28	5	9	27	12	3	Moderate	
Apilux 1G	35	24	5	9	12	8	3	Moderate	Figure 5
Apilux 1G	35	27	5	9	42	8	3	Moderate	
Apilux 1G	45	30	5	7	27	7	3	High	Figure 1
Apilux 2G	0	0	0	0	0	0	1	None	
Apilux 2G	20	9	3	9	27	8	1	Low	
Apilux 2G	35	21	3	9	27	8	1	Moderate	
Apilux 2G	35	22	3	9	12	8	1	Low	
Apilux 2G	35	26	3	9	42	8	1	Moderate	
Apilux 2G	35	22	3	7	27	9·9	1	Moderate	
Apilux 2G	45	31	3	9	27	8	1	High	
Apilux 2G	50	40	3	9	27	8	1	High	Figure 4
Apilux Smart Pro	0	0	0	0	0	0	1	None	
Apilux Smart Pro	14	9	6	4	15	5	1	Low	
Apilux Smart Pro	16	11	6	4	15	5	1	Low	
Apilux Smart Pro	18	12	6	4	15	5	1	Low	
Apilux Smart Pro	20	14	6	4	15	5	1	Low	
Apilux Smart Pro	22	14	6	4	15	5	1	Low	
Apilux Smart Pro	24	17	6	4	15	5	1	Low	
Apilux Smart Pro	26	18	6	4	15	5	1	Moderate	Figure 3
Apilux Smart Pro	28	20	6	4	15	5	1	Low	
Apilux Smart Pro	30	21	6	4	15	5	1	Moderate	Figure 3
Apilux Smart Pro	32	22	6	4	15	5	1	Moderate	

N, number of pulses; T1, first pulse duration; D1, delay between pulses; T2, duration of the second and later pulses; n, number of independent experiments. [a]Energy emitted measured by a power meter (FLASH Handheld Laser Probe). The estimated error in measurement is 20%. [b]Effects observed were regarded as none (0%), low (< 25%), moderate (25–49%) or high (≥ 50%) in terms of the extent of damage to hair follicles.

Karsten, Institute of Biological Sciences, University of Rostock, Germany) conjugated to Alexa Fluor 488 (Invitrogen, Carlsbad, CA, U.S.A.) was used. In some instances, Hoechst staining was performed to visualize nuclei.

Observations

The slides were examined under a Nikon Eclipse E600 microscope (Nikon, Tokyo, Japan) equipped with a SenSys® digital camera (Photometrics, Tucson, AZ, U.S.A.) for immunofluorescence pictures, and a Coolsnap™ digital camera (Photometrics) for colour pictures. Analysis was performed in a blinded fashion by three observers. Eight to 12 hair follicles were seen under the lower power objective in each slide. Effects observed in the hair follicle were regarded as none (0%), low (< 25%), moderate (25–49%) or high (≥ 50%) in terms of the extent of damage seen.

Results

To compare performance between the Apilux 1G, Apilux 2G and Apilux Smart Pro devices, the output energy was measured using a power meter. During testing of different configurations between devices, differences in measurement of emitted energy were noted (Table 1) but expected. The discrepancies can be explained as follows. The emitted energy from the xenon lamp is converted to thermal energy that is read by the sensing plate of the power meter. The fluence set value of IPL devices is the total energy emitted by the xenon lamp (400–1200 nm), while the energy measured by the power meter is that which has passed through the filter that limits the transmitted photons. In the case of our study, the HR (hair removal) filter was used, letting through the wavelengths from 620 to 1200 nm. As a result, the overall energy measured by the power meter was lower than the fluence set-

ting chosen, most notably with the Apilux 2G (Table 1), which presented an average of 11·9 J cm^{-2} lower, compared with 7·7 and 7·3 J cm^{-2} for the Apilux 1G and Apilux Smart Pro, respectively. The tested devices did not share the same size of handpiece. Different handpiece designs mean slight variation in the energy measured by the power meter, but, in spite of that, more energy seems to have been lost between the source and the output with the Apilux 2G device.

Highly pigmented cells are the principal target of intense pulsed light

To evaluate damage following IPL treatment, histological analysis was performed on treated scalp specimens. To maximize the photothermolysis effect, scalp skin was used, because > 85% of scalp hairs are in the anagen phase,[22] are thick and have, deep implantation and high density. When treating patients, it is suggested a train of pulses is applied. The duration of the emitted pulse and the delay between pulses depend on the thermal relaxation time (TRT) of the target, which is the time to diffuse about 50% of the acquired temperature to the surrounding tissues.[23] The skin TRT may vary from 3 to 10 ms, while the pigmented hair TRT may vary from 30 to 100 ms. So, the delay between pulses must be adjusted according to the skin phototype in order to infuse more heat to the pigmented hairs while protecting the skin and allowing a more comfortable treatment. In this study, trains of three to six pulses with 12–43 ms delay between pulses were tested. Microscopically, eight to 12 hair follicles were seen at the lower power objective in each slide. Obvious damage to the hair follicles was described as pigmentation in matrix cells or the hair shaft, or when the follicles differed from the sectioning- or processing-related artefacts present on control specimens, which appear as small holes or tears in the section.

The ratings of effects according to the different conditions tested are shown in Table 1. Even if the observations described are based on a limited number of complete hair follicles, similar effects were present for the same treatment conditions and absent from control skin sections. The most consistent finding observed was cellular damage to the pigmented hair shaft along its length, more pronounced in the upper part of the follicle (Figs 1b, 2a and 3b; arrows). Damage showed as distortion, coagulation necrosis and clumping of melanin in the pigmented hair shaft, which indicate that IPL photoepilation targets melanized hair structures. Cellular breaks were frequently observed in pigmented matrix cells, easily recognized by a brown coloration of the bulb region, and their severity increased with fluence (Fig. 1d vs. Fig. 2b, arrows, as an example). This cellular damage was not observed in matrix cells presenting pigmentation in the control sample (Fig. 1c, right hair follicle). In some cases, deformation of the bulb was also observed in addition to cellular damage (Fig. 3a,b). These results were expected, as these structures are highly pigmented. In contrast, no morphological change was observed in white hair, easily recognizable by the absence of pigmentation in matrix cells (Fig. 3b, left bulb), confirming the selective absorption of IPL-generated wavelengths by dark follicles.

The epidermis contains melanocytes and a significant amount of melanin pigment,[24] and thus represents a competing site for infrared absorption. We noted that the general appearance of the epidermis was normal (compare Fig. 1a with Fig. 1b) for all tested specimens, suggesting that the cooling procedures used (gel application and time between pulses) were appropriate to protect the epidermis. Furthermore, the sebaceous glands (Fig. 3), dermis and other structures surrounding hair follicles appeared to be unaffected by the treatments.

A large number of epithelial stem cells remain following intense pulsed light treatment

To evaluate whether destruction of epithelial stem cells occurred immediately following IPL treatments, we focused our attention on the bulge region of the hair follicles. In some cases, the cell layers of the inner root sheath were absent and damage extended down to the basal cell layer of the outer root sheath (Figs 1b and 2a, black arrowheads). This event was located mainly in the infundibulum region and generally did not extend to the bulge area (Figs 1b and 2a, white arrowheads). Again, the occurrence of this damage increased with fluence. To confirm stem cell localization, immunodetection of K19 was performed on skin specimens. As expected for hairy skin tissue,[14,15,18,25] K19-positive cells were detected in the basal layer of the outer root sheath, at the level of the hair follicle bulge in control samples (green staining in Fig. 4a,e; arrows). In all treated specimens, apparently intact K19-labelled cells were observed in several hair follicles (green staining in Fig. 4c,f; arrows), suggesting that a large number of epithelial stem cells were unaffected by the treatment.

Thermal damage in dermal papilla cells does not always occur following intense pulsed light treatment

The pioneering studies of Oliver and Jahoda *et al.*[26–28] showed the importance of dermal papilla cells for the orchestration of hair growth by performing the first demonstrations of their hair-inductive properties. Therefore, we hypothesized that permanent hair removal, close contact between the dermal papillae and the surrounding epithelial cells must be sufficiently destroyed to avoid mesenchymal–epithelial interactions leading to a new cycle of hair differentiation. However, dermal papilla cells are not highly pigmented, in contrast with matrix cells located around the dermal papilla (Fig. 1c), and are theoretically not directly targeted by the IPL wavelengths. We observed in histological slides that the thermal diffusion generated by IPL either did not affect (Fig. 1d) or partially destroyed dermal papilla cells, as shown by the presence of coagulation signs in treated samples (Figs 2b and 3) and the severity of these events increasing with fluence. However, the possibility that thermal diffusion will later lead to cell death

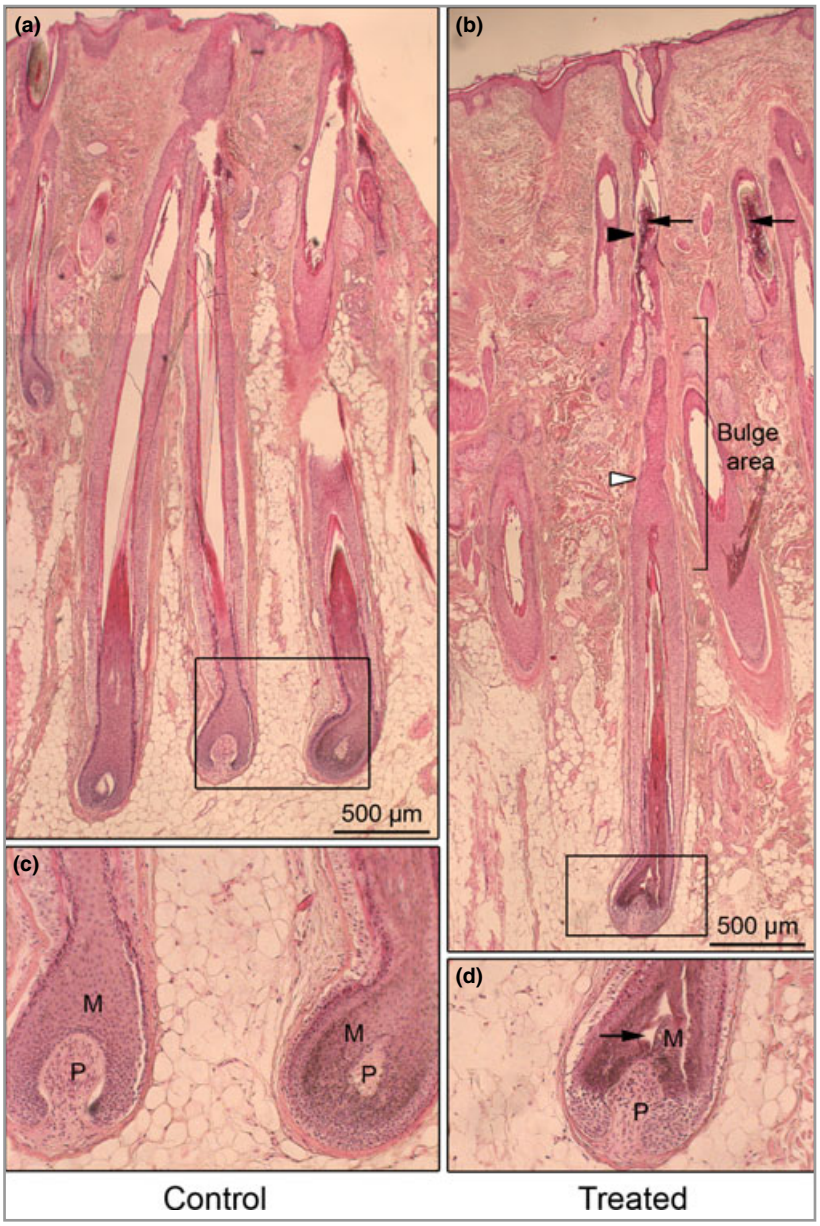

Fig 1. Histological pictures of a sample that was (a) not exposed or (b) exposed to 45 J cm^{-2} light delivered in 7-ms pulses using the Apilux device. The time interval between each pulse was 27 ms. (c, d) High magnification of the boxed areas in (a) and (b), respectively. Note cellular damage to the hair shaft, apparent in the upper third of the treated hair follicles (b, black arrows). In some cases, damage was observed down to the basal cell layer of the outer root sheath (b, black arrowhead) but did not extend to the bulge region (b, white arrowheads). M, matrix; P, dermal papilla.

by causing denaturation of essential proteins within dermal papilla cells cannot be ruled out.

To evaluate whether thermal diffusion following treatment caused protein denaturation, we next probed the activity of the alkaline phosphatase enzyme, which is highly expressed by dermal papilla fibroblasts.[20,29] Our results showed that in some cases, even if signs of destruction were observed in matrix or dermal papilla cells after IPL treatment, alkaline phosphatase activity was detected in the remaining dermal papilla cells (Fig. 5a–c, white arrows), indicating that the thermal diffusion was not great enough to denature the enzyme. It is likely that in some cases the dermal papilla was outside the zone of thermal damage induced by the IPL in the hair shaft and hair matrix. In other cases, alkaline phosphatase activity was not detected within dermal papilla cells (Fig. 5d, black arrows) that were exposed to five pulses of 35 J cm^{-2} with 12-ms intervals, compared with 27-ms intervals (Fig. 5a,c). Absence of alkaline phosphatase activity suggests that heat diffusion was sufficient to destroy dermal papilla cells.

Discussion

In this study, assessment of *ex vivo* IPL treatment was conducted on scalp specimens (phototype II skin on the Fitzpatrick scale) containing large terminal hairs, in order to determine which principal hair structures were targeted by the treatment. Even if hair follicle size, depth, angle and density, and the length of the hair cycle vary between scalp hairs and hairs of other body locations, follicle structure and growth are comparable.[22] So, we presume that the general understanding

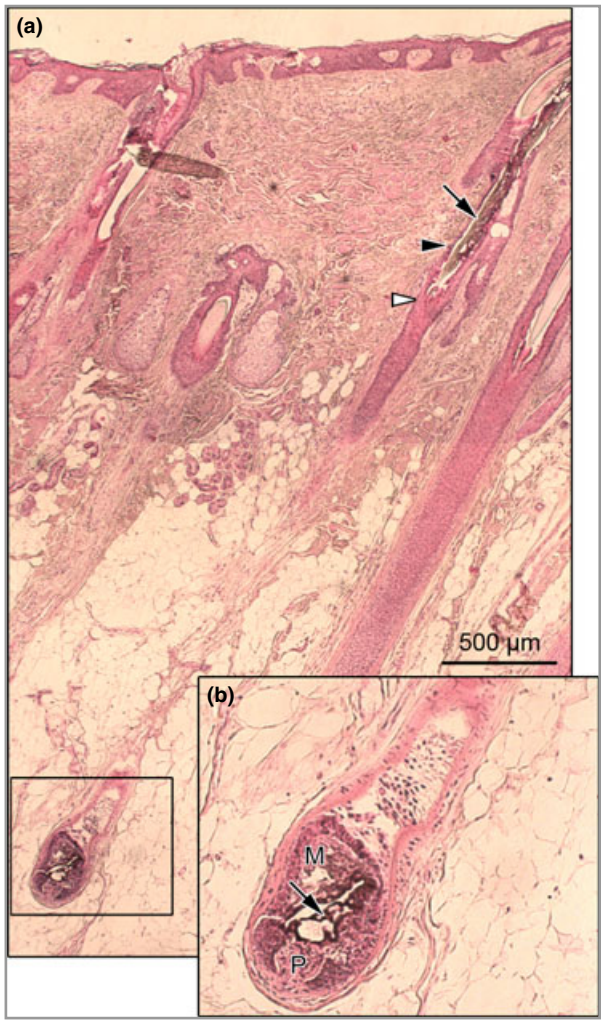

Fig 2. (a) Histological picture of a sample after exposure to 35 J cm^{-2} light delivered in three pulses using the Apilux device. The duration of the first pulse was 9 ms while the following pulses were 8 ms. The time interval between each pulse was 27 ms. (b) High magnification of the boxed area in (a). Note cellular damage to the hair shaft, apparent in the upper third of the treated hair follicles (a, black arrow) and at the level of the basal cell layer of the outer root sheath (a, black arrowhead). Damage does not extend to the full length of the bulge (a, white arrowhead). Note the presence of coagulation signs in matrix cells (b, black arrow) and the partial destruction of the dermal papilla cells. M, matrix; P, dermal papilla.

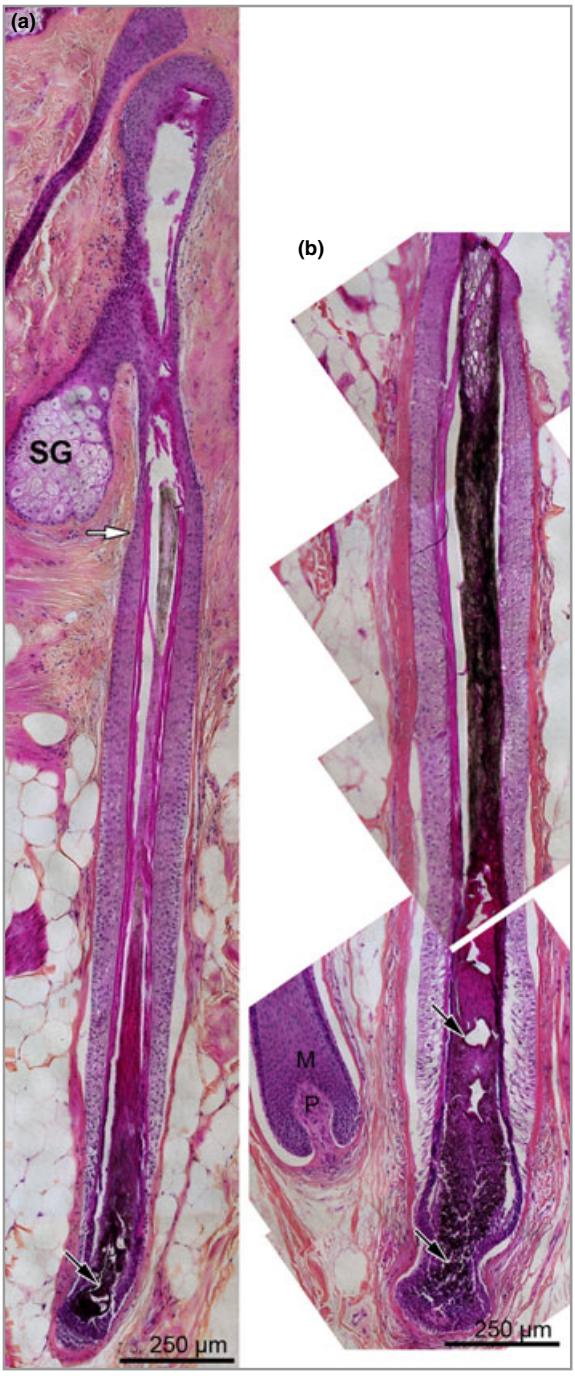

Fig 3. Histological pictures of a sample after exposure to (a) 26 J cm^{-2} or (b) 30 J cm^{-2} light delivered in six pulses using the Apilux Smart Pro device. The duration of the first pulse was 4 ms, while the following pulses were 5 ms. The time interval between each pulse was 15 ms. Note cellular damage of pigmented hair follicles (black arrows), while no morphological changes were present in white hair follicles, easily recognizable by the absence of pigment in the matrix cells (b, left bulb). No damage was observed at the level of the basal layer of the outer root sheath in the bulge region (a, white arrow), or in the sebaceous gland (a, SG). M, matrix; P, dermal papilla.

of the IPL effects on scalp hairs according to the different settings tested may be extrapolated to anagen hairs present on more frequently treated sites.

We worked with the hypothesis that permanent hair removal is obtained if epithelial stem cells of the bulge area or the dermal papilla-containing lower third of the hair follicle are damaged enough to prevent subsequent interaction between dermal papilla cells and the surrounding epithelial cells. Thus, we performed immunodetection of bulge epithelial stem cell-associated K19[14,18,25] and determination of alkaline phosphatase activity to assess protein denaturation in

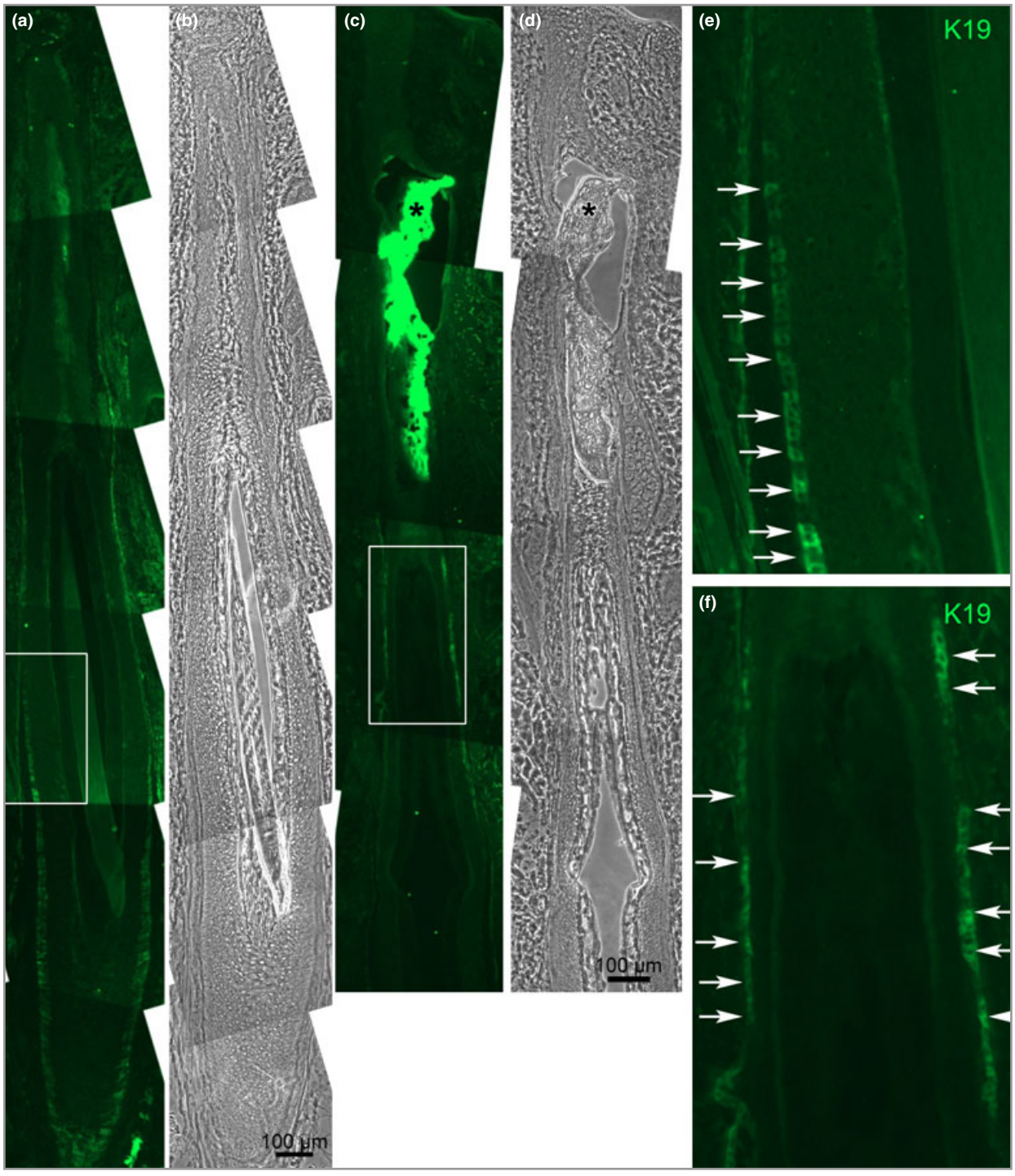

Fig 4. Epithelial cell staining in samples (a, b) not exposed and (c–f) exposed to 50 J cm^{-2} intense pulsed light (IPL) delivered in three pulses using the Apilux 2G device. The duration of the first pulse was 9 ms while the following pulses were 8 ms. The time interval between each pulse was 27 ms. (a, c, e, f) Immunofluorescence labelling of keratin 19 (K19). (b, d) Phase contrast micrographs corresponding to (a) and (c), respectively. (e, f) High magnification of the boxed areas in (a) and (c), respectively. Note that K19-positive cells were detected in the basal layer of the outer root sheath at the level of the hair follicle bulge in the control (e, arrows), as well as in IPL-treated samples (f, arrows). Note the presence of cellular damage in the upper third of the treated hair follicle (c, d, asterisks).

these specific locations. Lasers or IPL destroy hairs based on the principle of selective photothermolysis.[1] Because the major targets of permanent hair removal can be epithelial stem cells and the dermal papilla, we focused on both the hair bulge and the bulb. Histological observations confirmed that structures with greater optical absorption, such as the melanin-containing

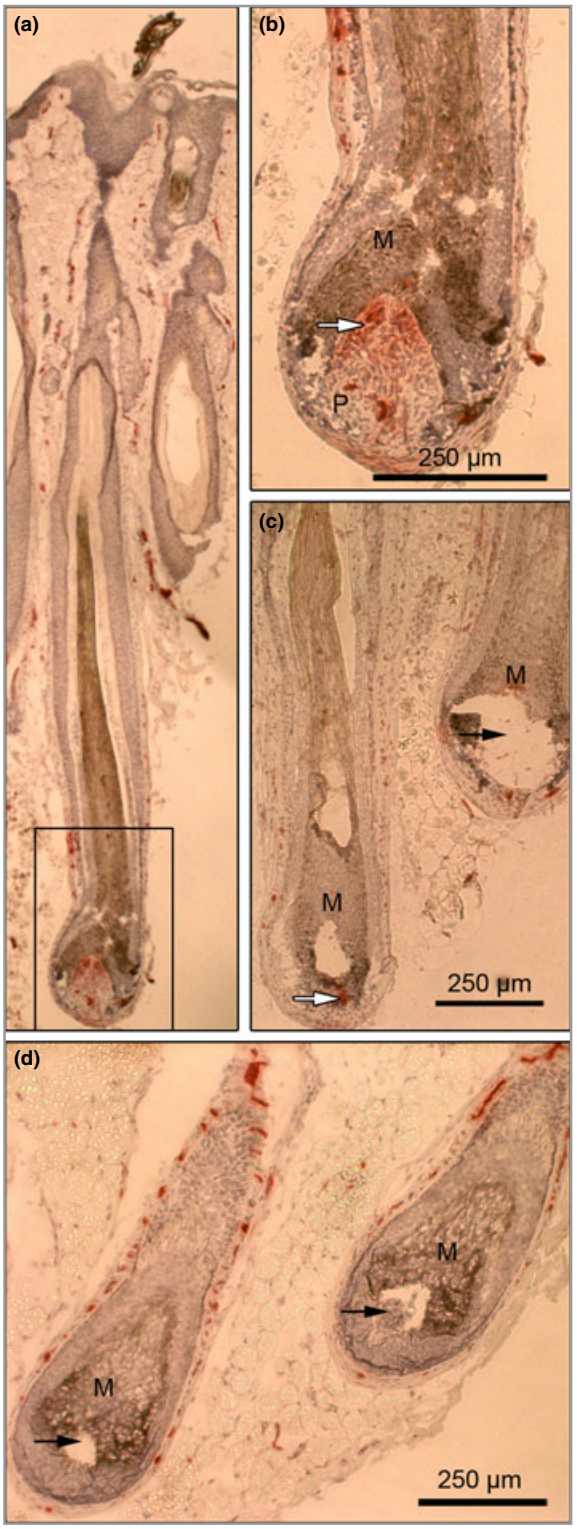

Fig 5. (a, c, d) Dermal papilla staining using alkaline phosphatase activity on samples after exposure to 35 J cm^{-2} light delivered in five pulses using the Apilux device. (b) High magnification of the boxed area in (a). For the skin sample seen in (a) and (c), the durations of the first pulses were 9 ms and 5 ms, respectively, while the following pulses were 8 ms and the time interval between each pulse was 27 ms. For the skin sample seen in (d), the duration of the first pulse was 9 ms, the following pulses were 8 ms and the time interval between each pulse was 12 ms. Note that alkaline phosphatase activity was detected in the remaining dermal papilla cells even if signs of destruction were observed in matrix cells (b, c; white arrows). Note that in other cases, alkaline phosphatase activity was not detected within dermal papilla cells (d, black arrows), suggesting that heat has denatured the enzyme. M, matrix; P, dermal papilla.

matrix cells surrounding the dermal papilla, and the pigmented hair shaft, are the principal targets of IPL treatment. However, cellular damage in inner and outer root sheath cells was also observed, suggesting that thermal diffusion extended outside pigmented areas. No significant effect was observed when the pulse duration and the delay between pulses were modified, probably because the specimens were treated *ex vivo* and the natural cooling mechanism of the skin does not come into action. No obvious coagulation signs were observed in treated epidermis, dermis and white hair follicles, suggesting that these structures do not absorb these wavelengths or are too far from thermal diffusion to be significantly affected.

Our results are in accordance with those of other groups who associated similar histological changes with the induction of a catagen-like state leading to temporary hair loss.[30,31] We noted damage in nonpigmented cells adjacent to targeted pigmented areas near to the bulge region. Although damage was observed mainly in pigment-bearing structures, immunofluorescence labelling revealed that many K19-expressing cells seemed unaffected by the treatments. Considering that the bulge is the lower permanent portion of the hair follicle during the hair cycle, the histological absence of obvious damage in the basal cells of the outer root sheath, combined with K19 immunoreactivity specifying the stem cell localization, suggests that stem cells were protected from IPL treatment under the tested conditions. However, the tissue was fixed after the treatment, thus we cannot draw conclusions regarding stem cell survival beyond the time of the treatment. Indeed, the possibility that K19-positive cells were lethally damaged but still expressed the K19 antigen following IPL treatment cannot be ruled out. To study whether K19-positive cells are preserved throughout the hair cycle, biopsies should be taken following short-term or long-term follow-up after IPL treatment. This was not possible in the present experiment on *ex vivo* skin specimens.

In many cases, even if damage was observed at the level of the dermal papilla, alkaline phosphatase remained active in the remaining cells. This observation, on the partial destruction of the dermal papilla, offers a relevant explanation to the clinical outcomes frequently obtained. Indeed, following photoepilation treatments, a progressive decrease in the number and size of hairs occurs (Dectro clinical experience, data not shown). The use of higher energy treatment modalities could, of course, destroy dermal papilla cells. In this case, excessive thermal damage with scar formation must be prevented.

It is known that there is a proportional relationship between the volume of the dermal papilla and the size of the hair produced.[29,32–34] It has also been reported that mesenchymal cells of

the peribulbar dermal sheath can regenerate the dermal papilla.[26,27,29,34–37] Dermal papillae provide essential stimuli for both follicle induction and hair growth. Thus, without a dermal papilla, or if the contact between the dermal papilla and surrounding epithelial cells is lost, a new round of hair cycling becomes impossible.[26–28,34,35,38] At the beginning of a new hair cycle, epithelial cells migrate from the bulge down to the bulb, where they become proliferating matrix cells, and under the influence of stimuli coming from the dermal papilla they differentiate into a new hair.[16,17] Therefore, it is logical to conclude that if the dermal papilla is not completely destroyed following the chosen IPL conditions, the follicle is likely to recover and new hair regrowth can occur some weeks after treatment. Therefore, based on the extended theory of selective photothermolysis,[39] the degree of melanization of the bulb matrix is of relative importance to the survival of the dermal papilla following IPL treatment. As the target, here the dermal papilla, exhibits weak photoabsorption potential, it has to be damaged by heat diffusion from a highly pigmented or strongly absorbing component.

In whole or in part, the cells of the basal layer of the outer root sheath in the bulge area remained unaffected under the IPL conditions tested. Hair pigmentation results from interactions between melanocytes and keratinocytes.[40] In hair follicles, melanocytes are dispersed among outer root sheath and matrix cells, but only bulbar melanocytes are melanogenic.[41–43] Moreover, hair is actively pigmented only during the anagen stage of the hair cycle, to which the melanogenic activity of follicular melanocytes is stringently coupled.[2] The end of the anagen stage is therefore the ideal phase at which to perform IPL treatment, as melanin formation is switched off in catagen and absent through telogen.[2,42]

In addition to epithelial stem cells,[15] the bulge area also houses melanocytic stem cells.[43–45] Our results suggest that melanocytic stem cells, which are amelanotic,[41,43] are protected from IPL radiation, which is absorbed mainly by the hair shaft and matrix cells rather than the bulge. Moreover, this observation helps to reduce concerns about cancer development caused by potential mutations in melanocyte stem cells following IPL treatment.

In addition to producing hair, bulge epithelial stem cells also give rise to epidermis.[16,46] The potential of bulge epithelial stem cells to generate the epidermis is particularly important in the case of severe trauma such as deep burns. Indeed, when skin damage is deep and extends over the bulge, regeneration of the epithelial barrier is possible only by the migration of intact epithelial cells from the remaining hair follicles or from around the wound. In the case of large wounds, the regeneration of the epidermis in a natural fashion is often compromised and the wound will not heal without skin grafting. The results reported here show that the integrity of epithelial stem cells in the anagen hair bulge appears to be preserved immediately after IPL treatment. Under the assumption that the treatment response of anagen scalp hair follicles can be extrapolated to anagen hairs of other body locations, we believe that this method is safe for the preservation of the healing potential of epithelial stem cells. Further studies should be conducted to support this affirmation.

In summary, even if our results show that a single IPL treatment causes local damage to the hair matrix that does not always extend to the dermal papilla, clinical data indicate that between 45% and 70% reduction of hair density may be reached, depending on the subject, following multiple IPL treatments.[3–7] For subjects with dark hair and pale skin, our results suggest that IPL epilation is a good and safe method to reduce the number and the size of hairs, without compromising bulge epithelial stem cell integrity. Thereafter, recalcitrant hairs can be removed using a complementary technique such as electrolysis.

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References

1 Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. *Science* 1983; **220**:524–7.
2 Slominski A, Paus R, Plonka P *et al.* Melanogenesis during the anagen-catagen-telogen transformation of the murine hair cycle. *J Invest Dermatol* 1994; **102**:862–9.
3 Fodor L, Carmi N, Fodor A *et al.* Intense pulsed light for skin rejuvenation, hair removal, and vascular lesions: a patient satisfaction study and review of the literature. *Ann Plast Surg* 2009; **62**:345–9.
4 Fodor L, Menachem M, Ramon Y *et al.* Hair removal using intense pulsed light (EpiLight): patient satisfaction, our experience, and literature review. *Ann Plast Surg* 2005; **54**:8–14.
5 Goh CL. Comparative study on a single treatment response to long pulse Nd:YAG lasers and intense pulse light therapy for hair removal on skin type IV to VI – is longer wavelengths lasers preferred over shorter wavelengths lights for assisted hair removal. *J Dermatolog Treat* 2003; **14**:243–7.
6 Toosi P, Sadighha A, Sharifian A, Razavi GM. A comparison study of the efficacy and side effects of different light sources in hair removal. *Lasers Med Sci* 2006; **21**:1–4.
7 Yaghmai D, Garden JM, Bakus AD *et al.* Hair removal using a combination radio-frequency and intense pulsed light source. *J Cosmet Laser Ther* 2004; **6**:201–7.
8 Yang CC, Cotsarelis G. Review of hair follicle dermal cells. *J Dermatol Sci* 2010; **57**:2–11.
9 Hardy MH. The secret life of the hair follicle. *Trends Genet* 1992; **8**:55–61.
10 Schneider MR, Schmidt-Ullrich R, Paus R. The hair follicle as a dynamic miniorgan. *Curr Biol* 2009; **19**:R132–42.
11 Ohyama M, Zheng Y, Paus R, Stenn KS. The mesenchymal component of hair follicle neogenesis: background, methods and molecular characterization. *Exp Dermatol* 2010; **19**:89–99.

12 Millar SE. Molecular mechanisms regulating hair follicle development. *J Invest Dermatol* 2002; **118**:216–25.
13 Schmidt-Ullrich R, Paus R. Molecular principles of hair follicle induction and morphogenesis. *BioEssays* 2005; **27**:247–61.
14 Michel M, Török N, Godbout MJ *et al.* Keratin 19 as a biochemical marker of skin stem cells *in vivo* and *in vitro*: keratin 19 expressing cells are differentially localized in function of anatomic sites, and their number varies with donor age and culture stage. *J Cell Sci* 1996; **109**(Pt 5):1017–28.
15 Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* 1990; **61**:1329–37.
16 Taylor G, Lehrer MS, Jensen PJ *et al.* Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell* 2000; **102**:451–61.
17 Oshima H, Rochat A, Kedzia C *et al.* Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* 2001; **104**:233–45.
18 Larouche D, Tong X, Fradette J *et al.* Vibrissa hair bulge houses two populations of skin epithelial stem cells distinct by their keratin profile. *FASEB J* 2008; **22**:1404–15.
19 Roberts WE. Skin type classification systems old and new. *Dermatol Clin* 2009; **27**:529–33, viii.
20 Handjiski BK, Eichmuller S, Hofmann U *et al.* Alkaline phosphatase activity and localization during the murine hair cycle. *Br J Dermatol* 1994; **131**:303–10.
21 Kasper M, Stosiek P, Typlt H, Karsten U. Histological evaluation of three new monoclonal anti-cytokeratin antibodies. 1. Normal tissues. *Eur J Cancer Clin Oncol* 1987; **23**:137–47.
22 Price ML, Griffiths WA. Normal body hair – a review. *Clin Exp Dermatol* 1985; **10**:87–97.
23 Yadav RK. Definitions in laser technology. *J Cutan Aesthet Surg* 2009; **2**:45–6.
24 Dupin E, Le Douarin NM. Development of melanocyte precursors from the vertebrate neural crest. *Oncogene* 2003; **22**:3016–23.
25 Commo S, Gaillard O, Bernard BA. The human hair follicle contains two distinct K19 positive compartments in the outer root sheath: a unifying hypothesis for stem cell reservoir? *Differentiation* 2000; **66**:157–64.
26 Oliver RF. The experimental induction of whisker growth in the hooded rat by implantation of dermal papillae. *J Embryol Exp Morphol* 1967; **18**:43–51.
27 Oliver RF. The induction of hair follicle formation in the adult hooded rat by vibrissa dermal papillae. *J Embryol Exp Morphol* 1970; **23**:219–36.
28 Jahoda CA, Horne KA, Oliver RF. Induction of hair growth by implantation of cultured dermal papilla cells. *Nature* 1984; **311**:560–2.
29 McElwee KJ, Kissling S, Wenzel E *et al.* Cultured peribulbar dermal sheath cells can induce hair follicle development and contribute to the dermal sheath and dermal papilla. *J Invest Dermatol* 2003; **121**:1267–75.
30 McCoy S, Evans A, James C. Histological study of hair follicles treated with a 3-msec pulsed ruby laser. *Lasers Surg Med* 1999; **24**:142–50.
31 Roosen GF, Westgate GE, Philpott M *et al.* Temporary hair removal by low fluence photoepilation: histological study on biopsies and cultured human hair follicles. *Lasers Surg Med* 2008; **40**:520–8.
32 Van Scott EJ, Ekel TM. Geometric relationships between the matrix of the hair bulb and its dermal papilla in normal and alopecic scalp. *J Invest Dermatol* 1958; **31**:281–7.
33 Van Scott EJ, Ekel TM, Auerbach R. Determinants of rate and kinetics of cell division in scalp hair. *J Invest Dermatol* 1963; **41**:269–73.
34 Horne KA, Jahoda CA. Restoration of hair growth by surgical implantation of follicular dermal sheath. *Development* 1992; **116**:563–71.
35 Oliver RF. Whisker growth after removal of the dermal papilla and lengths of follicle in the hooded rat. *J Embryol Exp Morphol* 1966; **15**:331–47.
36 Chi WY, Enshell-Seijffers D, Morgan BA. *De novo* production of dermal papilla cells during the anagen phase of the hair cycle. *J Invest Dermatol* 2010; **130**:2664–6.
37 Jahoda CA, Oliver RF, Reynolds AJ *et al.* Human hair follicle regeneration following amputation and grafting into the nude mouse. *J Invest Dermatol* 1996; **107**:804–7.
38 Cohen J. The transplantation of individual rat and guineapig whisker papillae. *J Embryol Exp Morphol* 1961; **9**:117–27.
39 Altshuler GB, Anderson RR, Manstein D *et al.* Extended theory of selective photothermolysis. *Lasers Surg Med* 2001; **29**:416–32.
40 Slominski A, Wortsman J, Plonka PM *et al.* Hair follicle pigmentation. *J Invest Dermatol* 2005; **124**:13–21.
41 Staricco RG. Amelanotic melanocytes in the outer sheath of the human hair follicle. *J Invest Dermatol* 1959; **33**:295–7.
42 Slominski A, Paus R, Costantino R. Differential expression and activity of melanogenesis-related proteins during induced hair growth in mice. *J Invest Dermatol* 1991; **96**:172–9.
43 Nishimura EK. Melanocyte stem cells: a melanocyte reservoir in hair follicles for hair and skin pigmentation. *Pigment Cell Melanoma Res* 2011; **24**:401–10.
44 Nishimura EK, Jordan SA, Oshima H *et al.* Dominant role of the niche in melanocyte stem-cell fate determination. *Nature* 2002; **416**:854–60.
45 Sotiropoulou PA, Candi A, Mascré G *et al.* Bcl-2 and accelerated DNA repair mediates resistance of hair follicle bulge stem cells to DNA-damage-induced cell death. *Nat Cell Biol* 2010; **12**:572–82.
46 Blanpain C, Lowry WE, Geoghegan A *et al.* Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* 2004; **118**:635–48.

