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Ruby laser-assisted hair removal: an ultrastructural evaluation of cutaneous damage

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SUMMARY. Ruby laser-assisted hair removal is thought to act via selective photothermolysis of melanin in the hair follicles. Although initial clinical trials of permanent hair removal using ruby lasers are promising, the exact mechanisms of hair destruction and the potential damage to other structures of skin are not known. The aim of this study was to evaluate the cutaneous ultrastructural changes following ruby laser hair removal.

Nineteen healthy Caucasian patients with dark (brown/black) hair were treated with the ruby laser and biopsies taken after 0, 2, 3, 5, 7, 14 and 21 days. Specimens were examined by light and electron microscopy.

Laser-treated specimens showed widespread coagulation and charring of subcutaneous hair shafts. These obviously damaged follicles were randomly dispersed amongst intact follicles within the same treatment sites. Microscopic changes were also seen in the basal epidermis where melanin was concentrated, irrespective of any obvious macroscopic damage. A low level of inflammatory response seen up to 2 weeks after treatment always followed laser treatment. Suprabasal epidermal necrosis was only seen in patients with blister formation after treatment.

Ruby laser irradiation results in selective damage to the hair follicles, with microscopic changes to the basal epidermis. The damage is probably compounded by the inflammatory response to the damaged hair. The normal appearance and distribution of collagen in the dermal layer supported the clinical evidence that laser-assisted hair removal, if performed correctly, does not lead to scar formation. © 1999 The British Association of Plastic Surgeons

Keywords: ruby laser, hair removal, hair follicle, ultrastructural changes, selective photothermolysis.

There is a clinical need for an efficient and reliable method of hair removal from the body. According to a survey on an unselected population of young Caucasian women, 9% were considered to be disfigured by their facial hair.[1] This problem is further compounded in patients with hormonal disturbances such as polycystic ovary disease and congenital adrenal hyperplasia. There is also a need to remove unwanted hair in patients with congenital hairy naevi and pilonidal sinuses and in some hair-bearing flaps used for reconstructive procedures.

There has been no ideal method of hair removal to date and the introduction of laser-assisted hair removal has generated much interest. Successful removal of ingrowing eyelashes has been achieved by precise aiming of argon laser at each hair follicle.[2–6] However, this method is non specific (causing considerable damage to the surrounding skin) and is both impractical and time consuming when attempting to remove hair from larger skin areas. Alternatively, rapid hair removal from large areas can be achieved using the process of selective photothermolysis.[7] This process uses ruby laser with a wavelength of 694 nm, which is preferentially absorbed by the melanin in hair and skin.[7] The ruby laser penetrates into the dermis where the only melanin-containing structures are the hair follicles, which are specifically targeted by preferential absorption of laser light.

The ruby laser in a long-pulsed mode has been used to remove unwanted body hair with encouraging results.[8–15] However, it has become clear from all the studies to date that one laser treatment to any specific treatment site does not usually lead to complete destruction of all hairs present. Variable regrowth rates have been reported which ranged from 40% to 80% with 12 weeks follow-up.[11,12] In addition to incomplete destruction of hairs, side effects of treatment, in the form of skin blistering, hyperpigmentation and hypopigmentation, have been observed in some patients, particularly those with darker skin.[8,12,13] These side effects, whilst producing no permanent damage, are distressing to the patient and prevent treatment at a higher fluence which might be more effective at destroying lightly pigmented hair.

Even though the induction of follicular injury is likely to be thermal in nature, the importance of other modes of follicular injury and the potential damage to other structures of skin are not known. It was the aim of this study to evaluate the ultrastructural changes in hair follicles and the surrounding tissues following ruby laser hair removal. By understanding the mechanisms of follicular injury, we may be able to develop

treatment regimes that reduce side effects without compromising the treatment results.

A prospective clinical study was performed to evaluate the cutaneous ultrastructural changes following ruby laser-assisted hair removal on patients who were most likely to benefit from the treatment, i.e. Caucasian individuals with dark coloured unwanted hair. Patients with dark coloured skin were excluded from the trial as they were judged to be more likely to sustain skin side effects after laser hair removal due to the absorption of laser energy by the greater amounts of melanin present in the epidermis. In addition, a highly pigmented epidermis may also reduce the penetration of radiant energy to the hair bulbs, and thus diminish efficacy of treatment.

Materials and methods

Nineteen healthy Caucasian volunteers were recruited for the study, all with fair white skin (Fitzpatrick skin type I to III) and unwanted dark hair (brown/black) on their body. All patients were treated with the Chromos 694 Depilation Ruby Laser (SLS/Biophile, Wales, UK), which has a wavelength of 694 nm, pulse width of 900 μs, repetition rate of 1 Hz, spot size of 7 mm, and a maximum fluence of 20 Jcm^{-2}. All patients were treated with a standard fluence of 11 Jcm^{-2}, which is considered to be the most efficacious in hair removal for Caucasian patients with dark coloured hair without causing excessive side effects (data not shown).

Punch biopsies were performed on patients (on arms or legs) at different intervals after laser-assisted hair removal, after ethical committee approval. Patients' skins were infiltrated with 1% lignocaine and adrenaline prior to biopsy. Two millimetre punch biopsies were performed on seven patients immediately after laser treatment and were examined by electron microscopy. Three millimetre punch biopsies were taken from the remaining 12 patients immediately and at 2, 3, 5, 7, 14 and 21 days after laser treatment. These specimens were examined by light microscopy. Two millimetre punch biopsies were performed on all patients before treatment as controls.

Electron microscopic examination

All specimens were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (Agar Scientific Ltd, Essex, UK) for 2 h. After two buffer washes, the secondary fixation with 1% osmium tetroxide in 0.1 M phosphate buffer was carried out for 1 h at room temperature. The specimens were then dehydrated through an increasing acetone series, prior to infiltration with 1:1 acetone:araldite CY212 resin overnight in the specimen rotator. After two changes of fresh resin for a minimum of 3 h each, samples were embedded in araldite CY212 resin and blocks were polymerised at 60°C for 18 h.

Ultra-thin (70–100 nm) sections were cut using a Diatome diamond knife on a Reichert-Jung Ultracut E ultramicrotome, floated onto distilled water, collected on formvar-coated copper grids and stained with 2% uranyl acetate and lead citrate for 10 min in each solution. The stained sections were viewed on a Joel 1200 CX electron microscope.

Light microscopic examination

All specimens were fixed immediately in formal saline and processed for routine wax impregnation histology. They were sectioned transversely in sequence at 4 μm until the deepest hair bulbs were reached and stained with haematoxylin and eosin (H&E). A systematic system was also used to assess the possible damage to each of the components of the skin by different staining methods:

1. Masson trichrome staining was used to demonstrate the morphology and distribution of the collagen in the specimens. The process of scarring is associated with the disruption and disorganisation of collagen.
2. Verhoeff's haematoxylin for elastin[16] staining was used to assess the morphology and distribution of elastic fibres.
3. Masson Fontana[16] staining was used to demonstrate both formed melanin and melanin precursors in the specimens, which are specifically targeted during laser irradiation.
4. Periodic acid-Schiff (PAS) reaction[16] was used to demonstrate the integrity of the basement membrane of the epidermis.
5. Modified Sacpic staining[17] technique was used to precisely identify the different components of a hair follicle and was especially useful in differentiating a growing from a resting hair follicle.

Results

Immediate gross changes

Fourteen patients had mild erythema of skin immediately after treatment, which resolved within a few hours. Three patients exhibited obvious erythema of skin immediately after treatment, which took 1–2 days to resolve completely. One patient developed serous crusting of the treatment site within 24 h of treatment. The skin healed in 4 weeks leaving a hypopigmented area, which resolved completely at 10 weeks. Another patient developed mild blistering of skin which healed completely without leaving residual scarring in 6 weeks.

Electron microscopic findings

Widespread intracellular vacuoles were seen in basal epidermal cells in all specimens examined (Fig. 1), with complete disruption of the cytoplasm in some cells. This change was not related to any gross macroscopic changes in the skin after laser treatment. Some of the most severely damaged basal epidermal cells were associated with minimal redness of skin macroscopically. Intercellular oedema was also seen resulting in separation of keratinocytes with collapsed desmosomes. This was seen in patients who had no blistering of skin after treatment.

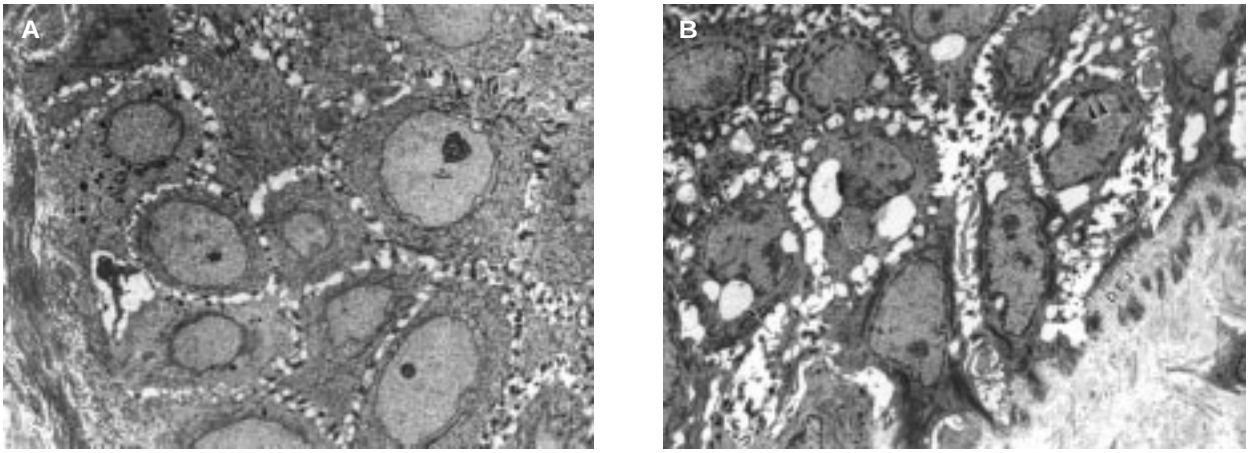

Figure 1—Electron micrographs of normal and laser-irradiated skin × 2500. (A) Control biopsy taken before laser irradiation, showing the normal ultrastructural appearance of sratum basale, stratum spinosum, dermo-epidermal junction and the dermis. The nuclei of the keratinocytes have a homogeneous appearance. (B) Laser-irradiated skin with large intercellular space due to oedema, intracellular vacuoles (arrowed) and margination of chromatin in the nucleus (double arrowheads). The dermo-epidermal junction (DEJ) and dermis are not damaged.

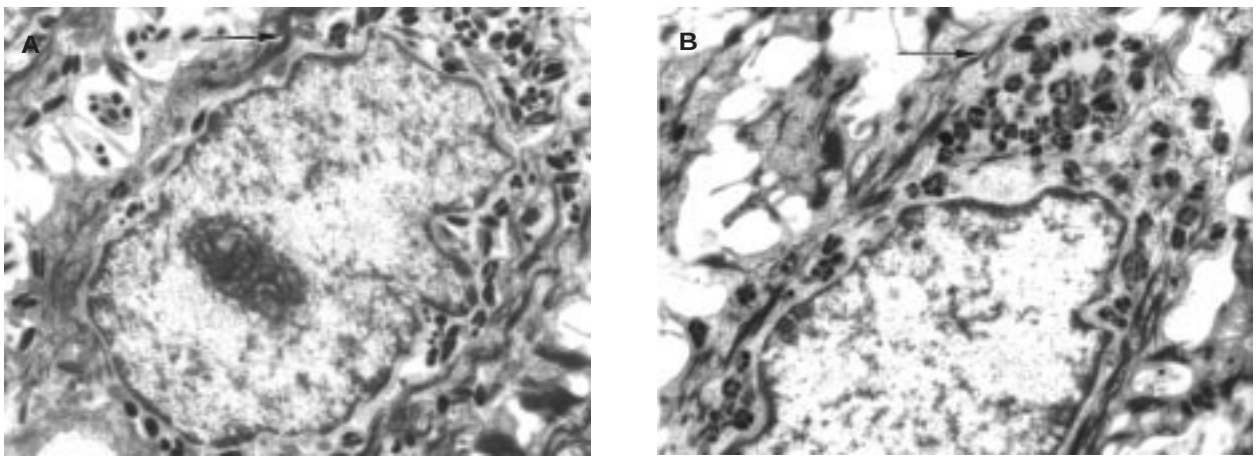

Figure 2—Electron micrographs of normal and laser-irradiated skin × 12 500. (A) Control biopsy taken before laser irradiation, showing the homogeneous appearance of the melanosomes (arrowed), which are distributed evenly around the nucleus. (B) Laser-irradiated skin showing melanosomes with a heterogeneous appearance (arrowed) as a result of disintegration and clumping. The melanosomes are also seen capping the nucleus as a response to radiation.

Melanosomes in laser irradiated skin had heterogeneous appearance. There was evidence of disintegration of the melanosomes into smaller fragments, whilst some melanosomes appeared completely empty. Clumping of melanin was also seen (Figs 2 and 3). This was in marked contrast with the normal melanosomes, which have a homogeneous appearance. In some instances, melanosomes were seen capping the nuclei, which was thought to be associated with a need to protect the nucleus against the harmful effects of irradiation.

Chromatin margination was seen in the nucleus (Fig. 1). Damaged mitochondria were occasionally seen, which were swollen and contained damaged cristae (Fig. 3). The inner and outer membranes, however, appeared intact. Golgi apparatus and endoplasmic reticulum were normal. Within the cytoplasm, clumping of tonofilaments was seen in some instances.

The dermo-epidermal junction was intact in all specimens examined despite the occasionally severe damage seen in the basal epidermal cells, with normal hemidesmosomes and anchoring filaments (Fig. 1). Follicular cells were not seen in all specimens examined, but in those specimens when follicular cells were seen, hair shafts and internal and external root sheaths appeared normal.

Collagen and fibroblasts in the dermis had normal distribution and appearance in all specimens examined.

Light microscopic findings

In normal transverse section of skin, the follicular unit consisted of 2–4 terminal follicles, with their sebaceous glands and arrector pilli muscles. An investing stroma and a perifolliculum surrounded each follicular unit. The perifolliculum consisted of small collagen fibres interspersed with elastic fibrils, and contained a vascular plexus and neural plexus.

In laser-treated specimens, the majority of hair follicles were obviously damaged, with charred material

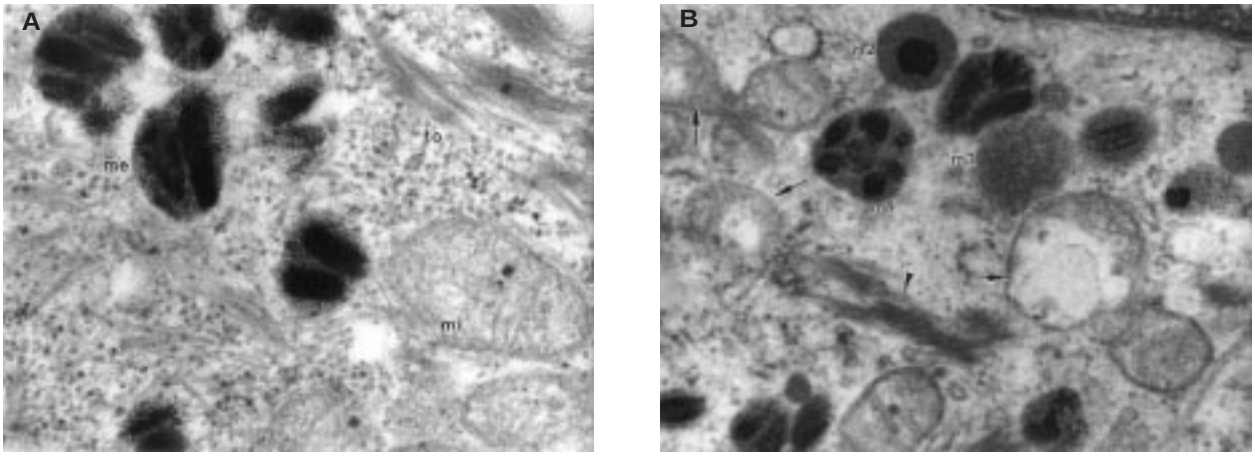

Figure 3—Electron micrographs of normal and laser-irradiated skin × 45 000. (A) Control biopsy taken before laser irradiation showing undamaged melanosomes (me), mitochondria (mi) and tonofilaments (to). (B) Laser-irradiated skin showing melanosomes with various stages of disintegration; fragmentation (m1), clumping (m2) and complete disappearance (m3). The surrounding mitochondria (arrowed) were swollen with the loss of cristae, but with intact inner and outer membrane. Clumping of tonofilaments was also seen (arrowhead).

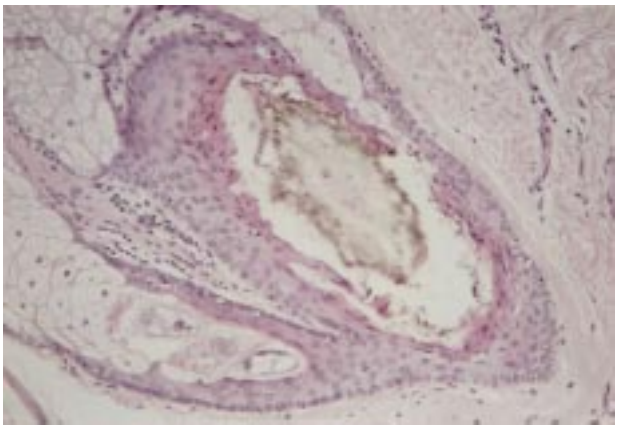

Figure 4—Transverse section of a damaged hair follicle after laser treatment. H&E × 200.

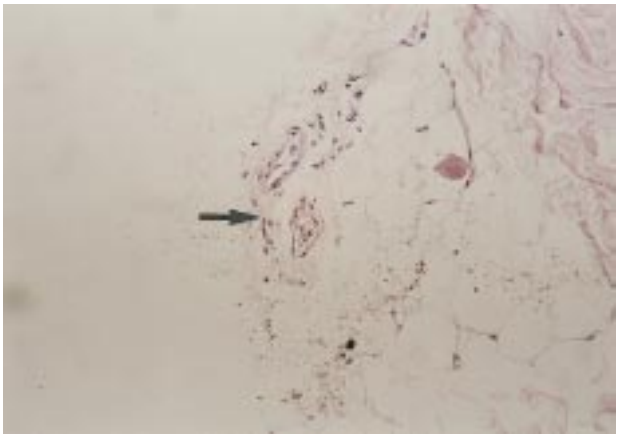

Figure 5—Necrotic hair bulb (arrowed) with collapsed outer root sheath and evidence of cell death. H&E × 100.

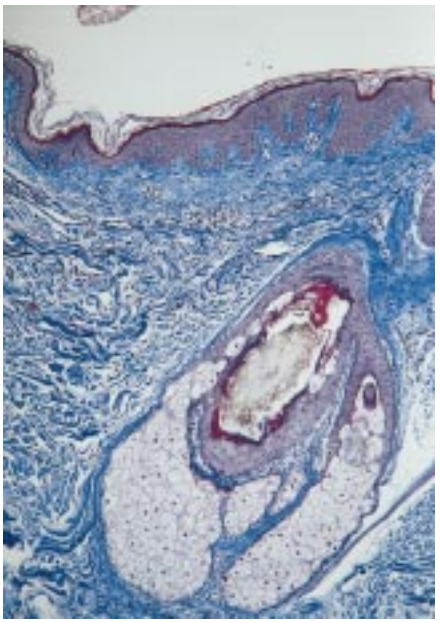

Figure 6—Longitudinal section of laser-treated skin. A thermal coagulated hair is seen, with surrounding normal collagen. Masson Trichrome × 100.

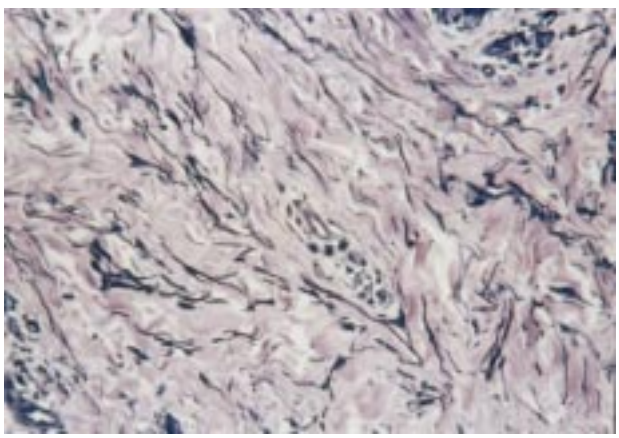

Figure 7—Normal appearance of elastin in laser-treated skin. Verhoeff's haematoxylin × 200.

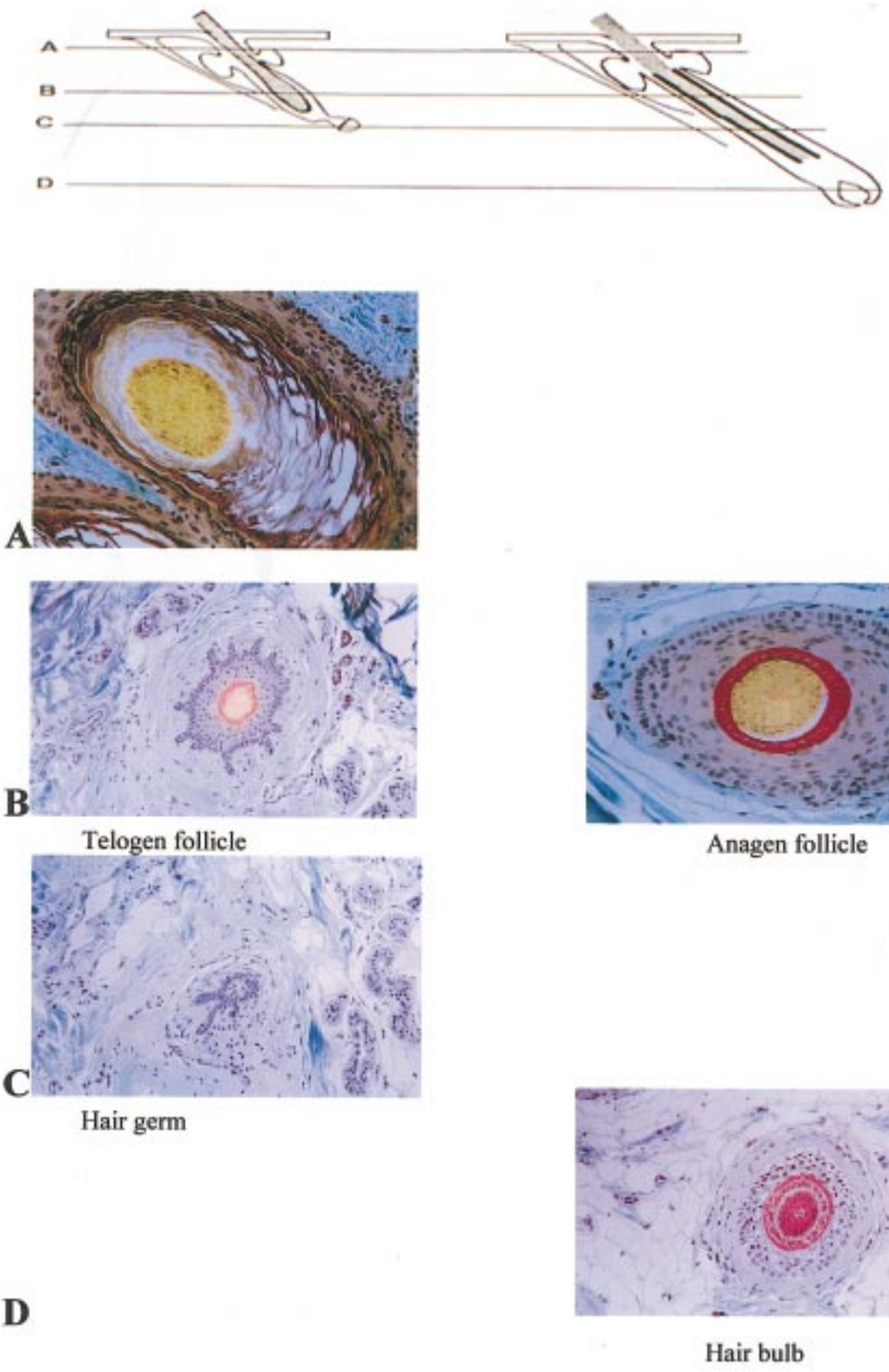

Figure 8—Hair growth cycle and modified Sacpic staining. A–D: transverse sections of hair follicles at various growth phases.

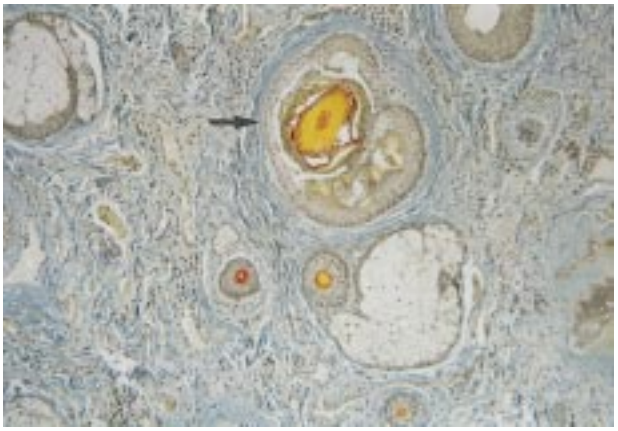

Figure 9—Transverse section of laser-treated skin at the level of the sebaceous glands. A thermally coagulated hair (arrowed) is surrounded by an anagen hair with bright red internal root sheath and a telogen hair with orange brush border. Modified Sacpic staining (keratinised structures stain yellow, internal root sheath bright red, outer border brush end orange, outer root sheath pale green, collagen blue, smooth muscle and erythrocytes green and nuclei blue/black) × 100.

around the periphery of the hair shafts and cracking and distortion of the shaft matrix (Fig. 4). In some instances, coagulated hair was seen surrounded by parakeratosis in a dilated and distorted ostium, with release of pigment or charred material to the keratin whorls surrounding the hair. There was also damage to the follicular epithelium (Fig. 4) seen up to 7 days after treatment, with increased eosinophilia and pyknotic nuclei. Occasional eosinophilic epidermal cells and colloid bodies representing apoptotic cells were identified. Some undamaged hair follicles were identified in the laser-treated area. Damaged hair follicles appeared in a random pattern throughout the measured areas.

The majority of hair follicles identified in the biopsy specimens up to 21 days were damaged by laser irradiation. Most of the damage extended down to the level of insertion of the arrector pilli muscle. However, in deeper sections, most of the hair bulbs seen appeared undamaged. Damaged bulbar follicular cells appeared shrunken with collapsed outer root sheath, absent nuclei and pale eosinophilic cytoplasm (Fig. 5).

Moderate lymphohistiocytic inflammation was present throughout the specimens at day 2 through to day 14. Specimens taken at day 21 did not show evidence of acute inflammation.

In a biopsy obtained from a patient with blister formation after laser treatment (2 days previously), there was suprabasal epidermal necrosis. Numerous melanin granules were seen in the eosinophilic necrotic epithelium, suggesting the involvement of melanosomes in the process of necrosis, and there was evidence of re-epithelialisation of the skin. Exaggerated lymphohistiocytic inflammation was seen in this instance. In cases with a less severe degree of skin damage, serous-cellular crusting was seen in the stratum corneum with normal epithelium.

In deeper sections, there was less florid inflammation. Fibroplasia was seen but there was no real evidence of increased collagen formation.

Masson trichrome staining revealed normal appearance and distribution of collagen throughout, despite the severe coagulation damage to nearby hair follicles (Fig. 6). Elastin in post laser-treated skin had normal appearance and distribution (Fig. 7).

Using the modified Sacpic staining method, nuclei are stained blue/black, keratin stained yellow, collagen stained blue, inner root sheath stained bright red, outer border of brush-ends stained orange, outer root sheath stained pale green and smooth muscle and erythrocytes stained green (Fig. 8). The Sacpic stain is well suited for visual assessment of follicle activity because it accentuates the inner root sheath, which accompanies a growing fibre. In contrast, with haematoxylin and eosin staining, some inner root sheaths appear eosinophilic while others appear basophilic.[17] Sacpic stained transverse sections are useful for estimating follicle activity because a suitable number of follicles can be observed simultaneously and the substructure of the hair follicles can be evaluated readily. The coagulation damage of hair follicles appeared to concentrate around the periphery of hair shaft keratin and the laser damage was not confined to anagen hairs (Fig. 9).

Periodic acid-Schiff (PAS) staining was used to demonstrate the integrity of the basement membrane (not shown). Consistent with the electron microscopic findings, no disruption of the basement membrane was seen (Fig. 1).

The Masson Fontana stain was used to demonstrate both formed melanin and melanin precursors. Melanin was distributed evenly in laser-treated specimens with no evidence of pigment found in the dermis.

Discussion

This study showed that the long-pulsed ruby laser caused selective damage to the hair follicles, which was likely to be mediated through the selective absorption of laser light by the melanin. It has been shown that ruby laser in a long-pulsed mode destroyed pigmented cells in vitro while leaving other non-pigmented cells intact.[18] Selective damage of melanosomes by the Q-switched ruby laser (40–50 ns) in both guinea pig and human skin has also been reported,[19–23] with minimal changes in the surrounding tissues.[19,20,24]

In addition to the damage to the melanosomes and hair follicles, sub-microscopic changes, including damage to the mitochondria, were also seen in the basal epidermal cells. Previous studies on photodamage to hepatocytes by visible light[25] suggested that the mitochondria damage might be due to the presence of photosensitisers such as flavin. Mitochondria play an important part in cellular metabolism and are therefore crucial for the survival of the cells. There was also evidence that tonofilaments and desmosomes, both of which provide structural support and cell–cell adhesions, were disrupted in some of the keratinocytes after laser irradiation.

Type 1 collagen constitutes the major type of collagen in the dermis and has a sharp melting transition for the fibrillar form between 60 and 70°C.[7] Its normal appearance and distribution in the dermal layer support

the clinical evidence that ruby laser-assisted hair removal, if performed correctly, does not lead to scar formation. The normal appearance and distribution of elastin in laser-treated skin also suggests that textural changes in skin are unlikely after ruby laser-assisted hair removal.

The precise mechanisms of melanosomal injury are not known. Photomechanical injury after absorption of laser energy has been postulated.[7,26] It is feasible that after selective absorption of laser light by the melanosomes, the large temperature difference between melanosomes and their surroundings produces localised rapid volume expansion, microvaporisation, or 'shock waves' in melanosomes causing structural damage. On the other hand, thermal denaturation leading to melanosomal damage is also a possible explanation. Histological study of lasered mouse skin showed evidence of both thermal coagulation and asymmetric focal ruptures of the follicular epithelium.[27] Secondary damage that is eventually seen in adjacent organelles could theoretically result from thermal diffusion or propagation of shock waves.

Although damage to the follicular cells is mainly thought to be thermal in nature, it is probably compounded by the presence of an inflammatory response, as seen in the histological sections.

The occasional capping of nuclei by melanosomes seen in this study is probably a shock response to laser irradiation. Similar findings have also been seen after freezing injury[28] and after split skin grafting.[29]

Suprabasal epidermal necrosis was seen in a patient with blistering of skin after laser irradiation. This change is characteristic of superficial burn.[30,31] The initial changes seen are thought to be due to direct effects of thermal injury and the subsequent inflammatory response. It has long been known that 2% of squamous cell carcinomata and 0.5% of basal cell carcinomata arise in burn scars, with most squamous cell carcinomata occurring after a long latent period, sometimes several decades. However, the frequency with which burn scars develop a neoplasm is not known. It is also not known how burns contribute towards carcinogenesis. It has been postulated that burning may act both as an initiator and a promoter of carcinogenesis. It is unlikely, however, that a burn of such a superficial nature as those seen after ruby laser irradiation will be carcinogenic, unless it is repeated many times.

Previous study has shown that there is a massive heterogeneity between patients where efficacy of laser-assisted hair removal is concerned.[9] This is consistent with the result of this study where apparently normal hairs are seen interspersed among coagulated hairs within the same biopsy of laser-treated skin. This differential susceptibility may be due to patient factors, which include skin and hair colour, hair thickness, growth phases and the anatomical sites of hair. Since all the hairs treated in this study are of the same colour to the naked eye, of approximately the same diameter and from the same anatomical sites, there must be some other intrinsic factors which make some hairs more susceptible to laser damage. We do not yet know what these properties are.

One intrinsic difference between the hairs within the same measurement site is their growth phase, since growth cycle in humans is mosaic. It has been shown that hairs in anagen phase may be more susceptible to laser injury due to the presence of melanogenesis.[27] The results of this study showed that obvious damage to the hair shaft is not restricted to anagen hairs. Whether this remains the case where damage is to the viable components of the hair follicle responsible for regrowth is unclear from our study.

Regeneration of a new hair follicle is feasible if the bulge region of the hair follicle, where the stem cells are believed to reside[32] is intact and if dermal papilla can be regenerated from the dermal sheath.[33–35] In this study, most of the laser damage extended to the follicular epithelium near to the insertion of the arrector pilli muscle, which is presumed to be the bulge region of the hair follicles. However, the majority of hair bulbs were not destroyed. The failure to destroy the hair bulb may explain the regrowth of hair after ruby laser irradiation.

In conclusion, it is obvious from this study that although the majority of damage occurs within certain hair follicles after ruby laser irradiation, subcellular damage also occurs within the overlying epidermis, even in the absence of clinically observed macroscopic changes. Furthermore, obvious damage to hair follicles appears to occur in a random fashion with damaged follicles interspersed between intact follicles. The reason behind the differences in susceptibility to laser treatment is unclear but remains an obvious goal of further study to enable us to manipulate either the patients' hairs or the treatment in order to achieve greater treatment efficacy.

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