- SHORT COMMUNICATION -

# The effect of diode laser and intense pulsed light on the histological structure of hair follicles in mice

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Skin exposure to laser or intense pulsed light results in differentiation on the surface and deep down depending on the parameters used. It has been proposed that lasers and intense pulsed light sources can cause follicular damage leading to the miniaturization or full destruction of the hair follicle. The aim of this study was to evaluate histologically the hair follicle damage in the skin of mice caused after exposure to diode laser and intense pulsed light. The results of this study show that the laser treatment, compared to intense pulsed light, is more effective in mice skin.

Key words: diode laser, intense pulsed light, mouse model, hair cycle stages.

## INTRODUCTION

The presence of unwanted hair has a negative effect on the quality of life. Photo-hair destruction with laser devices is one of the most efficient methods of long-term hair destruction currently available (Freedman & Earley, 2000). Upon treatment with a laser or intense pulsed light device, light is absorbed over millisecond pulse durations by melanin contained within melanosomes in the hair matrix and within keratinocytes in the hair shaft. Heat energy is transferred from the follicular matrix to the surrounding non-pigmented follicular epithelium and perifollicular dermis. Sufficient thermal injury to the follicle and its surrounding tissue results in miniaturization of follicles such that they become clinically unapparent for a variable duration of time (Willey *at al.*, 2007).

Several hair destructive photo-systems have been

shown to be effective (Haedersdal & Wulf, 2006; Leontaridou & Stalika, 2006). Ruby laser (694 nm) (Topping *et al.*, 2001), alexandrite laser (755 nm) (Lehrer *et al.*, 2003.), diode laser (800 to 810 nm) (Cameron *et al.*, 2005; Zins *et al.*, 2008), neodymium: yttrium-aluminium-garnet (Nd:YAG) laser (1064 nm) (Levy *et al.*, 2001) and intense pulsed light sources (590 to 1200 nm) (Schroeter *et al.*, 2004; Fodor *et al.*, 2005) are commonly used. The parameters used with each system vary considerably (Liew, 2002).

The methods for laser and intense pulsed light-assisted hair destruction are based on the principle of selective photothermolysis, with the melanin in the hair follicles serving as chromophore (Anderson & Parrish, 1983). Selective absorption of hair chromophores from lasers and broad band light sources results in destruction of hair follicles while leaving the skin undamaged (Lask *et al.*, 1999; Liew, 2002). In the present study, we compared the efficacy of this phenomenon using a diode laser and an intense pulsed light (IPL) device in a mouse model.

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# MATERIALS AND METHODS

## Subject animal

The black-haired mice (C57Bl) used in this study were obtained from the laboratory of Theagenio Hospital of Thessaloniki, Greece. The experiments were carried out on the Laboratory of Zoology, School of Biology, Aristotle University of Thessaloniki, Greece and all the approved experimental methods were followed.

Forty eight female, 6-8 weeks-old black-haired C57Bl mice, in the telogen phase of the hair growth cycle, were selected (Muller-Rover *et al.*, 2001).

### Test procedure

Photo-assisted hair destruction in mice is not successful for hairs in the telogen phase. Mature anagen was induced by plucking hair from the skin on the back (Müller-Röver *et al.*, 2001). Ten days post-epilation hair on the back of the mice was carefully shaved using an electric razor before exposing to the light sources.

The mice were divided into twelve groups, each of four subjects, and were anaesthetized; the first eight groups were exposed to the light of a diode laser (810 nm, spot size  $0.6 \times 0.6$  cm, pulse duration 50-60 msec) and the other four groups were treated with the light of an intense pulsed light source (600-900 nm, spot size  $1 \times 5$  cm, pulse duration 30-45 msec). When the mice were exposed to the light sources, only the hair on one side (48 flanks) was exposed to the laser and intense pulsed light fluences. The contra-lateral untreated side served as control. Before exposing to the light an appropriate low temperature was established by exposing the mouse skin to a cooling gel. Then the mice were sacrificed and skin samples were taken from both the treated and the control areas.

Four mice were irradiated by the diode laser at 24 joules  $cm^{-2}$  (one shot), four mice at 24 joules  $cm^{-2}$  (two shots), four mice at 28 joules  $cm^{-2}$  (one shot), four mice at 33 joules  $cm^{-2}$  (one shot), four mice at 33 joules  $cm^{-2}$  (two shots), four mice at 40 joules  $cm^{-2}$  (one shot), four mice at 45 joules  $cm^{-2}$  (one shot) and four mice at 45 joules  $cm^{-2}$  (two shots).

The corresponding irradiation of the intense pulsed light was: four mice at 12 joules  $cm^{-2}$  (one shot), four mice at 14 joules  $cm^{-2}$  (two shots), four mice at 14.75 joules  $cm^{-2}$  (one shot) and four mice at 16 joules  $cm^{-2}$  (one shot).

For the morphological examination the specimens of treated mice skin, as well as the mice control skin,

were fixed in formalin, embedded in paraffin, cut into sections and stained with haematoxylin and eosin stain. Specimens (skin and hair follicles) were then examined histologically for the site and extent of cellular damage using an Olympus BH-2 optical microscope. Photos were taken with an Olympus SP-565 digital camera.

#### RESULTS

Hair destruction in this animal model system was successful in growing-hair regions. The damage, after the application of laser or intense pulsed light extended in skin and underneath the skin surface. In the control, untreated, mice skin the hairs are extended normally from the skin surface, while the hair follicles are full of epithelial cells with intact maturation (Fig. 1).

Our current photo-hair destruction protocol included the use of a number of different fluences. In general, the damage, within the treated skin area, was increased accordingly the fluence used, for both treatments. Thus, for the first group of 32 mice that underwent laser-assisted hair destruction, at 24, 28 and 33 joules cm<sup>-2</sup> (one shot), the skin remained uninfluenced while the laser damage in hair shafts was complete but did not seem to extend far enough down to the hair bulbs. When 24 and 33 joules cm<sup>-2</sup> (two shots) was used, the laser damage was extended deep into



FIG. 1. Longitudinal sections of untreated mice hair follicles. The bulbs of the hair follicles appear full of epithelial cells with intact maturation.  $\times 40$ .



FIG. 2. Mouse skin irradiated at 45 joules cm<sup>-2</sup> (one shot). The damage caused by laser beam is severe in hair shafts and the hair bulbs (arrows) while the skin is slightly affected.  $\times 40$ .



FIG. 3. Mouse skin irradiated at 16 joules cm<sup>-2</sup> (one shot). The damage is severe in hair shafts but not in the hair bulbs (arrow) where many cells with morphologic features of hair follicle stem cells are observed. The skin appears unaffected.  $\times 40$ .

the hair bulbs and the skin seemed to be intact. At 40 and 45 joules  $cm^{-2}$  (one shot), the laser damage was severe in hair shafts and the hair bulbs while skin was slightly affected (Fig. 2). These findings suggest that laser should produce safe permanent hair destruction.



FIG. 4. The average number of the undamaged hair follicles was reduced in laser treated group, compared to normal and pulse light treated animals.

The skin of 16 mice of the second group was similarly irradiated by the intense pulsed light. At fluences below 16 joules  $cm^{-2}$  (12 and 14.75 joules  $cm^{-2}$ ), no significant alterations were observed in the skin, compared to untreated skin, while the hair bulbs seemed undamaged even after two shots at 14 joules cm<sup>-2</sup>. At 16 joules cm<sup>-2</sup> (the maximum intensity of light the device can emit) the damage was severe in hair shafts but not in the hair bulbs, where many progenitor cells (cells with morphologic features of hair follicle stem cells) remained undamaged and the skin appeared intact (Fig. 3). These findings suggest that intense pulsed light should produce limited damage in hair follicle. The average score of damaged hair follicles was greater in diode laser treated group than in intense pulsed light group (Fig. 4).

## DISCUSSION

Traditional hair follicle destructive techniques have included mechanical, chemical and electrical destruction. The theory of selective photo-thermolysis led to the development of a variety of different laser systems (Sadighha & Mohaghegh Zahed, 2009). Considering that laser and intense pulsed light devices efficacy is superior to conventional treatments, photohair destruction is commonly used. Despite the widespread use of lasers and intense pulsed light devices for hair reduction, the question concerning their efficacy still remains (Marayiannis *et al.*, 2003).

The aim of the present study was to estimate the extent of damage to the hair follicles of mice after a diode laser and an intense pulsed light treatment and to determine whether the laser or intense pulsed light source destroyed the bulbs of hair follicles. Even if irradiation of skin has now become an acceptable method of producing hair destruction, side effects, which include superficial burning and changes in skin pigmentation, still occur and, although temporary, can be distressing to the patient (Topping *et al.*, 2000; Liew, 2002). Methods to reduce the incidence of adverse effects include lightening of the skin and sun avoidance prior to laser treatment, cooling of the skin during treatment, and sun avoidance and protection after treatment. Successful preconditioning of mouse skin prior to laser or intense pulsed light exposure appears to reduce irradiation-induced skin side effects.

In the current study the appropriate low temperature allowed for the superficial skin cells to be protected and undamaged, leaving the target hair-producing cells unprotected. Then the mice were exposed to the different treatments. Most of the intense pulsed light damage involved the hair shafts but fell short of the hair bulbs. Changes were found to a greater depth (to the bulb) and greater extent (beyond the bulge) in those follicles treated with diode laser.

Previous reports suggest that although there is no obvious advantage of one laser system over another in terms of treatment outcome, laser parameters may be important when choosing the ideal laser for a patient. Regardless of the type of laser or intense pulsed light source used multiple treatments are necessary to achieve satisfactory results (Schroeter et al., 2003; Leontaridou & Stalika, 2006). Several clinical studies on the efficacy of photo-assisted hair destruction have reported that re-growth of hair after treatment is common. One of the reasons for the re-growth of hair is the incomplete destruction of progenitor hair cells, which may be due to the insufficient penetration of irradiation in the skin. Multiple treatments are also necessary due to the nature of the hair growth cycle (Leontaridou, 2006). It is generally believed that hair follicles are more responsive to treatment while they are in the growing (anagen) phase (Speroff et al., 1994), while it is not successful for hairs in the telogen phase (Liew, 2002). Growth of mouse hair can be artificially induced by hair plucking, allergic contact dermatitis and skin irradiation (Müller-Röver et al., 2001; Li et al., 2003). We applied photo-assisted hair destruction in mice, in mature anagen, that was induced by plucking hair from the skin on the back and examined the specimens of treated mice skin after they were once irradiated. Efficacy is improved when repeated treatments are given, suggesting that more

treatments are required if hair-free appearance is to be achieved. In laser-assisted hair destruction clearance, after repeated treatments, from 40 to 60% is generally reported 6 months after the last treatment.

Several investigators have attempted to determine the efficacy of laser and intense pulsed light treatment for unwanted hair (Cameron *et al.*, 2005). Based on the present best available evidence it was concluded that photo-hair destruction with laser and light source induces a partial short-term hair reduction (Cameron *et al.*, 2005). Evidence exists for a partial long-term hair removal efficacy after repetitive treatments with lasers whereas evidence is lacking for longterm hair removal after intense pulsed light treatment (Haedersdal & Wulf, 2006).

The results of the present study in mice skin indicate that the best available results for hair destruction was found for the diode laser, whereas limited evidence was available for the intense pulsed light source. We suggest that both laser and IPL could reduce the hair even though the intense pulsed light-assisted damage did not seem to extend far enough down to the hair shafts to result in permanent hair destruction. In any case, patients are recommended to be pre-operatively informed of the expected treatment outcome.

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