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Journal of Cosmetic and Laser Therapy

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ijcl20

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JOURNAL OF COSMETIC AND LASER THERAPY
https://doi.org/10.1080/14764172.2023.2284640

Effects at cellular level of epilation with various laser wavelengths applied simultaneously on different skins: two case reports

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ABSTRACT

This study evaluated the effects of laser application of diverse wavelengths applied simultaneously and on different skins. The sample included two participants, a woman with light skin with abdominal hair and a woman with dark skin and hair on the inner part of the lower limbs, who received a laser therapy session. After 45 days from laser application, abdominoplasty and thigh dermolipectomy surgery were performed. In the control sample, the hair follicles were in the anagen phase, showing the presence of Bcl-2 expression. In the treated areas, follicles were observed in an advanced phase (telogen), with the presence of CK-18 and negativity of Bcl-2, highlighting the phase of hair loss at that moment and the complete apoptosis of the investigated follicle. Significant difference was observed in the comparison of the anagen phase ($p = .00$) and it similarly occurred in the comparison of the telogen phase ($p = .00$). The presence of a greater amount of follicles in the anagen phase in the control area and follicles in the telogen phase in the treated area demonstrates the efficiency of the laser at different wavelengths when reaching different skin phototypes and hair thickness, being reinforced by apoptosis and cell proliferation markers. Therefore, the hair-removal process has been optimized with various laser wavelengths.

ARTICLE HISTORY
Received 21 December 2022
Revised 17 August 2023
Accepted 13 November 2023

KEYWORDS
Laser and light sources; surgery; hair removal

Introduction

Laser (Light Amplification by the Stimulated Emission of Radiation) is one of the many epilation techniques. Its application will depend on the chosen objective. The intensity and duration of the pulse produce colored light beams, which vary from ultraviolet to infrared light. To achieve the proposed epilation goal, the laser has an action mechanism that uses longer wavelengths (range 600–1100 nm), which penetrate more deeply into the skin. Thus, its absorption by the melanin can be used for selective photothermolysis, promoting a thermal injury that generates the weakening or destruction of the hair follicle (1,2).

For epilation, some types of laser are considered more effective, them being the ruby laser (695 nm), alexandrite (755 nm), diode (800 nm), and Nd: YAG (1,064 nm). The treatment time is related to the natural hair growth cycle in different phases, namely anagen (growth), catagen (stability), and telogen (hair loss). The action of the laser is more efficient in the anagen phase because at that moment, the hair is susceptible to the aggressions caused by the laser. This is related to the fact that melanin is found in the hair bulb only during this phase. For this reason, regular sessions are required for a certain period. The average hair reduction rate is between 70 and 90% for at least six months of treatment (3–5).

For selective photothermolysis of the hair follicle to occur, radiation must penetrate at least 3 mm into the skin. For phototypes elevated IV to VI, laser diodes 800 nm and Nd: YAG 1,064 nm are recommended, whereas for skins phototypes I, II, and III the alexandrite laser is the most effective, followed by diode laser, and lastly, Nd: YAG. It is also emphasized that white hair does not respond to laser when compared to darker and thicker hair (5,6).

The literature points the diode laser as one of the most suitable for the epilation technique. Its wavelength ranges from 800–810 nm, being a parameter with good absorption by melanin and reliable for not causing epidermal injuries. Its application leads to follicle overheating, distributing heat to the surrounding structures, such as the vascular structures that nourish the follicle and places close to the erector hair muscle, making the procedure even more effective (7,8).

Davoudi et al (9). compared the effectiveness and safety of Nd: YAG and alexandrite lasers, individually and in combination, in reducing leg hair in the long term. After treating 15

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volunteers of skin phototypes III and IV with four laser sessions, it was noticed that after 18 months, the average hair reduction was 75.9% with the alexandrite laser (12 mm), 84.3% with the alexandrite laser (18 mm), 73.6% for the Nd: YAG laser, and 77.8% for the combined therapy. However, the statistical analysis did not show significant differences ($P > .05$). The authors highlight adverse effects (hyperpigmentation) and pain intensity, especially in the areas that received the combined therapy ($P = .001$).

Variations in skin type, hair characteristics, and the choice of wavelength can lead to different results. Vast literature has been found on the effects of laser therapy for epilation using wavelengths in isolation. Although there are studies that have analyzed these in a combined manner, their actual effects on skin types and distinct hair characteristics and the possible changes at the histological level are still unclear. Thus, the purpose of this study was to cover the effects of laser therapy of different wavelengths applied simultaneously and on different skins, showing their action at cellular level. Based on the hypothesis that the use of laser therapy at different wavelengths and skin types presents changes at the cellular level.

Methodology

Research characterization and sampling

A case study was carried out with two volunteers under the approval of the Ethics Committee (code: 4.486.400). All volunteers signed an informed consent form before the start of the study, and the treatment was carried out from October to November 2021.

Two volunteers of different skin phototypes and different types of hair were selected. For the inclusion criteria, individuals should have to be between 30 and 50 years of age, willing and able to undergo abdominoplasty surgery or thigh dermolipectomy, with the ability to understand and preserve local sensitivity.

The exclusion criteria were applied to all participants who presented changes in sensitivity of the area to be treated, contraindication to the use of the device: tanned skin, having been exposed to sun or UV light one week before the treatment or less; photosensitivity to Jod's disease or to the drugs that produce it; treatment with cis-retinoic acid; neoplasia in the region; Hx of allergy or hives; tattoos or permanent makeup, pregnant women, diabetes, and coagulopathies.

Procedures

Data collection instrument

The identification and anamnesis data were collected using an adapted version of the Facial Evaluation Protocol – PAF (10), then the skin phototype of each volunteer was measured by the SkinUp evaluator (HS MED™, São Paulo, Brazil). The laser was applied with Laser Crystal 3D™ (BodyHealth™, Minas Gerais, Brazil), which features three wavelengths that are applied simultaneously: 755 nm (alexandrite), 810 nm (diode), and 1064 nm (Nd:Yag).

The safety and efficacy of using the combination of three different wavelengths in a single device was previously verified by the study by García et al (11). Where a wide range of absorption and penetration has been observed, with promising results on the most resistant hair type. The chosen parameters were 7 J/cm2 and 26 KJ or 8–9 J/cm2 and 28–30 KJ for skin type IV and skin type II/III, respectively. Another study (12) also saw a significant reduction in hair count between baseline and the 3-month follow-up visit for all skin types using treatment parameters 7–8 J/cm2 and 13–14 kJ of total energy for skin types I – III and 4–6 J/cm2 and 12–14 kJ of total energy for skin types IV – VI. The results of these two studies were used in the choice of parameters.

Forty-five days after the laser application, the volunteers underwent surgery for the removal of skin flaps (five flaps were analyzed for each region). They were prepared in blocks and histological and immunohistochemical analyses of the hair follicles were carried out using HE staining, in order to capture the general hair follicle morphology, quantification of cells in cell proliferation (Bcl-2), and the detection of apoptosis-suggestive cells (Cytokeratin 18 — CK18).

Case presentation

Volunteer 1, female, 32 years old, with thin hair in the linea alba and the pubic crest areas. In the phototype evaluation, type 2 was found, indicating that it is white skin, sensitive to the sun. The left infraumbilical region was chosen to receive the treatment, while the right side was maintained as a control (Figure 1). The parameters chosen for the application were: energy of 10.8 joules, frequency of 5 Hz, using the scanning technique. Forty-five days after the laser session, the abdominoplasty procedure was performed, and five flaps were removed from each region.

Volunteer 2, female, 49 years old, with thick hair in the groin and inner thighs. In the phototype evaluation, type 4 was found, indicating moderate brown skin with normal sensitivity to the sun. The inner region of the right lower limb (contour of the groin and inner thigh) received the laser application, and the contralateral side was maintained as a control (Figure 2). The parameters chosen for the application were: Energy of 9.4 joules, frequency of 5 Hz,

(a) (b)

Figure 1. Abdominal region of volunteer 01: (A) control region; (B) treated region.

Figure 2. Region of the lower limbs of volunteer 02: (a) treated region; (b) control region.

using the scanning technique. After the procedure, a dermolipectomy of the thighs was performed, with the removal of the flaps of the limbs.

Data analysis

Histological data were analyzed by GraphPad Prism (Version 8.0, GraphPad Software, San Diego, California, USA). The Mann-Whitney U test was used to assess the statistical significance of the results (p values <0.05 were considered statistically significant), only on parametric data. In addition, qualitative data were described based on the pathologist's reports (descriptive analysis of histological images and quantification of cells).

Results

Figure 3 shows some aspects of the control abdominal region sample. The histological analysis (3A and 3B) showed that the hair follicles were in the growth phase; that is, they were in the anagen phase, showing that there was normal follicle development. In addition, Figure 3c-d revealed the presence of Bcl-2 expression in a normal hair follicle, as expected for the phase.

Figure 4a shows the presence of a follicle at advanced telogen phase, highlighting the phase of hair loss at that time and the complete apoptosis of the investigated follicle, which can be ratified by the absence of Bcl-2 (Figure 4c). The presence of CK-18 (Figure 4b) was also identified, supporting the existence of an advanced apoptosis process.

When investigating the characteristics of the hair, it was observed that volunteer 01 presented a large amount of growing hair on the control side. About 72.8% of the follicles were in the anagen phase, whereas only 13.3% were in the telogen phase. Regarding the treated side, 20.2% of the hair was found in the anagen phase and 35.6% in the telogen phase. When performing the statistical analysis, the comparison of the anagen phase (control versus treated) showed significant difference ($p = .00$) and the same occurred in the comparison of the telogen phase ($p = .00$).

In the second skin sample collected (volunteer 02), results similar to the ones found in the abdominal region were

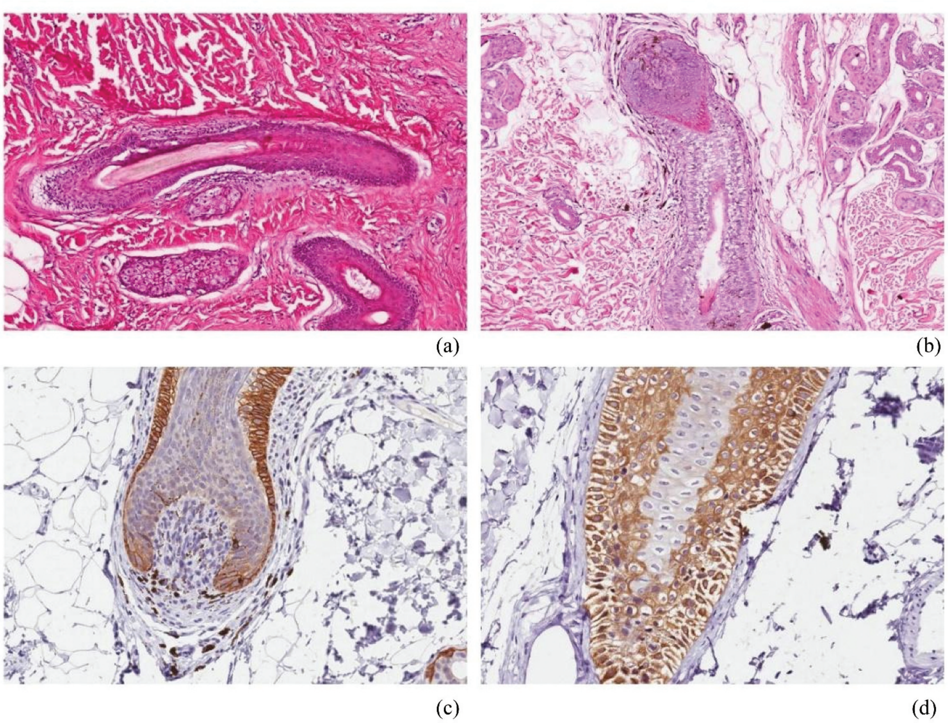

Figure 3. Histological analysis of abdominal tissue in the control area: (A and B) hair follicles of the anagen phase, showing normal follicle development; (C and D) expression of Bcl-2 in normal hair follicle.

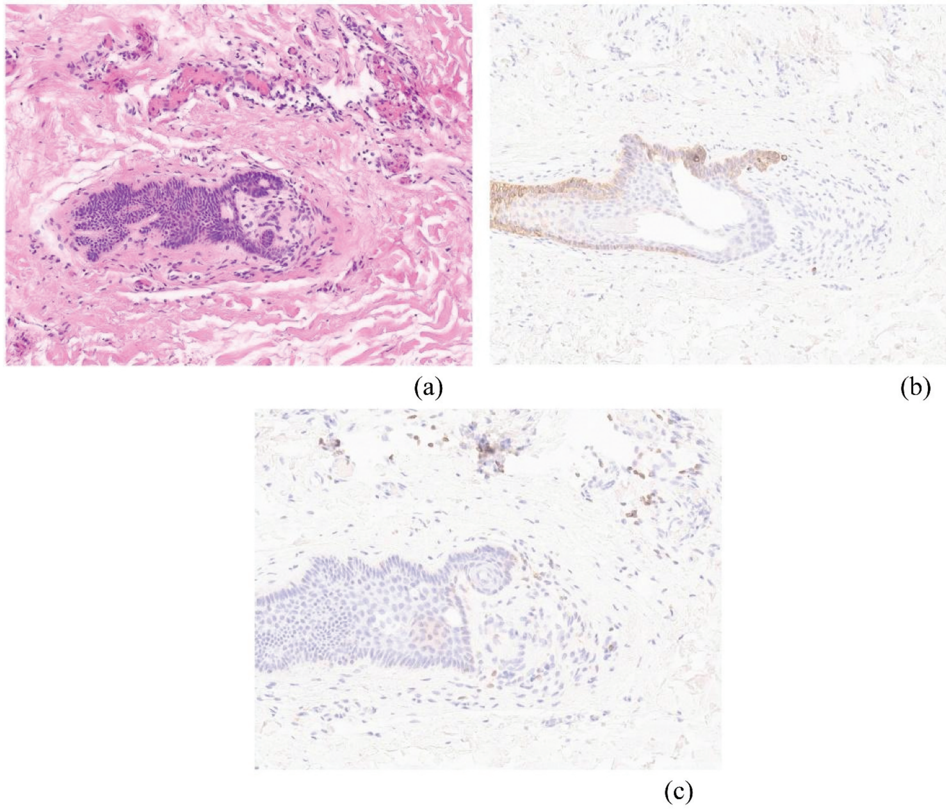

Figure 4. Histological analysis of the treated area: (a) follicle in the advanced telogen phase. Demonstrating complete apoptosis of the analyzed follicle. (b) CK-18 positive, indicating advanced apoptosis process. (c) Bcl-2 negative, indicating advanced apoptosis process.

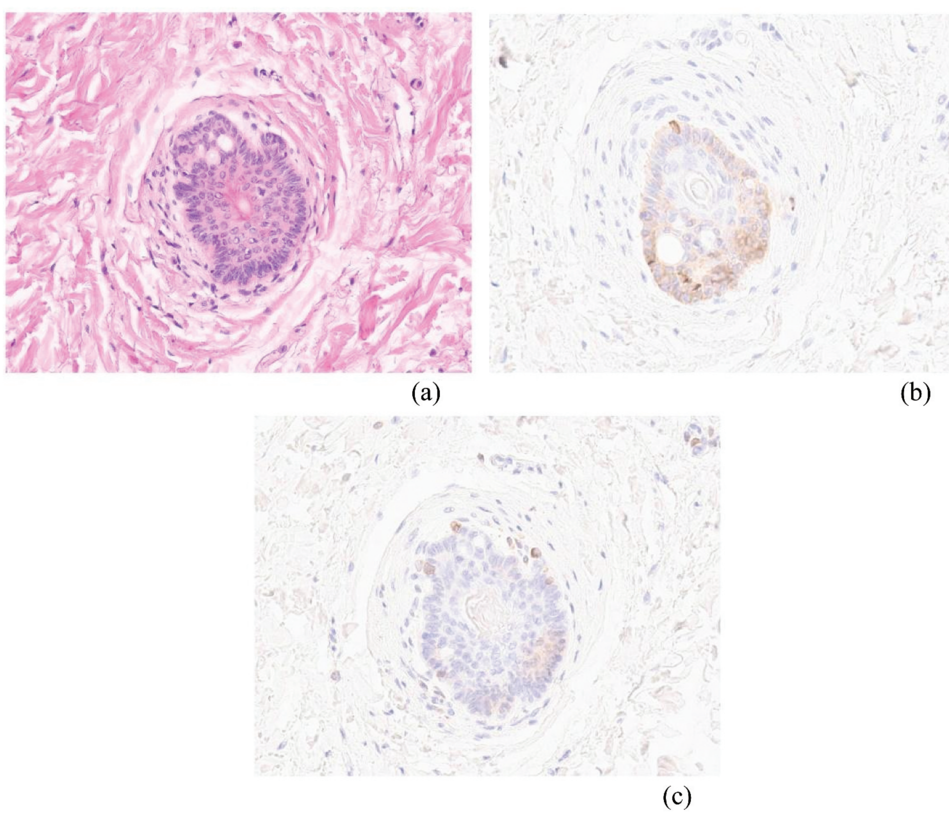

Figure 5. Histological analysis of the groin tissue of the treated area: (a) follicle in the advanced telogen phase. Complete apoptosis of the analyzed follicle. (b) CK-18 positive, indicating advanced apoptosis process. (c) Bcl-2 negative, indicating advanced apoptosis process.

observed. In the treated area, the found follicles were in an advanced stage of telogen (Figure 5a), showing complete apoptosis, which can be confirmed by the presence of CK-18 (Figure 5b) and by the negativity of Bcl-2 (Figure 5c).

The specificities of the hair of the second volunteer, which had a thicker appearance and a higher phototype, in the control sample revealed 53% of hair growth in the anagen phase and only 4% in the telogen phase. On the other hand, in the treated sample, only 3% of the follicles were in the anagen phase, whereas approximately 36.8% were in the telogen phase. In a statistical analysis, when comparing the values of the anagen phase (controls versus treated), a significant difference was observed ($p = .00$) and the same occurred in the comparison of the telogen phase ($p = .00$).

Discussion

Recent evidence points to the clinical effectiveness of laser use to remove unwanted hair (11–14). In contrast, studies that report the effects histological and immunohistochemical of these devices using different wavelengths in a single application and the behavior of the structure and function of the hair follicles in different skin types are scarce. Therefore, this study looked into histological and immunohistochemical aspects of laser epilation in different skin phototypes and hair with distinct characteristics to elucidate some of the involved mechanisms, making it different from other studies already carried out.

Depending on the region, hair development cycles can present themselves in different ways. While in the scalp, the anagen phase, considered an active growth phase, can last for several years, in the body, the hair follicles are identified by a greater frequency and duration in the telogen phase. For the arms region, the duration of the anagen phase is between 6–12 weeks, and the telogen phase is 7–13 weeks. The leg region has 19–26 weeks for the anagen phase and 13–34 weeks for the telogen phase. Body hair is also characterized by being irregularly medullated and presenting fine tips, with a typical length that ranges from 5 to 60 mm (15,16).

In this study, the control samples had more follicles in the anagen phase. In addition, they were considered normal and showed the presence of Bcl-2, which was expressed in portions of the follicle that grow more actively. The anagen phase in most hair made them more susceptible to effectively receiving therapy (17–19). However, it is essential to note that for some authors (20), capillary cycles, such as anagen or telogen, do not affect the effects of laser epilation.

The results of this therapy may suffer interference depending on some factors, such as skin color, hair appearance, and hormonal status. The ideal patient to receive the laser would be the one with thick and dark hair and white skin with normal hormone levels. So, for individuals who are not in this expected pattern, wavelengths are applied individually to make the therapy more effective. However, it is not always possible to achieve the proposed objective (21,22). Therefore, technological advances must be focused on developing a unique device that meets the purpose of epilation for different types of skin, being also efficient for hair of specific characteristics. The idea of combining three wavelengths to be applied simultaneously goes to meet these needs and disregards the differences found in each individual to be treated.

Rao and Goldman (23) analyzed the effectiveness, tolerability, and subjective satisfaction of three different laser systems used individually and together to remove axillary hair. Twenty women with type II skin phototype (the axillary hair varied between brown, red, or light) were selected. Each armpit was graphically divided in half to produce four distinct areas that were treated by the following lasers: 1. 3 sessions with a 755 nm Alexandrite laser, 2. 3 sessions with an 810 nm diode laser, 3. 3 sessions with Nd laser: 1.064 nm YAG and 4. rotational treatment consisting of a single session for each of the three laser systems (sequentially). Sequential application of lasers was statistically more effective than treatments with Nd: YAG alone, but considerably less effective than treatments with alexandrite or diode alone. In corroboration, this study applied laser not only sequentially but simultaneously, using the alexandrite and Nd: Yag laser together, in addition to the diode laser, seeking to treat variations of hair and skin color with greater efficiency.

Currently, it is understood that the increase in the effectiveness of laser therapy may be related to the interruption of the balance of epithelial cell proliferation and apoptosis. Through a detailed analysis of the ratio Bcl-2 (apoptosis inhibitor) and Bax (apoptosis promoter) during anagen-catagen transformation, a sudden, progressive decline in Bcl-2 expression is observed, associated with a simultaneous increase in Bax in the proximal capillary matrix during the stages of catagen II to VIII, in comparison with that of the anagen VI. After applying the laser, the sample with both the lighter-skinned phototype and that of phototype 4 showed protein negativity. This change can also be seen in the telogen phase percentage increase in the treated samples. As mentioned, Bcl-2 is an anti-apoptotic protein, and when it is found to be reduced or absent, the hair tends to move to the telogen phase, where all its epithelial expression ceases, and complete apoptosis of the follicle occurs (17).

Preventing the development of cells within the hair follicle base, especially in the bulge region and dermal papilla (part of the isthmus that produces follicular stem cells), seems to be the critical factor for permanent hair removal (24,25). Previous studies (26,27) performed biopsies in three individuals, taken from a total of 208 volunteers, three months after the last treatment with a long low-power pulse Nd: YAG Laser, and confirmed the complete loss of hair follicles. It was also observed that six hours after the first session, there was extensive necrosis of the hair follicle and sebaceous gland epithelium. After 25 minutes of a long pulse Nd: YAG Laser application, there was nuclear elongation and cytoplasmic degeneration in the outermost portion of the external root sheath at the level of the hair bulb.

The literature points to a reduction in efficacy in treatment intervals of less than four weeks or more than eight weeks regarding the most effective epilation interval. 45-day intervals are more effective when compared to 60 or 90-day intervals when the goal is to remove facial and neck hair. Usually, intervals of 4 to 8 weeks are chosen for laser epilation, and the results of hair removal can be seen in an average of five or more treatments, where most patients will perceive subjectively that the number of hair decreases (28). The combination of the wavelengths in this study led to results after a single session analyzed 45 days after application.

Epithelial cells are characterized by specific groups of intermediate filaments called cytokeratins (CKs). Among them, CK 18 can be found in hair follicles. In immunohistochemical analysis, it is possible to see that simple epithelial keratins are the first targets of caspase activity in apoptosis. The cleavage of keratin destabilizes the filament network due to its hetero polymeric nature, so the role of fragmentation by caspases can facilitate the apoptotic elimination of cells, allowing the keratin network to be dismembered (29,30). In this study, CK-18 showed complete apoptosis of the follicles in the abdominal and groin regions.

Laser devices safety levels have already been observed in several studies (31). For people with higher skin phototypes, the use of a method for cooling the skin surface can be combined with an increase in pulse duration for effective hair removal, leaving the epidermis stabilized and protected, thus avoiding thermal lesions on the surface of the skin (32,33). It is also important that the therapy promotes hair removal without causing collateral damage and subsequent cosmetic complications. Usually, perifollicular erythema and edema without blisters or purpura are found, being considered a good immediate clinical response, demonstrating safe and effective heating of the hair follicle (22). The device used in this study had a built-in cooling system, making it safe to apply the therapy, even in higher phototypes. Thus, despite being exposed to three wavelengths in a single application, the volunteers did not experience any adverse reactions beyond what is expected, which are mild heat discomfort, mild pain sensation and mild hyperemia after application (6,21).

Orringer et al (34). demonstrated the immunohistochemical staining properties of human hair follicles after applying 800 nm wavelength diode laser and 1064 nm Nd: YAG laser wavelength in unwanted axillary hair. The researchers used the cytokeratin 15, cytokeratin 19, and CD34 markers, the post-treatment follicles examined in this study remained, for the most part, structurally intact, despite the positive clinical response. The authors concluded that the use of the laser does not seem to work through the frank destruction of follicular stem cells. However, this study was limited only to observe the acute changes. In addition, the resulting changes may not have been detectable by the selected markers.

Unlike the previous literature, the present study used a device intended for laser epilation that provides the union of different wavelengths (Crystal Alexandrite 755 nm, Crystal Diode 810 nm, and Crystal Nd Yag 1064 nm) in a single application, which led to apoptosis of the hair follicle after 45 days in volunteers with different skin phototypes, seen by immunohistochemical analysis of the treated area. This may lead to more effective therapies in different phototypes and hair with different characteristics. However, further clinical studies are required, so that several series of systematic histological evaluations over a more extended period and with a higher number of phototypes are carried out.

Conclusion

The presence of a greater number of follicles in the anagen phase in the untreated site and follicles in the telogen phase demonstrates the efficiency of the laser at different wavelengths when reaching different skin phototypes and hair thickness. Therefore, the hair-removal process has been optimized with various laser wavelengths. There is a significant lack of histological studies that present the effects of lasers with the combination of wavelengths for body hair removal, especially regarding immunohistochemical markers. In this study, the apoptosis markers and cell proliferation coincide with the follicle behavior in the different phases and they reinforce the action of the laser on the follicle. Therefore, modulating the balance of follicular proliferation and apoptosis can be a crucial strategy for controlling hair growth and regression, and the combined use of wavelengths can lead to better effects of therapies for different phototypes, however, this needs to be better investigated by larger studies.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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