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Effectiveness of Light-Emitting Diode Epilation on Different Skin Types: A Pilot Study

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Abstract

Objective: This study analyzed the histological and immunohistochemical changes in hair follicles submitted to epilation with light-emitting diode (LED).
Background: The use of specific wavelengths of LED leads to the absorption of photons by chromophore tissues, enabling different photophysical and photochemical events, bringing therapeutic benefits such as removing body hair.
Methods: The sample included five participants, with phototypes II–V, divided into two groups. The volunteers received a session of epilation with the Holonyak® device on the pubic region and right groin, whereas the contralateral side was kept as a control. An energy of 10 J and a cooling temperature of −5°C were used, after which the pain provoked by the equipment was questioned using the analogue pain scale. After 45 days, the punching procedure was performed in the region where skin samples were taken for histological and immunohistochemical analysis.
Results: For all phototypes, in the treated area, the follicles and sebaceous glands were in a stage of involution, showing perifollicular inflammatory infiltrate with changes suggestive of apoptosis. The apoptosis process was confirmed by the increase in markers cytokeratin-18 and cleaved caspase 3, in addition to the reduced expression of Blc-2, and the lower cell proliferation (Ki67), reinforcing the action of LED based on the definite involution and resorption of the follicle, through macrophages (CD68) triggered by the inflammatory process.
Conclusions: The preliminary results of this study found relevant histological changes and immunohistochemical markers in the epilation process, which may indicate the efficacy of LED in permanent hair removal.

Keywords: light-emitting diode, epilation, skin types, histological

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Introduction

THE CLINICAL PROCEDURES that promote the progressive removal of hair make use mainly of laser and intense pulsed light, which can be classified as phototherapeutic resources, having its basic principle the selective photothermolysis, represented by light radiation with a predilection for a particular type of chromophore or group of atoms that give color to certain body structures. The response to treatment will depend on some individual characteristics, such as the darker and thicker the hair, the more effective the treatment.[1,2]

With the technological developments of recent years, new technologies, beam sources, and treatment concepts have emerged. The latest is the light-emitting diode (LED)-based wavelength emission, which enables the combination of various effects on the tissue and allows better results. Therefore, the treatment with LED enables the use of wavelengths that can offer advantages over different treatments, including epilation.[3] The use of LED is classified as a nonthermal therapy, where the phototherapeutic effects are stimulated by nonthermal physiological processes, generating photochemical and photobiological processes. Recent studies have shown that the use of LEDs can be used in patients with all types of skin pigmentation, safely preventing excessive heating of the tissue.[4]

Despite the scarcity in the literature, the use of LED is based on some assumptions. One is that repetitive treatment of the skin area with hair using low energy density (fluence) allows the hair to be continuously stimulated to the root, avoiding unwanted temperature rise of the surrounding tissue, the most common cause of side effects and complications. This way, less epidermal cooling would be required, and the pain would be dramatically reduced.[5,6]

The principle of photothermolysis and the theory of extended photothermolysis underlie the process of light absorption for photoepilation to occur. During this process, energy is selectively delivered leading to a rapid increase in temperature to transfer heat to adjacent tissues, causing only local thermal necrosis of the regenerative structures of the follicles.[7] The study by Altshuler et al.[8] report a new theory of selective thermal damage, called the extended theory of selective photothermolysis. This theory is important for biological targets with space between all or part of the target and the pigmented tissue area. In this way, the target is destroyed by diffusion of heat from the pigmented area to the target. Shirkavand et al.[9] also highlighted that pulse duration longer than thermal relaxation time are considered more appropriate for laser hair removal.

The characteristic of electroluminescence makes this component emit light with less heat generation, implying in the photoepilation a higher electronic cooling capacity of the SPOT (up to −15°C) and a higher stability in maintaining a cryogenic temperature of the SPOT because the heat exchange between the LED component and the Peltier cells is very low. It also implies, and this is a technical specification that differs it from LASERS, the higher pulse width capability (up to 750 ms, whereas on average, in photoepilation lasers, it is 400 ms). Perhaps this is the assumption for the acceptance of LED photoepilation treatment in high phototypes.[10]

In the literature, the use of LED for epilation is practically nonexistent, and it is not possible to determine the effects of its use in different types of skin, as the results of LED at the histological and immunohistochemical level for epilation remain uncertain. Thus, the purpose of this study was to analyze the effects of LED epilation on different skin types, showing its action at a cellular level.

Materials and Methods

A pilot intervention study was conducted with five volunteers for body hair removal with LED equipment. Women were selected in the age range of 30–50 years and presented the following characteristics: one volunteer with phototype II, one with phototype III, one with phototype IV, and two volunteers with phototype V of the Fitzpatrick scale. The Research Ethics Committee approved the study (code: 5.462.018), and all volunteers signed a written informed consent form and underwent anamnesis, clinical evaluation, photography, skin biopsy, LED epilation treatment, and follow-up.

Clinical evaluation

Each volunteer was clinically evaluated before treatment and underwent surgery to remove a skin fragment (punch technique) 45 days after applying LED. The clinical evaluation included the collection of identification and anamnesis data using an adapted version of the Facial Assessment Protocol,[11] the measurement of the skin phototype with the SkinUp evaluator from HS MED™ (São Paulo, Brazil). Photography was taken for each volunteer to register the cases. The laser was applied using the Holonyak™ device from Adoxy™ (São Paulo, Brazil), the area chosen to receive treatment was the pubic region and right groin, whereas the left side was kept as a control.

Skin biopsy and histopathological examination

Skin flaps of ∼4 mm were removed 45 days after the application of the LED session. The area where the punch was made was cleaned with alcohol and received local anesthesia. Then with a scalpel blade, the material was removed and the area was punctured. The biopsies were stored in 10% formalin and fixed in paraffin. The materials were cut transversally and stained with hematoxylin and eosin to show the general morphology of the hair follicle, quantification of proliferating cells and lymphocytes (Ki67), antiapoptotic marker (Bcl-2), detection of cells suggestive of apoptosis of the epithelium [cytokeratin 18 (CK-18)], inflammatory infiltrate (CD68), and another marker of apoptosis (caspase 3-cleaved).

LED treatment

All volunteers received one session of LED-assisted epilation. The device uses NEIR LED, having a variable wavelength of 780–850 nm, with the adjustable energy density of 5–100 J/cm^2 and pulse width of 5–750 ms adjusted to the skin type and hair thickness. This device also features cooling technology ranging from 10°C to −15°C. The laser was applied to the pubic region and right groin of the volunteers using an energy of 10 J and a cooling temperature of −5°C, and then the pain caused by the equipment was questioned using the analogue pain scale (AAS), ranging

from 0 to 10, 0 being no pain and 10 being unbearable pain. All parameters used are described in Table 1.

Data analysis

Histological data were analyzed by GraphPad Prism (version 8.0; GraphPad Software, San Diego, CA), Mann–Whitney *U* test was adopted to evaluate the statistical significance of the results (p values <0.05 were considered statistically significant). In addition, qualitative data were described based on the pathologist's reports (descriptive analysis of histological images).

Results

For quantitative data analysis, the volunteers were divided into two groups: light phototype I, II, and III and dark phototype IV and V. For both types of phototypes, the follicles were in a stage of involution (telogen phase), where a perifollicular inflammatory infiltrate is observed and also involution of sebaceous glands, showing changes suggestive of apoptosis, confirmed by immunohistochemistry (Fig. 1A, B).

The light phototype control side (Fig. 1C) showed well-developed follicles with associated sebaceous glands, absence of inflammation and involution. All structures were within normal range, hair mainly in the anagen phase and similarly was observed in the dark phototype (Fig. 1D).

Other relevant findings for the treated area were the rare presence of basal cells positive for Ki67 (Fig. 2A, red arrow), indicating lower proliferation and, consequently, higher apoptotic rate since there was the low expression of the antiapoptotic marker Blc-2, with negative expression in the hair, and positive expression only in some adjacent lymphocytes (Fig. 2B, red arrows).

In the region kept as control, there was a high expression of Ki67, which can be observed in Fig. 2C. As expected in the control group, there was some demarcated nuclear expression in the basal region of the hair (red arrow) and sebaceous glands (yellow arrow). In the dark phototype (Fig. 2D), high Blc-2 expression was observed, with nuclear and cytoplasmic expression in the hair (in green) and in adjacent lymphocytes (yellow) characteristic of the control group.

There was a high cytoplasmic expression in the basal and suprabasal regions of the follicular epithelium (Fig. 3A, in red) of CK-18, denoting the apoptosis process. On the control side, the presence of cells showing expression for

Table 1. Parameters Used in Applications

NIR light wavelength	780–850 nm (not fixed)
Power	1200 W (peak)–840 W (output)
Operation mode	Sweep
Wrist width	Buttons are color scale Six buttons, one for each phototype: 1–77 ms 2–77 ms 3–77 ms 4–82 ms 5–86 ms 6–91 ms
Energy density:	10 J/cm^2
Spot cooling:	–5°C

NIR, near infrared.

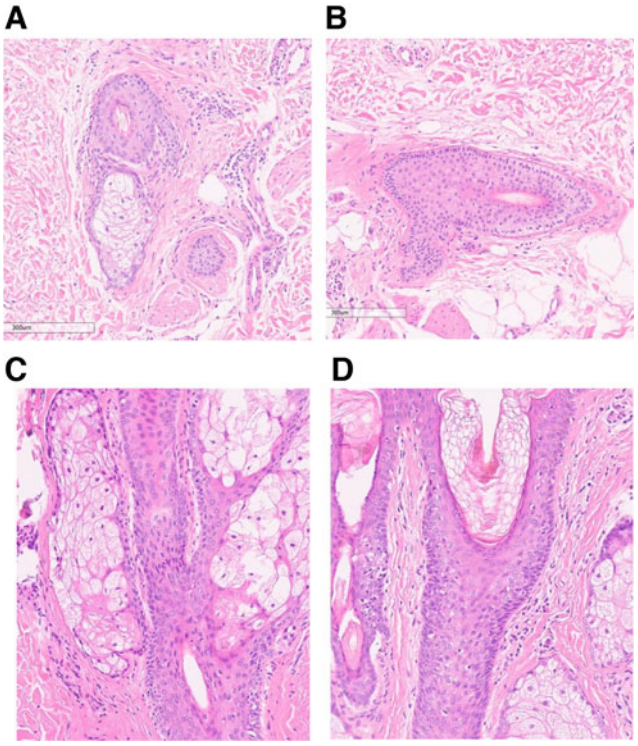

FIG. 1. Histological analysis of the tissue. Treated area: **(A)** light phototype and **(B)** dark phototype; control area: **(C)** light phototype and **(D)** dark phototype.

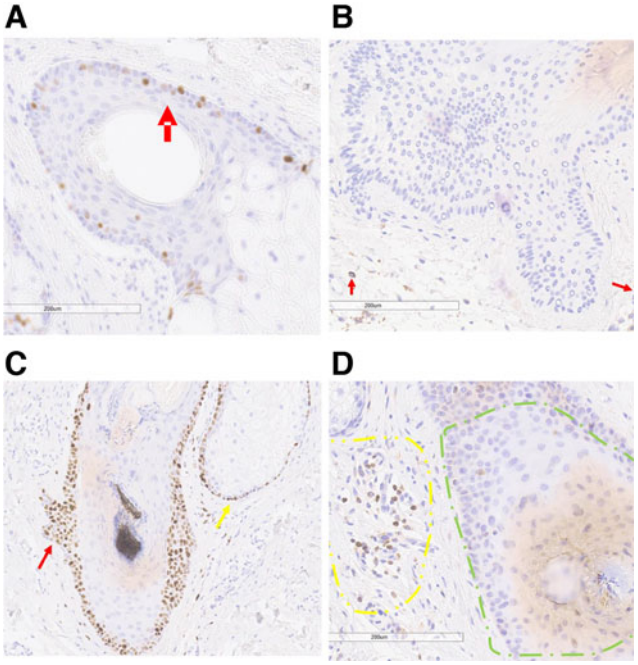

FIG. 2. Histological analysis of Blc-2 **(A, B)** and Ki67 **(C, D)**. **A:** *Arrow:* Rare presence of basal cells positive for Ki67. **B:** *Arrows:* Low expression of the anti-apoptotic marker Blc-2. **C:** *Arrow:* High expression of Ki67. **D:** High Blc-2 expression was observed, with nuclear and cytoplasmic expression in the hair and in adjacent lymphocytes characteristic of the control group.

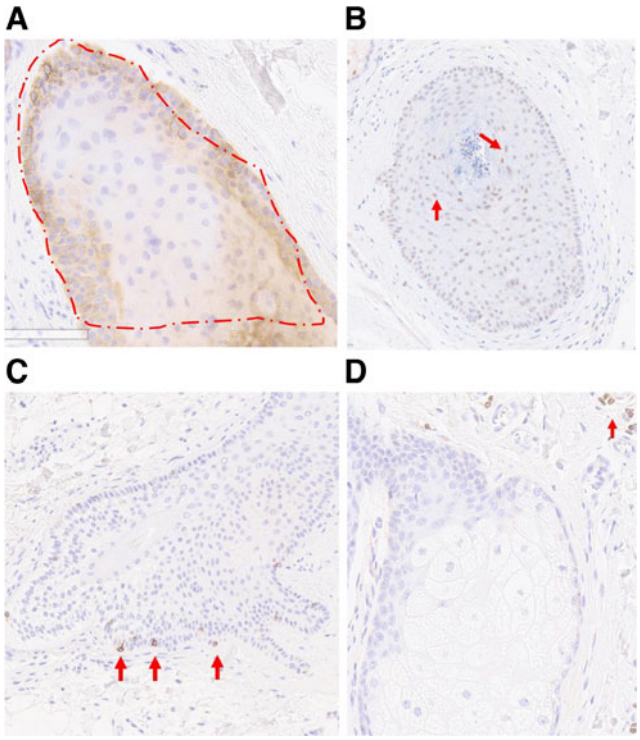

FIG. 3. Histological analysis of cytokeratin-18 **(A, B)** and caspase 3 **(C, D)**. **A:** High cytoplasmic expression of CK-18. **B:** *Arrows:* The presence of cells showing expression of CK-18 was rare in the control group. **C:** *Arrows:* High nuclear expression of cleaved caspase 3. **D:** *Arrow:* Rare presence of lymphocytes positive for cleaved caspase 3 was observed.

CK-18 was rare, restricted to basal hair region (Fig. 3B, red arrows), as expected in the control group.

Cleaved caspase 3, known as a more reliable marker of apoptosis, showed high nuclear expression in basal and suprabasal regions of the follicular epithelium (Fig. 3C, red arrows), corroborating the confirmation of the apoptosis process. For the control side, the rare presence of lymphocytes positive for cleaved caspase three was observed, restricted to these cells (Fig. 3D, red arrow) and negative in the fur.

Inflammation was seen by the presence of perifollicular infiltrate, demonstrating cells positive for CD68 and showing high cytoplasmic expression (Fig. 4A, demarcated in red). For the control side, few cells positive for the same marker were found, with low expression, demonstrating few macrophages (Fig. 4B, red arrows).

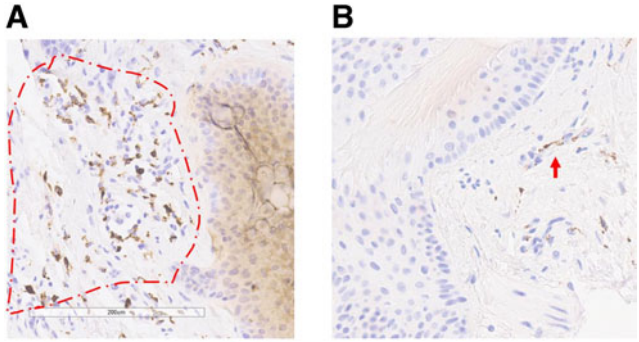

FIG. 4. Histological analysis of the CD68 marker. **A:** Demarcated. Presence of perifollicular infiltrate demonstrating cells positive for CD68. **B:** *Arrows:* Few cells positive for the CD68 marker.

Statistical analysis of the markers showed significant differences when comparing the treated side of the light phototype with its respective control. For all markers, the p value was <0.05. Similarly, when comparing the treated side of the dark phototype with its respective control, the p value was <0.05 in all analyses. The values found for each marker are described in Table 2. In the analysis using the comparison between the phototypes (light vs. dark), the values were not significant ($p > 0.05$).

When asked about the pain felt during the LED epilation procedure, it was seen that lighter phototypes had higher scores on the visual analog scale (VAS), with an average of 6.6 points, when compared with dark phototypes, which had an average of 5.5 points. No adverse events were reported during the applications.

Discussion

A hairless body is part of the aesthetic standard of modern society. To achieve this goal, photoepilation methods are used, where different light sources are employed for the permanent removal of hair. LED, invented in 1962 by Nick Holonyak Jr, is a technology that has recently joined the ranks of methods that promote epilation. This study is pioneering in the histological and immunohistochemical analysis of this technology applied to body hair.[3,12,13]

The use of specific wavelengths of LED leads to the absorption of photons by chromophore tissues, presenting similar effects to the laser.[14] With this absorption, different photophysical and photochemical events occur in various biological scales without causing thermal damage, bringing therapeutic benefits.[15] In this study, when we observed the histology of the hair for both types of phototypes, the follicles and sebaceous glands were in involution, with perifollicular inflammatory infiltrate, characterizing the apoptosis process.

Although there is no evidence focused on LED epilation, the studies direct the possible findings to this area. The use of LED stimulated apoptosis induced by direct mitochondrial damage, with increased oxidative stress in retinal cells.[16] In this study, an almost negative expression of the antiapoptotic marker Blc-2 was found in hair, and this reduction or absence is considered an inducer of the telogen phase, interrupting all epithelial expression and leading to follicle apoptosis.[17]

Immunohistochemical analyses were positive for the Ki67 marker and revealed less cell proliferation after using LED in the regions with hair. These findings are also found in other

TABLE 2. RESULTS OF THE STATISTICAL ANALYSIS OF THE MARKERS

	Light phototype Treated × control	*Dark phototype Treated × control*
Markers	p	p
Ki67	0.0001	0.0001
Blc-2	0.04	0.01
Caspase 3 cleaved	0.009	0.009
CK-18	0.007	0.03
CD68	0.02	0.008

studies with LED, where the correct choice of wavelength led to inhibition of proliferation in different cell types, such as gingival fibroblasts[18] and embryonic mitotic proliferation.[19] Therefore, findings with Blc-2 and Ki67 may be related to the effectiveness of the therapy since it promotes disruption of the balance of epithelial cell proliferation and apoptosis.

After applying LED, other markers that signalize the apoptotic process in the hair were found, such as the high cytoplasmic expression of CK-18 in the basal and suprabasal regions of the follicular epithelium and cleaved caspase 3, which also showed high nuclear expression in the same regions. No studies were found that use these markers in epilation. However, it is known that CK-18 are proteins found in the intracytoplasmic cytoskeleton of epithelial tissue, which concentrate in the cytoplasm and are involved in important signaling pathways after cell death, such as apoptosis.[20]

Cleaved caspase 3, meanwhile, is essential for cell death in a remarkable, tissue-specific manner. Cleavage of keratin destabilizes the filament network due to its heteropolymeric nature. Thus, the role of fragmentation by caspases may facilitate the apoptotic elimination of cells by allowing the keratin network to be dismembered.[21] Although these are preliminary results, the histological and immunohistochemical findings cited earlier open up several possibilities for future research with LED in epilation.

In a clinical aspect, a good response to the epilation procedure is obtained when perifollicular erythema and edema without blisters or purpura are found because this denotes that the heating process of the hair follicle occurred safely and effectively.[22] The application of LED produced inflammation in the region, with the presence of a perifollicular infiltrate, with a high expression of cells positive for CD68, indicating the reabsorption of the hair follicle and a good clinical response to the equipment. The photoactivation of epidermal keratinocytes can explain this inflammatory process, an important source of cytokines and other chemokines, which by sequential reaction, attract cells to the region, including macrophages, and these, when photoactivated, tend to internalize phagocytic materials more quickly.[23]

Moreover, the device used in this study uses a method for cooling the skin surface, providing greater convenience to users, which may be related to relatively low averages on the pain scale (cooling capacity and greater pulse width), corroborating the previous study,[24] where most volunteers reported only mild pain during epilation with LED. It is known that maintaining lower temperatures on the skin surface, especially for people with higher phototypes, provides stabilization and protection of the epidermis, thus avoiding thermal lesions in the region or subsequent cosmetic complications.[25]

The statistical analyses did not show significant differences between the treatment in light and dark phototypes. This comparison allows the initial understanding that LED epilation can be performed in different phototypes and that the presence of melanin or melanocytes may not interfere in this process. However, this hypothesis should be better studied later for a period and with a more significant number of phototypes.

Conclusions

The preliminary results of this study found relevant histological changes and immunohistochemical markers in the epilation process, which may indicate the effectiveness of epilation with the use of LED. The apoptosis process was observed by increasing CK-18, cleaving caspase 3, and reduced Blc-2 expression. These findings are related to lower cell proliferation (Ki67), reinforcing the action of LED based on the definite involution and resorption of the follicle through macrophages (CD68) triggered by the inflammatory processes. Effects at the cellular level were observed after using LED in light and dark phototype skin, and these showed significant differences in markers when compared with the control group. This study will be continued with a more significant number of volunteers to confirm and elucidate the findings.

Authors' Contributions

P.F.M., R.M.V.S., and R.R.M. analyzed the data and wrote the article. E.M.C., S.L.Q.F., M.M., and A.L.M.B. performed the experiments. M.A.M., F.C.P.B., and C.E.M. designed the study, revised the article, and supervised the study.

Author Disclosure Statement

No competing financial interests exist.

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