A New Thermographic and Fluorescent Method for Tuning Photoablative Laser Removal of the Gingival Epithelium in Patients with Chronic Periodontitis and Hyperpigmentation

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Abstract

Objective: The purpose of this study was to optimize gingival laser photoablation by thermographic and autofluorescent feedbacks. Background data: Photoablative laser treatment is commonly used for gingival de-epithelization in patients with chronic periodontitis or hyperpigmentation. The reduction of collateral thermal damage of periodontal tissues is crucial for optimal treatment outcome. Methods: Nineteen patients with chronic periodontitis, seven of whom showing gingival hyperpigmentation, were subjected to de-epithelization with an 810 nm diode laser used in continuous (1 W, 66.67 J/cm²) or pulsed wave mode (69 µJ, 18 µs, 8000 Hz, corresponding to peak/mean power of 3.8 W/0.6 W, 401 J/cm²), depending on individual gingival features. Photoablation was controlled in real time with a 405 nm violet light probe, which stimulated a yellow autofluorescence of the laser-coagulated tissue. The temperature at the target tissue was controlled with an infrared thermographic probe. When appropriate, small biopsies were taken to evaluate epithelial ablation and thermal effects. Results: The energy density transferred to the treated tissue surface was computed based on the irradiation modality of the target tissues. Laser photoablation performed under thermographic control yielded complete removal of the gingival epithelium with minimal injury to the underlying lamina propria. Irradiation-evoked autofluorescence, conceivably the result of epithelial keratins, allowed very sharp recognition of the borders between laser-ablated and intact epithelium, thus preventing repeated irradiation. Conclusions: This study further supports the favorable characteristics of photoablative diode laser for gingival de-epithelization. Concurrent thermographic and fluorescent analysis can provide substantial help to the setup of a safe and well-tolerated protocol.

Introduction

In recent years, the use of medical laser for surface photoablation of abnormal gingivas has been recognized as one of the most effective and safe techniques. Removal of the gingival epithelium represents a major therapeutic indication in patients with chronic periodontitis, however, the persistence of periodontopathogenic bacteria within the epithelial lining of the pocket and outer gingiva represents a key factor for disease chronicization and relapses after conventional mechanical debridement. Recently, we have offered evidence for the clinical efficacy of a photoablative diode laser emitting at 810 nm wavelength in periodontal therapy. Using appropriate settings, this laser was proven equal or superior to other medical lasers, such as Nd:YAG, Er:YAG or CO₂ lasers, in removing the contaminated gingival epithelium without causing damage of the underlying dental/periodontal tissues. We also provided evidence that diode laser offered some advantages over the others, such as easy gingival reshaping, reduced need of local anesthesia, and excellent hemostasis associated with a significant decrease of pain and inflammatory postoperative score and satisfactory clinical outcome in the long term.

Another promising field for therapeutic photoablation is physiologic gingival hyperpigmentation (PGH), an aesthetic abnormality caused by excess melanin deposition by basal and suprabasal epithelial melanocytes. Although PGH is not a health concern, an increasing number of persons are seeking a suitable treatment, especially when black gingivas are clearly visible during speaking and smiling. In the past, gingival depigmentation has been performed by various techniques, including mechanical and chemical abrasion and

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oral surgery, with different degrees of success and undesired complications. More recently, however, laser ablation has been identified as the most appropriate approach. Different photoablative laser types have been used for this purpose, including CO₂, Er:YAG, Nd:YAG, and diode laser. In principle, epithelial ablation depends upon the photothermal effect of laser irradiation. However, conversion of photon energy into heat may cause overheating and injury of the targeted tissues, with adverse consequences on treatment outcome. For example, when the temperature rises >80°C for 2-5 sec, there is a high risk for irreversible damage of the periodontal alveolar bone and dental components. The overall tissue effects induced by laser treatment basically depend upon the characteristics of the treated tissue (i.e., pigmentation, inflammation) and the laser system (wavelength, power, emission mode, power density), as well as on the operator’s skills. Such parameters cannot be standardized to achieve a consensus, widely accepted therapeutic protocol. Therefore, over time, dental practitioners will adjust their protocols to fit the characteristics of their laser instruments and patients. In this context, the present study has exploited the recent development of a novel laser device equipped with 405 nm violet light and thermographic probes combined with a photoablative laser diode (λ 810 nm) emitting in continuous or pulsed modes, to obtain an exact control of gingival epithelial photoablation through the detection of autofluorescence and thermographic feedback during laser irradiation.

Materials and Methods

Patients

Nineteen patients (7 males, 12 females; ages 18-65, mean 51.6) affected by slight to severe chronic periodontitis, 7 of whom (2 males and 5 females, ages 18-40, mean 26.5) showed bilateral PGH of the upper and lower gingiva, were included in this study. Gingival hyperpigmentation was assessed using the criteria reported by Dummett, namely: mild (pink to light brown), moderate (deep brown to black), or severe: mixed color. Four patients were classified as severe and three as moderate. The protocol was designed in compliance with the guidelines of the Declaration of Helsinki, as amended in Edinburgh, 2008, and approved by the Ethical Committee of the Faculty of Medicine, University of Florence, Italy. Written informed consent for their enrolment in the study was given by all the subjects. Exclusion criteria were: (1) history of systemic diseases (diabetes mellitus, cancer, HIV, metabolic and endocrine diseases), (2) pregnancy or lactation, or (3) heavy smoking habit (>10 cigarettes/day). All the treatments described below were administered by the same experienced dentist (M.G.).

Before the laser treatment, the patients underwent full-mouth supragingival prophylaxis by ultrasound and/or hand instrumentation. The patients received oral hygiene instructions and appropriate motivation. When required for therapeutic purposes, that is, to reduce periodontal pockets, small biopsies, 2x2 mm, were taken after the laser treatment to determine the extent of de-epithelization and the possible thermal damage of the gingival lamina propria (n = 4). The PGH patients also underwent small gingival biopsies on first observation for histopathological diagnosis. Seven days later, upon histological assessment of benign melanin pigmentation, the patients were subjected to laser photoablation. When deemed appropriate, that is, to reduce hypertrophic gingivitas, a second biopsy was taken after the laser treatment to determine the histological features, as mentioned (n=3).

Photoablative laser treatment

To remove the gingival epithelial lining, the patients were laser-irradiated with a λ 810 nm diode laser (4 x 4 Dental Laser, General Project, Montespertoli, Italy) equipped with a 0.6 mm optical fiber. In some patients (n = 12) the laser was set in continuous mode, 1 W output power, corresponding to 66.67 W/cm² irradiation energy. These settings were chosen on the basis of those previously reported to achieve gingival de-epithelization in periodontopathic subjects. In other patients, namely those with moderate or severe hyperpigmented gingivas (n = 7), the laser was set in pulsed mode, 69 μJ pulse energy, 18 μs pulse duration, 8000 Hz pulse repetition frequency, corresponding to 0.6 W mean power (peak power 3.8 W) and 40 J/cm² irradiation energy. These settings were chosen based on the empirical observation that a mean power >0.6 W resulted in pain and tissue injury, whereas one of <0.6 W resulted in insufficient photoablative effect. Energy output of the lasers was measured with a power meter before each procedure. Irradiation was performed in contact mode, the fiber tip touching the gingiva, to remove the epithelium around the teeth from the free gingival margin and interdental papilla to the mucogingival junction. The fiber end was controlled at every irradiation to check for a carbonized tip (hot tip), required to generate enough thermal energy to cause tissue vaporization. When the fiber came in contact with tissue, debris immediately accumulated on its tip and intensely absorbed infrared laser energy, thus heating the tip and carbonizing the debris and the optic fiber end. As laser energy continued to be absorbed by carbon deposits, the tip reached a red hot temperature (~760°C), causing tissue vaporization. Excess carbonized debris was removed with wet gauze. The tip was moved at a constant speed of 2.5 mm/sec under air flow cooling to prevent overheating. During the treatment, the actual speed was checked using a digital chronograph. Eye protection of the operator, assistant, and patients was assured by wearing safety glasses. Anesthesia (Articain HCl, Ultracain, Frankfurt, Germany) was usually not needed and only used on patient demand. After the photoablative laser treatment, the patients were subjected to supragingival and subgingival scaling and root planning with a combined use of hand (Hu-Friedy, Chicago, IL) and ultrasonic instruments, under local anesthesia (Articain HCl). Patients were instructed to discontinue tooth brushing on the day of laser therapy to prevent mechanical trauma at the treated sites and facilitate re-epithelization. From day 2 onwards, normal personal tooth hygiene with manual toothbrush and interproximal instruments was encouraged. Chlorhexidine digluconate and other topical medications were not prescribed.

Safety and efficacy controls of photoablative treatment

The device used was equipped with a violet light probe emitting at λ 405 nm and stimulating a yellow biofluorescence by the laser-treated tissues, which could be observed using yellow-green filters integrated into the
protective eyeglasses. Moreover, thermographic assessment was performed during laser irradiation using an infrared thermal camera (Ti9, Fluke, Brugherio, Italy). It is of note that the diode laser was equipped with a thermographic feedback probe that activated an alarm alerting the operator when the tissue temperature approached the damage threshold and stopped laser emission if the temperature exceeded such value.

**Histological and ultrastructural analysis**

The biopsies were fixed by immersion in 4% (w/v) formaldehyde in 0.2 M phosphate-buffered saline, pH 7.4, dehydrated in graded ethanol and embedded in paraffin. Six μm thick sections were stained with hematoxylin and eosin, viewed, and photographed under a light microscope (Nikon, Tokyo, Japan).

**Results**

**Computations of photoablative laser-tissue interaction**

The laser–tissue interaction depends upon the laser beam characteristics, the irradiation modality, and the cromophores in the target tissue, but not on the treated surface area. The epithelial surface was treated maintaining the fiber tip in contact with the tissue; therefore, the relevant surface (S1) was treated in successive, nonoverlapping stripes, the height of which was equal to the fiber diameter (d = 0.6 mm), whereas their length (l) and number (N) depended upon the extension of the diseased tissue. The scheme in Fig. 1A depicts a graphic representation of the treated surface, whereas Fig. 1B shows the movement of the fiber tip during the treatment. The total time of the treatment (tT) was given by the following formula:

\[ t_T = N \cdot t_S = N \cdot \frac{1}{v} \]

where \( t_S \) was the time required to treat a stripe and \( v \) was the constant fiber speed, 2.5 mm/sec.

The total energy transferred to the surface (ET) was computed starting from the laser beam mean power (P) set for the treatment (1 W in continuous emission, 0.6 W in pulsed emission) and the treatment time (tT):

\[ E_T = P \cdot t_T = P \cdot N \cdot t_S = N \cdot P \cdot t_S = N \cdot E_S = P \cdot N \cdot \frac{1}{v} \]

where \( E_S \) was the energy transferred to each stripes (ES).

The fluence (F) was given by the following formula:

\[ F = \frac{E_T}{S_T} = \frac{N \cdot E_S}{S_S} = \frac{E_S}{S_S} = \frac{P}{v} \cdot \frac{1}{l} \cdot \frac{1}{d} \cdot \frac{1}{d} = \frac{P}{v} \cdot \frac{1}{d} \cdot \frac{1}{d} \cdot \frac{1}{d} \]

As F depends upon the laser beam characteristics (mean power and diameter) and irradiation modality (fiber movement speed), whereas it is independent from the geometric characteristics of the surface to be treated, when the laser was applied in continuous mode it was estimated to be:

\[ \Phi = \frac{1}{\delta} \cdot \frac{1}{v} = \frac{1}{0.6 \cdot 2.5} = 66.67 \text{ J/cm}^2 \]

when the laser was applied in pulsed mode it was estimated to be:

\[ \Phi = \frac{1}{\delta} \cdot \frac{1}{v} = \frac{1}{0.6 \cdot 2.5} = 40 \text{ J/cm}^2 \]

These data are summarized in Table 1.

**Execution of photoablative laser treatment**

Figure 2A shows the typical clinical features of hyperpigmented gingival epithelium from a representative PCH subject. It is well known that the main absorption of laser energy is caused by the presence of tissue cromophores.

![FIG. 1. Schematic representation of laser irradiation over time: (A) Representative gingival surface area subjected to laser treatment: irradiation is delivered to consecutive, not overlapped tissue stripes. (B) The overlapping circles represent the succession of the laser beam footprints on the target surface.](image-url)
threshold. We first showed that the 810 nm diode laser used in contact mode caused minimal thermal spreading to the tissues nearby, especially upon air spray cooling. We then demonstrated that the energy transfer changed substantially among normal, inflamed and hyperpigmented gingival mucosa. Under constant laser settings, the maximum temperature of the targeted tissue was substantially higher in the presence of inflammation and hyperpigmentation (Table 2). In these circumstances, to overcome the risk for undesired mucosal injury, it was sufficient to shift the diode laser from continuous to pulsed mode (Table 2). Moreover, the thermographic probe integrated in the laser instrument offered the possibility to properly set the temperature threshold alarm and laser irradiation switch-off when the tissue temperature reached a dangerous value, thus substantially reducing the possibility of undesired gingival damage.

The histopathological analysis performed on gingival biopsies after laser irradiation confirmed the thermographic findings. Figure 3 shows representative images of hyperpigmented gingiva with slight periodontal inflammation (A) and normally pigmented gingiva with severe periodontitis before laser treatment (B). When applied to normally pigmented gingivae, the 810 nm diode laser operating in continuous mode yielded a complete removal of the squamous epithelium without causing appreciable changes to the stromal and microvessel components of the lamina propria (Fig. 3C). By contrast, when applied on hyperpigmented gingivae it induced a diffuse heat-induced coagulation of the papillary connective tissue and microvessels (Fig. 3D). However, no signs of stromal injury were observed when the irradiation was performed in pulsed mode (Fig. 3E). In all instances, laser irradiation was associated with prominent microvessel constriction, which was consistent with the clinical observation of minimal intraoperative bleeding.

Finally, the clinical outcome was satisfactory in all the cases. No patients complained of pain during laser irradiation or of delayed healing or complications after the treatment. In the long term (up to 6 months), no recurrence of hyperpigmentation was observed in the subjects treated for PGH.

**Monitoring of photoablative laser treatment by autofluorescence detection**

The diode laser device used in our study was also equipped with a violet light probe emitting at λ 405 nm and stimulating a yellow autofluorescence by the laser-treated tissues, which could be viewed using yellow-green filters.

<table>
<thead>
<tr>
<th>Laser mode</th>
<th>Gingiva</th>
<th>Continuous wave</th>
<th>Pulsed wave</th>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Hyperpigmented</td>
<td>Normal</td>
</tr>
<tr>
<td>Airflow cooling</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>45 ± 3</td>
<td>170 ± 12</td>
<td>50 ± 5</td>
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<tr>
<td>T&lt;sub&gt;min&lt;/sub&gt;</td>
<td>40 ± 2</td>
<td>140 ± 9</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>ΔT</td>
<td>5</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>42 ± 3</td>
<td>113 ± 9</td>
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<td></td>
<td>5</td>
<td>23</td>
<td>8</td>
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Values are expressed as °C measured at the target tissue.
FIG. 3. Representative gingival biopsies taken in the different conditions: (A) hyperpigmented gingiva, showing a continuous basal layer of melanized keratinocytes and sparse perivascular inflammatory infiltrate. (B) Normally pigmented gingiva with severe periodontitis, showing a dense inflammatory infiltrate in the lamina propria. (C) Laser treatment in continuous wave mode on normally pigmented gingiva, showing complete removal of the surface epithelium and negligible thermal damage of the underlying connective tissue, whose cells appear normal (inset). (D) Laser treatment in continuous wave mode on hyperpigmented gingiva, also showing complete epithelial removal, but with signs of heat-induced damage of the lamina propria, consisting in a superficial band of collagen hyalinosis (H-bars and inset). (E) Laser treatment in pulsed wave mode on hyperpigmented gingival, showing complete epithelial photoablation in the absence of heat-related damage of the lamina propria (inset). When present, arrows point at the margins of the photoablated tissue. Scale bars, 100 μm.

(Fig. 2B). During the photoablative treatments, this optical phenomenon allowed a very sharp recognition of the borders between laser-ablated and intact epithelium (compare with Fig. 2A).

Discussion

Because of the different intrinsic characteristics and wide range of setting possibilities offered by the medical lasers available for photoablative purposes, a comparison among the numerous studies existing in the scientific literature aimed at defining consensus therapeutic protocols for oral/dental diseases is practically impossible and meaningless. For this reason, it is particularly important to set up reliable methods to get a feedback control over laser irradiation. In the present study, we have used a mathematical model to calculate the energy density, or fluence, transmitted to the target tissue during photoablative laser treatment. This model indicates that the appropriate irradiation procedure consists in photoablation of the gingival mucosa by contiguous stripes by a fiber hot tip, 2.5 mm/sec, under airflow cooling.

From a practical aspect, during photoablative treatment it is important to carefully discriminate between laser-ablated and untreated gingival mucosa to avoid repeated irradiation. Correct photoablative treatment should lead to an effective removal of the surface epithelium without causing thermal damage of deep periodontium; this can ensure fast and complete wound healing, avoid bleeding at treatment, and reduce the risk for delayed complications such as gingival scarring. To these purposes, real-time thermographic measurement can represent a useful tool to maximize irradiation of the target tissue and minimize thermal spreading. We also noted that, with the same laser settings, the maximum target temperature was substantially higher in inflamed and hyperpigmented gingiva. To overcome the risk for undesired mucosal injury, it was sufficient to shift the diode laser from continuous to pulsed mode: in this way the tissue irradiation energy also varied slightly from 66.67 to 40 J/cm², with no substantial outcome differences. To our knowledge, the used instrument is among the few commercial diode lasers capable of operating both in continuous mode and high frequency (short pulse width) pulsed mode. Moreover, this is the only dental laser equipped with a 405 nm light to detect autofluorescence and a thermographic feedback probe to automatically switch off laser irradiation when tissue temperature exceeds the programmed damage threshold. These features make it a particularly versatile therapeutic tool.

Besides real-time thermography, the peculiar autofluorescent phenomenon observed upon illumination with λ 405 nm violet light offers a valuable indication to achieve accurate removal of the gingival lining and minimize the risk for overlapped irradiations. The exact
mechanism underlying the laser-induced autofluorescence remains a matter of speculation: it may likely depend on thermal modifications of epithelial prekeratins, keratin being the main fluorophore in the stratum corneum. Moreover, as an anecdotal observation, we have obtained a similar autofluorescent effect after laser irradiation of a nail lamina, which is almost exclusively composed of hard keratins.

Histological analysis performed on selected biopsies after the photoablative laser treatment show that the adopted photoablative procedures allowed for complete removal of the gingival epithelium with no signs of microvascular and extracellular matrix injury, as we previously reported in a similar study. This is consistent with the overall subjective and objective clinical amelioration of the patients observed after the laser treatment. It is of note that in PGH, radical ablation of the pigmented epithelium greatly reduces the probability of re-invasion by melanocytes from the surrounding gingival and interdental papilla. Most patients perceived very little discomfort during treatment, even in the absence of local anaesthesia, and had an overall preference for the laser modality, as was also recently reported elsewhere. Moreover, the clinical course was uneventful in all cases and no complications arose throughout the follow-up.

Conclusions

This study further supports the favorable characteristics of photoablative λ 810 nm diode laser for selective photoablation of the diseased gingiva, in keeping with previous reports. Although a higher number of studied patients would be desirable to draw definitive conclusions, the present findings strongly suggest that real-time thermographic and fluorescent analyses can provide substantial help to the setting of safe and well-tolerated therapeutic protocols.

Gingival photoablation performed with a λ 810 nm diode laser can yield complete removal of the surface epithelium with minimal injury to the underlying lamina propria. The irradiation mode needs to be properly adjusted in the presence of inflamed or hyperpigmented gingivae. To this purpose, thermographic control can provide substantial help. Moreover, irradiation-evoked tissue autofluorescence, conceivably caused by epithelial keratins, allows sharp recognition of the borders between treated and intact epithelium, and prevents repeated irradiation.

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Author Disclosure Statement

Dr. Massimo Lasagni is employed in the Research and Development section of General Project Ltd. Drs. Marco Giannelli, Lucia Formigli, and Daniele Bani have no competing financial interests.

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