Comparative Evaluation of Photoablative Efficacy of Erbium: Yttrium-Aluminium-Garnet and Diode Laser for the Treatment of Gingival Hyperpigmentation. A Randomized Split-Mouth Clinical Trial

Marco Giannelli,* Lucia Formigli, † and Daniele Bani †

Background: The use of lasers in periodontology is a matter of debate, mainly because of the lack of consensual therapeutic protocols. In this randomized, split-mouth trial, the clinical efficacy of two different photoablative dental lasers, erbium:yttrium-aluminum-garnet (Er:YAG) and diode, for the treatment of gingival hyperpigmentation is compared.

Methods: Twenty-one patients requiring treatment for mild-to-severe gingival hyperpigmentation were enrolled. Maxillary or mandibular left or right quadrants were randomly subjected to photoablative deepithelialization with either Er:YAG or diode laser. Masked clinical assessments of each laser quadrant were made at admission and days 7, 30, and 180 postoperatively by an independent observer. Histologic examination was performed before and soon after treatment and 6 months after irradiation. Patients also compiled a subjective evaluation questionnaire.

Results: Both diode and Er:YAG lasers gave excellent results in gingival hyperpigmentation. However, Er:YAG laser induced deeper gingival tissue injury than diode laser, as judged by bleeding at surgery, delayed healing, and histopathologic analysis. The use of diode laser showed additional advantages compared to Er:YAG in terms of less postoperative discomfort and pain.

Conclusions: This study highlights the efficacy of diode laser for photoablative deep epithelialization of hyperpigmented gingiva. It is suggested that this laser can represent an effective and safe therapeutic option for gingival photoablation. J Periodontol 2014;85:554-561.

KEY WORDS
Clinical trial; gingiva; hyperpigmentation; lasers, semiconductor; lasers, solid state; melanins.

P hysiologic gingival hyperpigmentation (PGH), caused by excessive melanin deposition by melanocytes mainly located in the basal and suprabasal cell layers of the epithelium, affects numerous people of different ethnic backgrounds. Although PGH is definitely benign and does not represent a health concern, complaints of dark gums are common, particularly among individuals with excessive gingival display during smiling or talking, which compels them to seek appropriate cosmetic treatment. Gingival depigmentation has been performed using various methods and techniques, including mechanical abrasion, surgical removal, cryosurgery or electro-surgery, and chemical etching, with different degrees of success. Moreover, some of these techniques are prone to side effects and complications. In recent years, the use of laser photoablation has been recognized as one of the most effective, pleasant, and reliable techniques for this purpose. The commonly used lasers for gingival deep epithelialization include semiconductor diode, erbium: yttrium-aluminium-garnet (Er:YAG), neodymium:yttrium-aluminum-garnet

* Odontostomatologic Laser Therapy Center, Florence, Italy.
† Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Florence, Italy.

doi: 10.1902/jop.2013.130219
(Nd:YAG),\(^{13}\) and CO\(_2\).\(^{13,14}\) High-power lasers (CO\(_2\), \(\text{Nd:YAG}\), diode \(\lambda\) 810 to 980) are preferred for soft-tissue surgery because they cause tissue ablation/vaporization, hemostasis, and sterilization.\(^{13,14}\) Instead, Er:YAG laser is commonly used to target hard tissues, such as bone, enamel, cementum, and dentin, and it is recently gaining importance and interest for gingival de-epitelization.\(^{15}\) Recent research has centered on pulsed diode laser (\(\lambda\) 810) used in photoablative mode in periodontal surgery; this has been primarily used for oral surgery of the tongue and gingiva and in chronic periodontitis to remove the infected epithelium inside and around periodontal pockets.\(^{16}\) Indeed, this laser has some advantages compared to the others, such as easier gingival reshaping, reduced need for local anesthesia, excellent hemostasis, minimal thermal injury of the deeper tissues, and negligible postoperative pain and inflammation.\(^{16,17}\) All these characteristics may also be important in the use of diode laser for cosmetic purposes, including the removal of benign gingival hyperpigmentation. In keeping with this hypothesis, there is evidence in the recent literature of successful depigmentation using diode lasers.\(^{10,18,19}\)

In the present study, the authors want to further expand the knowledge on the issues discussed above by analyzing and comparing the effects of a diode laser (\(\lambda\) 810 nm) and an Er:YAG laser (\(\lambda\) 2,940 nm) on gingival depigmentation in terms of the following: 1) clinical outcome in the short term and midterm (up to 6 months of follow-up); 2) histologic response of gingival tissue to laser photoablation; and 3) patients’ discomfort during treatment and preference for either treatment modality.

**MATERIALS AND METHODS**

**Patients**

The study was designed in compliance with the guidelines of the Declaration of Helsinki, as amended in Edinburgh 2008, and it was performed in keeping with the CONSORT (Consolidated Standards of Reporting Trials) consolidated standards of reporting trials,\(^{20}\) as summarized in Figure 1. It was approved by the Ethical Committee of the University of Florence Faculty of Medicine, Florence, Italy. The clinical part of the study was conducted at the Odontostomatologic Laser Therapy Center, Florence, Italy, from March 2012 to February 2013. Twenty-five patients were assessed for eligibility, and 21 of them (10 males and 11 females, aged 18 to 40 years; mean age: 26.5 years) enrolled in the study and signed a written informed consent. They presented hypopigmentation of the mandibular/maxillary vestibular gingiva at the first clinical observation. Exclusion criteria included the following: 1) history of systemic diseases; 2) pregnancy and lactation; and 3) heavy smoking habit (\(\geq\)20 cigarettes/day). The inclusion criterion was moderate-to-severe bilateral melanin hyperpigmentation of the upper and lower gingivae, as detailed below. Before treatment, small samples of gingival soft tissue, \(2 \times 2\) mm, were taken with a biopsy punch for histopathologic purposes to confirm the clinical diagnosis of benign melanin pigmentation. Seven days later, the patients were recalled and subjected to laser treatment. Then, a second biopsy was performed to exactly determine tissue ablation and thermal damage. When deemed appropriate to reduce hypertrophic gingivae, additional biopsy specimens were taken from six quadrants in three patients after 6 months to determine the midterm histologic response of gingival tissue to laser ablation.

**Pretreatment and Post-Treatment Clinical Assessment**

The degree of gingival pigmentation was scored according to the Dummet Oral Pigmentation Index:\(^{21}\) 1 = pink tissue, no clinical pigmentation; 2 = mild light brown tissue, mild clinical pigmentation; 3 = medium brown or mixed brown and pink tissue, moderate clinical pigmentation; 4 = deep brown/blue–black tissue, heavy clinical pigmentation.

The treatment outcome was semiquantitatively evaluated by the following clinical parameters. Wound healing included the following: 1) complete reepithelialization, 2) incomplete reepithelialization, 3) ulcer, and 4) tissue defect or necrosis. Assessment was performed with the aid of a high-resolution digital video microscope\(^\dagger\) at \(\times50\) magnification, which allowed the authors to easily distinguish between intact epithelium and fibrin-coated connective tissue. Bleeding at surgery included the following: 1) none, 2) slight, 3) moderate, and 4) severe.

Scoring values of the upper and lower quadrants were merged. The clinical parameters were objectively evaluated and scored by the operator (MG) at admission (day 0) and at days 7, 30, and 180 after the treatments.

**Patient Randomization and Allocation**

In each patient, the upper and lower quadrants were randomly subjected to one or the other treatment, i.e., diode or Er:YAG laser. Allocation concealment was performed by sequentially numbered, opaque sealed envelopes. For each patient, the randomization envelope was opened immediately before the treatment. Treatment assignment was registered by a non-clinical investigator (LF) and kept concealed.

\(^\dagger\) Dino-Lite, Iteleco, Turin, Italy.
to the investigator who performed the statistical analyses (DB) until completion of the study. The non-clinical investigator was also in charge of administration to the patients of a satisfaction questionnaire, reported in Table 1, for subjective evaluation of treatment.

**Photoablative Laser Treatment**

All laser procedures were performed by the same expert operator (MG). The patients were subjected to photoablative treatment using the following devices: 1) Er:YAG laser, λ 2,940 nm, pulsed-wave mode; and 2) diode laser, λ 810 nm, pulsed-wave mode.

The detailed irradiation parameters, chosen on the basis of those reported previously for the treatment of gingival epithelial ablation, are given in Table 2.

Laser energy output was measured with a power meter before each procedure. To minimize harmful photothermal effects, photoablation was performed under either air/water flow (Er:YAG) or airflow (diode) cooling. Photoablation began at the free gingival margin and proceeded toward the mucogingival junction, including the interdental papilla. Irradiation was performed in contact mode, with the fiber tip touching the gingival epithelium. For the diode laser, 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 coagulation at the incision line. Excess carbonized debris was removed with wet gauze. To prevent heat-induced tissue damage, the treatment was performed under continuous infrared thermographic monitoring, setting an 80°C thermal threshold as the target. The tip was moved at a constant speed of 2.5 mm/second, evaluated visually, to minimize gingival thermal damage, as demonstrated previously. The diode laser was also equipped with a violet-light-emitting diode probe emitting at λ 405 nm, which stimulated autofluorescence of the photoablated tissue and was clearly visible when wearing yellow-green filtered goggles, allowing precise targeting of the photoablative laser beam; both probes were included in the 4 × 4 dental laser.

Local anesthesia was usually unnecessary and was only performed upon patient demand. Eye

---

**Figure 1.** CONSORT flowchart of the clinical trial.
protection of the patients and the operator was ensured by wearing safety glasses.

Post-Treatment Instructions
Patients were instructed to discontinue toothbrushing on the day of laser photoablation to prevent mechanical trauma at the treated sites and facilitate reepithelialization. From day 2, normal tooth hygiene with toothbrush and interproximal instruments was encouraged. Local use of chlorhexidine digluconate or other local medications was not prescribed.

At the 1-day postoperative visit, patients were asked to grade the treatment modalities in their order of preference. One day and 1 week after surgery, the patients were asked to respond, based on visual analog scores, about their perceived degree of pain/discomfort.

**Table 1. Satisfaction Questionnaire**

<table>
<thead>
<tr>
<th>Question</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was the treatment painful?</td>
<td>1, no pain; 2, mild pain; 3, severe pain</td>
</tr>
<tr>
<td>Did you experience pain on the day of the treatment?</td>
<td>1, no at all; 2, mild; 3, severe</td>
</tr>
<tr>
<td>Did you experience pain during the first week after the treatment?</td>
<td>1, no pain; 2, mild pain; 3, severe pain</td>
</tr>
<tr>
<td>Did you notice a cosmetic change 1 week after the treatment?</td>
<td>1, not at all; 2, moderate; 3, marked</td>
</tr>
<tr>
<td>Did you notice a cosmetic change 6 months after the treatment?</td>
<td>1, not at all; 2, moderate; 3, marked</td>
</tr>
<tr>
<td>Did the treatment meet your expectations?</td>
<td>1, no; 2, yes; 3, over and above</td>
</tr>
<tr>
<td>Would you repeat the treatment if necessary?</td>
<td>1, no; 2, yes; 3, over and above</td>
</tr>
</tbody>
</table>

Modified from McGill Pain Questionnaire.²²

**Table 2. Laser Irradiation Parameters**

<table>
<thead>
<tr>
<th>Laser beam characteristics</th>
<th>Diode</th>
<th>Er:YAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>810 nm</td>
<td>2,940 nm</td>
</tr>
<tr>
<td>Irradiation mode</td>
<td>Pulsed wave</td>
<td>Pulsed wave</td>
</tr>
<tr>
<td>Pulse energy</td>
<td>69 mJ</td>
<td>100 mJ</td>
</tr>
<tr>
<td>Pulse frequency</td>
<td>8,000 Hz</td>
<td>10 Hz</td>
</tr>
<tr>
<td>Pulse duration</td>
<td>18 μs</td>
<td>400 μs</td>
</tr>
<tr>
<td>Power (beam, average)</td>
<td>0.6 W</td>
<td>1 W</td>
</tr>
<tr>
<td>Fiber diameter</td>
<td>0.6 mm</td>
<td>0.8 mm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surface treatment data</th>
<th>Contact tip</th>
<th>Contact tip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser spot at target diameter/area</td>
<td>0.6 mm/0.283 mm²</td>
<td>0.8 mm/0.503 mm²</td>
</tr>
<tr>
<td>Fiber movement speed</td>
<td>2.5 mm/s</td>
<td>2.5 mm/s</td>
</tr>
<tr>
<td>Cooling</td>
<td>Airflow</td>
<td>Air/water flow</td>
</tr>
<tr>
<td>Total energy density (fluence)</td>
<td>40 J/cm²</td>
<td>50 J/cm²</td>
</tr>
</tbody>
</table>

**Biopsy Collections and Morphologic Analyses**
After admission and 180 days after the laser treatments, small samples of gingival tissue, ≈2 × 2 mm, including surface epithelium and the underlying lamina propria, were taken under local anesthesia using a 2-mm-diameter biopsy punch, paying attention not to expose the periosteum (n = 13, seven taken before and six after the treatment). The biopsies collected at the 180-day follow-up were taken at sites in which there was a therapeutic indication to reduce the periodontal pockets (three patients). For conventional histology, samples were fixed by immersion in 4% (weight/volume) formaldehyde in 0.2 M phosphate-buffered saline (pH 7.4), dehydrated in graded ethanol, and embedded in paraffin. Five-micrometer-thick sections were stained with hematoxylin and eosin (H&E) and photographed under a light microscope.
Statistical Analyses
The patient’s quadrant was assumed as the test unit for statistical comparison. Values were expressed as mean ± SEM. The clinical parameters, which varied depending on treatment and time, were first analyzed by two-way repeated-measures analysis of variance (ANOVA) to assess whether the interaction between the two variables was significant. If so, differences between each time point were assessed by paired t test, followed by Bonferroni multiple comparison test.25 The clinical parameters, which varied depending on treatment alone, namely those of the satisfaction questionnaire, were analyzed by Student t test for paired values. P < 0.05 was considered significant.

RESULTS
All the enrolled patients successfully completed the study. The results of the semiquantitative analysis of the clinical parameters, e.g., gingival color, wound healing, and bleeding at surgery, performed before or soon after the treatments (day 0) and during the follow-up are reported in Figure 2. Both Er:YAG and diode laser treatments caused a significant improvement of gingival hyperpigmentation. No recurrence of gingival pigmentation was found in any of the patients during the follow-up period (Fig. 2A). Er:YAG laser induced deeper gingival mucosal injury than diode laser, as judged by the persistence of scattered deepithelialized areas 7 days after treatment that were not found in any of the quadrants treated with diode laser (Fig. 2B). Moreover, bleeding at surgery was common with Er:YAG laser, although it was never observed in the mucosa treated with diode laser (Figs. 2C and 3C). Representative clinical images taken before, during, and after the treatments are shown in Figures 3A, 3C, and 3F.

The histopathologic analysis performed on gingival biopsies after laser irradiation confirmed and extended the clinical findings. Before laser treatment, the hyperpigmented gingiva showed numerous melanin deposits in the basal and suprabasal epithelial cell layers (Fig. 3B). The diode laser yielded a complete, uniform removal of the squamous epithelium with no appreciable changes of the stromal and microvessel components of the lamina propria (Fig. 3D). Conversely, Er:YAG laser irradiation often caused an incomplete ablation of the gingival epithelium, with the deeper epithelial ridges remaining in place (Fig. 3E). This required repeated passages and increased the risk of causing damage to the lamina propria. Moreover, Er:YAG laser irradiation was often accompanied by microvessel dilation, likely accounting for the marked bleeding at surgery. With the diode laser, the hyperpigmented epithelium

Figure 2.
Outcome of the noted clinical parameters during follow-up after the treatments with either Er:YAG or diode lasers. Differences were assessed by two-way repeated-measures ANOVA and paired t test, followed by Bonferroni multiple comparison test. n.s. = not significant.
appeared to absorb laser energy with higher efficacy than the normally pigmented one. In fact, histologic signs of coagulation of the superficial stroma were prominent when the instrument was set in continuous mode (data not shown). For this reason, it was found that a pulsed-wave irradiation mode was required for optimal photoablative results with both of the lasers used. The biopsies taken at the 180-day follow-up showed a normal gingival mucosa, with no features of residual hyperpigmentation (Fig. 3G).

As a final clinical note, the post-treatment course was uneventful in all patients, and no complications, such as ulcers, persistent bleeding, or infections, were observed throughout the follow-up. Moreover, all patients except one perceived slight or no pain and discomfort during the laser treatments and did not require local anesthesia. Subjective perception of Er:YAG versus diode laser treatment evaluated by the patients’ responses to a satisfaction questionnaire showed an overall preference for the diode laser (Table 3).

**DISCUSSION**

Despite their indisputable merits in oral surgery, the complexity of medical lasers in terms of different wavelengths, energy output modes, and setting parameters has produced a multiplicity of clinical protocols with different outcomes, thus hampering comparison of the results and identification of univocal guidelines for their use. In the present study, two different lasers widely used for oral surgery, namely Er:YAG and diode laser, were compared with the purpose to define suitable irradiation protocols for successful removal of hyperpigmented gingival epithelium with minimal undesired mucosal damage. It was found that Er:YAG laser irradiation, although it was able to induce complete epithelial photoablation and improve PGH in the long term, required caution to be properly executed. Indeed, it often caused an incomplete ablation of the gingival epithelium, with the deeper epithelial ridges remaining in place, thus requiring repeated passages and increasing the risks of damaging the lamina propria. It also caused marked blood vessel dilatation, accounting for delayed gingival healing and bleeding at treatment. This was likely attributable to its mode of action, causing a sudden vaporization of water contained in the targeted tissues. Better results were obtained using the diode laser; with the noted irradiation parameters, it yielded a complete removal of the gingival surface epithelium without causing stromal damage and microvessel dilatation. Rather, epithelial photoablation was accompanied by microvessel narrowing, possibly related to direct vasomotor...
effects and/or deactivation of local proinflammatory mediators by the diode laser light. These features may likely be attributed to heat-induced coagulation of the targeted tissues induced by this laser type. During the follow-up, the quadrants treated with the Er:YAG laser showed delayed healing, with a higher bleeding score during the treatment and a higher injury score at day 7 compared to the diode laser. Thus, the diode laser offers the advantage of a successful and safe application by being able to prevent bleeding, limiting postoperative inflammation and pain, and favoring healing of the gingival mucosa. These findings confirm and extend the previous data on the successful application of laser techniques for the treatment of gingival hyperpigmentation and provide novel evidence that the diode laser fulfills the clinical rationale for minimally invasive approaches that reduce intraoperative and postoperative trauma.

Using either Er:YAG or diode laser, it should be pointed out that complete deep epithelialization requires that the instruments are used in contact mode to get optimal control of the laser beam without damaging the neighboring tissues, such as teeth and alveolar bone, and are set in pulsed-wave mode to reduce light absorption by chromophores, such as melanin and oxyhemoglobin, and to limit harmful thermal energy accumulation.

At the 6-month follow-up, both Er:YAG and diode lasers gave satisfactory clinical results, with no recurrence of PGH. It has been suggested that recurrence of gingival hyperpigmentation may initiate from epithelial resettlement of residual melanocytes migrated from the gingival areas that are less easily accessible to surgical maneuvers, such as the gingival margins and interdental papilla. Therefore, the radical ablation of hypermelanized gingivalae, as afforded by both laser treatments, can greatly reduce the probability of PGH relapse in the long term.

CONCLUSIONS
The results of the present study highlight the efficacy of dental lasers, especially the diode laser, for photoablative deep epithelialization of PGH. Compared to Er:YAG lasers, which are among the most diffuse oral-care laser devices, diode lasers produce similar clinical results in terms of esthetic outcome, plus the major advantage of minimal injury of the targeted gingiva. These features can favor an ever-increasing diffusion of diode lasers among dental practitioners to expand their therapeutic repertoire.

ACKNOWLEDGMENTS
The authors are grateful to Dr. Alessia Tani, Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Florence, Italy, for skillful help in the preparation of histologic specimens. The authors report no conflicts of interest related to this study.

REFERENCES


