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Research Article

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Gingival Depigmentation Using 445, 940, and 2780 nm Lasers at Various Settings Evaluated By Optical Coherence Tomography and Confocal Laser Microscopy

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Abstract

Several different techniques including lasers have been used for gingival depigmentation to date. The diversity of the wavelengths, application methods, and settings used in laser treatments has necessitated the development of application standards that meet the biological requirements of the tissues while ensuring the esthetic effects of the used methods. Thus, the aim of our study was to evaluate the tissue effects of lasers with wavelengths of 445, 940, and 2780 nm and various laser settings by using optical coherence tomography (OCT) and confocal laser microscopy.

Fifty-four dark pigmented gingival biopsy specimens were randomly divided into 9 groups and treated using a 445 nm diode laser (200-400 mW, 320 µm tip, continuous wave [cw], non-contact mode), 940 nm diode laser (600-800 mW, 400 µm tip, cw, contact mode) or 2780 nm laser (1.5-2.5 W, MC3 chisel tip, 30 Hz, 30% air, 40% water, contact mode). Every setting was repeated in 5 depigmentation procedures. The treated gingival samples were examined using the CIRRUS OCT 5000 device (Carl Zeiss Meditec Inc., Germany) and the laser confocal microscope Olympus LEXT OLS5000 (Olympus Corporation, Tokyo, Japan) immediately after laser irradiation.

All examined wavelengths could effectively remove gingival melanin pigmentations. The Er,Cr:YSGG laser (2780 nm) was especially effective and safe for gingival depigmentation because of its very thin effective laser trace and power range between 1.5 and 2.0 W together with the continual scanning movement of the laser tip, which yielded optimal depigmentation for gingival tissue. With the 940 nm diode laser, the effective power range that did not involve damage to the lower structures ranged between 600 and 700 mW, cw, with continual movement of the laser tip. Moreover, non-contact application of the 445 nm laser using only 200 mW power in the continuous mode appeared to be a promising method for gingival depigmentation.

Keywords: Depigmentation, Diode laser, Erbium laser, Gingival pigmentation

Introduction

Gingival hyperpigmentation can be defined as a darker-than-normal gingival color [1]. This condition has an adverse effect on esthetics and also causes psychological negativity. Gingival color is an important parameter of visible oral esthetics, and hyperpigmentation is an indication for correction for many people. The gingiva participates in the harmony of the facial features during verbal communication and smile, and its color plays a crucial role in this function. Therefore, unusual pigmentation of the gingiva is considered to be unaesthetic by patients and may have a psychological impact.

Hyperpigmentation can be attributed to several endogenous and exogenous etiological agents. The common causes of hyperpigmentation are melanin, melanoid, reduced hemoglobin, carotene, bilirubin, and iron. Melanin pigmentation occurs as a result of melanin granules produced by melanocytes [2]. The deposition of these granules results in diffuse, irregular patches of purple and brown, necessitating treatment of the gingival hyperpigmentation [3].

Gingival depigmentation can be defined as a periodontal cosmetic procedure whereby gingival hyperpigmentation is removed or reduced by various techniques [4]. Depigmentation is not a clinical indication but a treatment of choice where esthetics is the primary concern, and correction of the pigmentation is desired by the patient [5,6]. To date, several different techniques have been used for gingival depigmentation, including the scalpel technique, cryosurgery, electrosurgery, chemical methods, masking the pigmented gingiva with less pigmented gingival areas (free gingival

graft-acellular dermal matrix allograft), and lasers [7].

Various types of lasers, including argon, CO2, erbium, diode, Nd:YAG, Ho:YAG, and KTP lasers, have been used for the treatment of gingival hyperpigmentation. The corresponding chromophores of each laser are located at various layers of the gingival epithelium, and their mechanisms of pigment removal are slightly different. For procedures involving the CO2, erbium family, and Ho:YAG lasers, water plays a crucial role since water molecules are also present in the most superficial layers of the gingival epithelium. The laser photon energy absorbed by the cellular water content produces micro-explosions in a process called water-induced ablation, which affects the surface of the gingival epithelium. When the laser photon energy is absorbed by the melanin or hemoglobin chromophores, the laser exhibits thermal effects as a result of heat accumulation, thereby causing excision and coagulation [8]. When high-power lasers are used in gingival depigmentation, their effects are based on the thermal interactions that produce heat and increase the kinetic energy of irradiated tissue. These effects are subsequently detectable as coagulation, denaturation, vaporization, necrosis, and carbonization.

The depigmentation techniques listed above involve different mechanisms of effectiveness and are associated with various potential adverse effects. Chemical methods destroy the gingival epithelium and inhibit melanin formation, but the unpredictable extent of these destructive activities is their main adverse effect. Mechanical removal of gingival epithelium is associated with pain and postoperative discomfort and is contraindicated in people with a thin gingival biotype. Grafting methods are more complex surgical procedures associated with postoperative discomfort and frequently involve problems associated with color mismatches of the tissue grafts. Electrosurgery is based on epithelial coagulation, but the accumulated heat can cause damage to the surrounding structures and result in a painful healing period. Freezing of the gingiva and subsequent cryonecrosis is a therapeutic mechanism based on cryosurgery that is associated with the same limitations as electrosurgery in addition to the uncontrolled penetration of the temperature applied and postoperative swelling. Although laser therapy has been reported to show optimal efficacy in the correction of gingival hyperpigmentation, inappropriate laser application can damage the gingiva and underlying alveolar bone with negative outcomes for the normal anatomy of the dento-gingival complex [8].

Recurrence of gingival pigmentation is also a very important parameter to be considered in the evaluation of different therapeutic methods. Although laser application is reported to be a superior technique with excellent esthetic results and low rates of recurrence, the diversity of used wavelengths, application methods, and settings has necessitated the development of application standards that simultaneously address the biological requirements of the tissues and the esthetic effects of the used method. In this regard, the aim of the present study is to assess the tissue effects of lasers with wavelengths of 445, 940, and 2780 nm and various laser settings by using optical coherence tomography (OCT) and confocal laser microscopy.

Optical coherence tomography is a noninvasive imaging technique that can be employed for both real-time and in situ investigations and can perform a cross-sectional evaluation of tissue microstructure on the basis of the specific intensity of backscattered and reflected light [9]. Optical coherence tomography utilizes near-infrared low-coherence interferometry to gate the detection of ballistically backscattered photons at varying tissue depths [10]. Depth-resolved images are generated from light-based echoes analogous to ultrasound resolved from the interference of a reference and the sample arm beam [11]. Although OCT generally provides higher-resolution images than high-frequency ultrasound, its depth of penetration is limited to 1.3-1.5 mm [12]. Nevertheless, OCT can allow visualization of the oral epithelium, basement membrane, lamina propria, submucosa, submucosal salivary glands, and blood vessels [11]. Depending on the location within the oral cavity, the epithelium shows variable thickness (285 ± 33 µm in the case of the gingiva) [13]. Thus, in the treatment of gingival hyperpigmentation, OCT can detect in vivo clinical changes in the epithelium directly after laser irradiation. Therefore, we used this method to determine the optimal settings of lasers with different wavelengths with respect to pigmentation removal and the biological quality of gingival tissue for healing processes.

Methods

Fifty-four patients exhibiting dark melanin pigmentation in the anterior area of the dental arch indicated for removal were included in this study. These periodontally healthy individuals were between 24 to 37 years of age, and their pigmented lesions were categorized as moderate or heavy clinical pigmentations according to the Gingival Pigmentation Index (GPI) proposed by Kumar [14]. Smokers, patients taking medication that was expected to have a negative influence on healing processes, and individuals with a thin gingival biotype were excluded from the list of potential patients. All patients received information about the therapeutic process and provided informed consent before participating in the study.

Before the therapeutic removal procedure, histological verification of the clinical diagnosis was performed. Under local infiltration anesthesia (Ultracain D forte, Sanofi Aventis, Germany), a piece of gingival tissue containing the epithelium and the underlying connective tissue was excised. After separation, one part of the gingival tissue was placed in a formalin solution and sent for histological processing. The second piece of tissue was used for evaluations with different laser procedures. The obtained specimens were randomly divided into 9 groups. The depigmentation activity of the following lasers was tested in this study:

1. Diode laser, 445 nm, Sirolaser Blue (Dentsply Sirona, Germany)
2. Diode laser, 940 nm, Epic (Biolase, USA)
3. Er,Cr:YSGG laser, 2780 nm, (Biolase, USA).

For every wavelength, three different settings were used, and every setting was repeated in five depigmentation procedures.

In all procedures, rapid movement of the tips over the gingival surface was applied to minimize the thermal effect, especially in procedures using diode lasers. Laser irradiation was stopped in all gingival specimens when the dark pigmentation clinically completely disappeared. Treated samples of gingival tissue were examined using the CIRRUS OCT 5000 device (Carl Zeiss Meditec Inc., Germany) immediately after laser irradiation. Simultaneously, the laser confocal microscope Olympus LEXT OLS5000 (Olympus Corporation, Tokyo, Japan) was used for scanning and visualization of the surface details in 3D space.

During OCT examinations, the following criteria were evaluated:

1. Morphologic structure of the superficial epithelial layer.
2. Depth of structural changes in the epithelium and lamina propria.
3. Accessibility of the basement membrane region with pigment deposition for the used laser systems.

Results

Evaluation of Depigmentation Methods with Reference to OCT Images of Normal Gingiva

The typical gingival structure consists of keratinized multilayer squamous epithelium and lamina propria. The width of both components ranges from 200 to 300 µm depending on individual disposition and localization. The total width of measurable tissue is thus 600-650 µm. The border between the epithelium and lamina propria – basement membrane is not consistently detectable on the OCT image (Figure 1). Color OCT images allow better identification in comparison with black-and-white images (Figure 2).

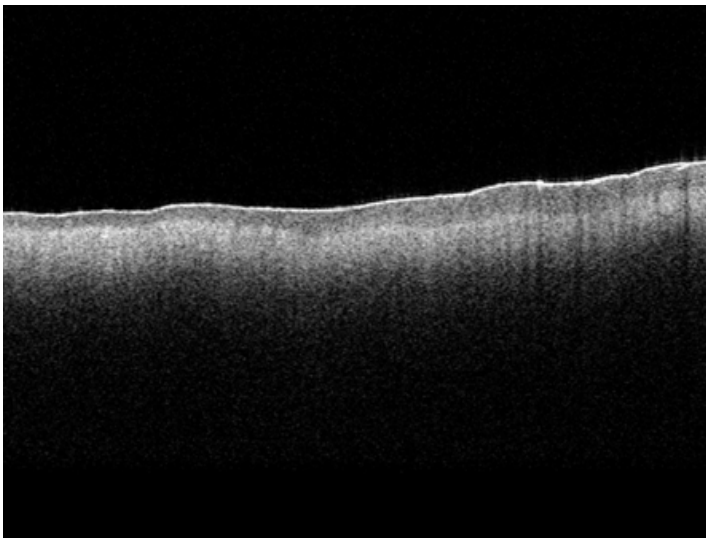

Figure 1: OCT image of normal gingiva

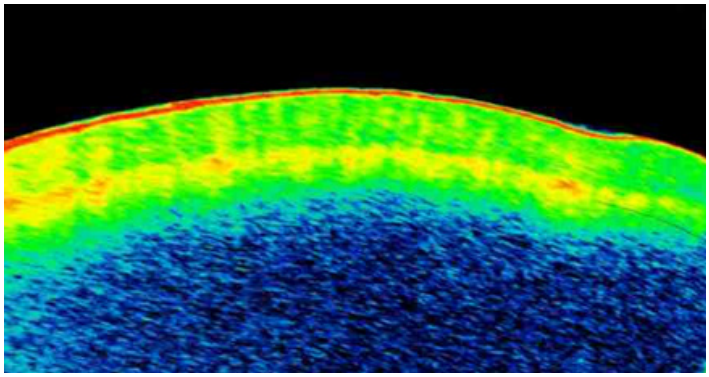

Figure 2: Color OCT image of normal gingiva

Treatment with the 445-nm Laser

Application of 200 mW laser power resulted in depigmentation without morphological changes in the superficial layer. Moreover, the OCT examinations suggested that the keratinized layer was as intact as the epithelial layers. Disorganization of the typical cellular architecture was observed only in the deepest epithelial layers on the border with the basement membrane, since this location corresponds to the anatomical location of melanin and the target of the laser energy (Figure 3). Confocal laser microscopy revealed a completely intact surface of multilayer squamous epithelium (Figure 4). The same tissue findings were noted in all tested gingival tissues treated with 200 mW laser.

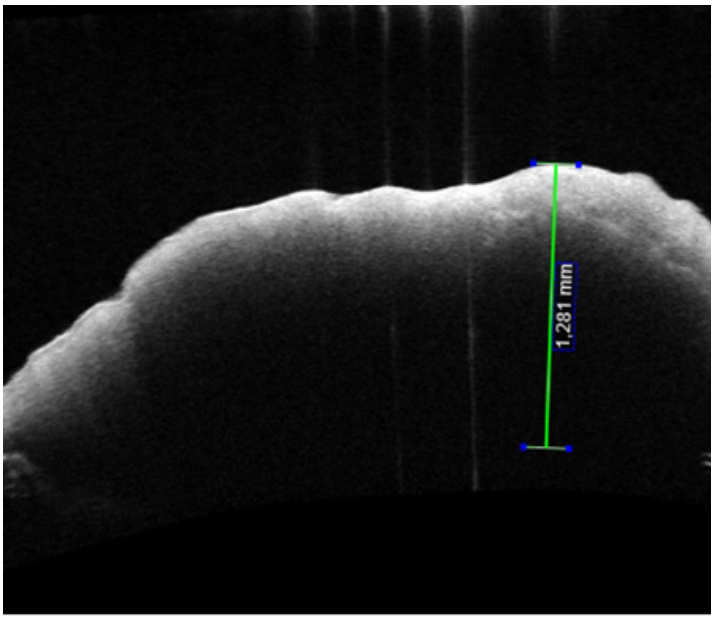

Figure 3: Pigmented gingiva after treatment by 445 nm laser (200 mW power - right side of the image in relationship with untreated gingival tissue – (left side) (OCT examination)

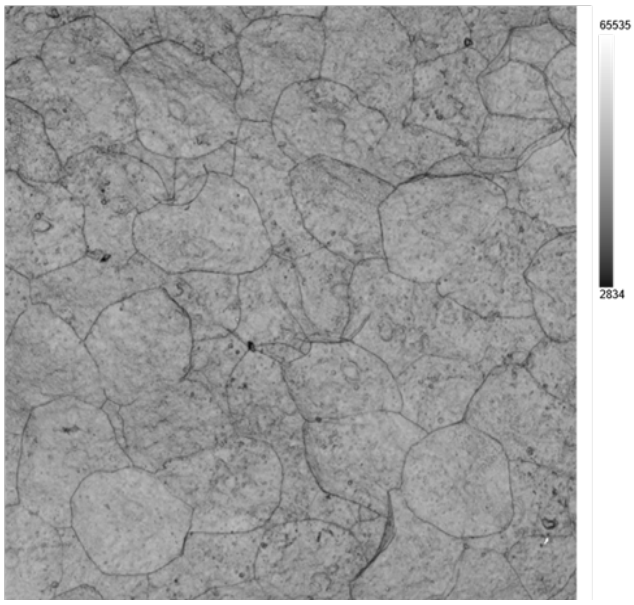

Figure 4: Intact surface of multilayer squamous epithelium after 445 nm laser irradiation (200 mW) (confocal laser microscopy examination)

Application of two-fold higher laser power (400 mW) was also very effective in pigmentation removal and caused no clinical damage to the gingival tissue. However, OCT evaluation showed advanced manifestations of tissue destruction, such as shrinkage of the epithelial layer and basement membrane, partial absence of the keratinized layer, and localized cell destruction inside the epithelial layer (Figure 5a and 5b). Detailed evaluation of the superficial epithelial layer with OCT examinations showed a distorted surface and small spots as traces of the laser tip (Figure 6). Thus, on the basis of the evaluation criteria, the 445 nm laser, especially at 200 mW, can be considered to be safe and non-traumatic for the superficial epithelial layers. Laser energy is then utilized directly and selectively in the zone of melanin deposits with minimal disorganization of the typical architecture of multilayer squamous epithelium. Moreover, the accessibility of target structures is excellent due to the specific wavelength for melanin, and the intact epithelial surface determines the possibility of visual control of pigmentation removal.

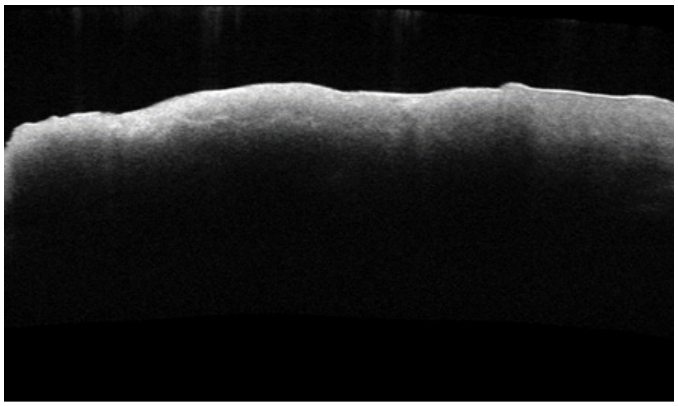

Figure 5a: Pigmented gingiva after treatment by 445 nm laser (power 400 mW)- left side of the picture compared with untreated gingival tissue- (right side) (OCT examination)

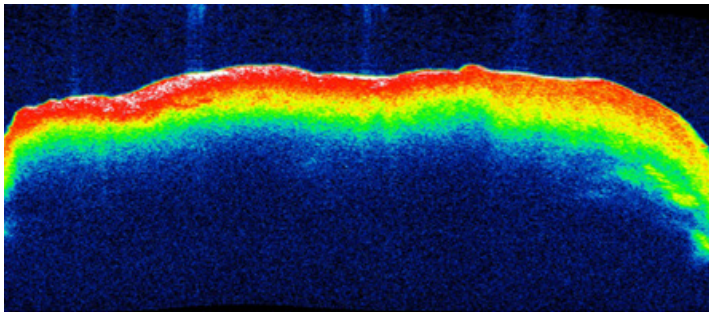

Figure 5b: The same situation from Figure 5a in color OCT modification

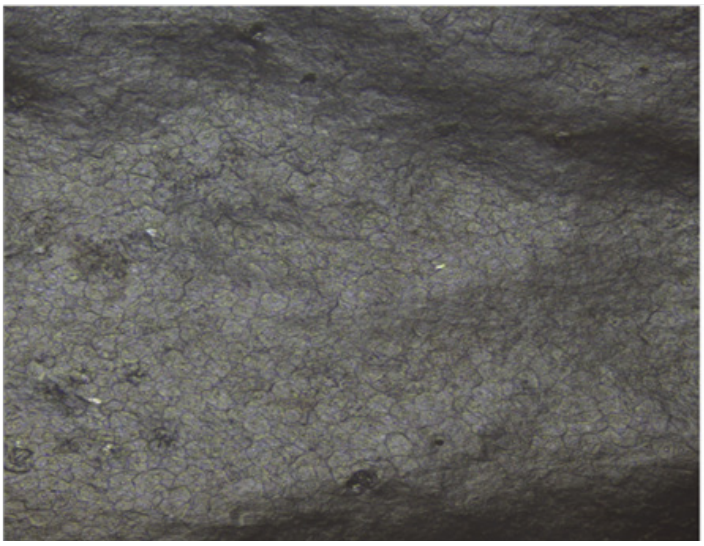

Figure 6: Evaluation of gingival surface using confocal laser microscopy after 445 nm (400 mW) laser irradiation. Slightly distorted surface and small spots as traces of laser tip

Treatment with the 940 nm Laser

This type of treatment is characterized by direct contact of the laser tip with the epithelial surface and penetration of the laser beam to the target structures. The final effect is related to tissue evaporation and the adjacent thermal effect. The typical features observed in OCT examinations were similar across all three settings. Irregular epithelial defects reaching the basement membrane manifested with fuzzy borders and various shapes. OCT examinations also revealed different depths of epithelial defects and a non-homogenous base of the treated area (Figure 7). The mechanism of epithelial cell and melanin removal was confirmed using confocal laser microscopy. Figure 8 documents the interaction between the laser beam and the most superficial layer of epithelial cells and also presents the deeper crater-shaped results of the thermal laser effect. In cases involving higher levels of power density, more locations with carbonized surfaces were detectable. The use of this wavelength in contact mode resulted in destruction of the epithelial layers, which essentially depends on the energy density and exposure duration. The 940-nm wavelength showed good interaction with melanin, thereby improving the quality of accessibility. The base of the lesion after laser irradiation is irregular, and in combination with potential carbonization, can make exact visual control

extremely difficult. Treatment at the higher power level (800 mW) caused morphological changes in the lamina propria but showed protective effects against recurrence on the other side.

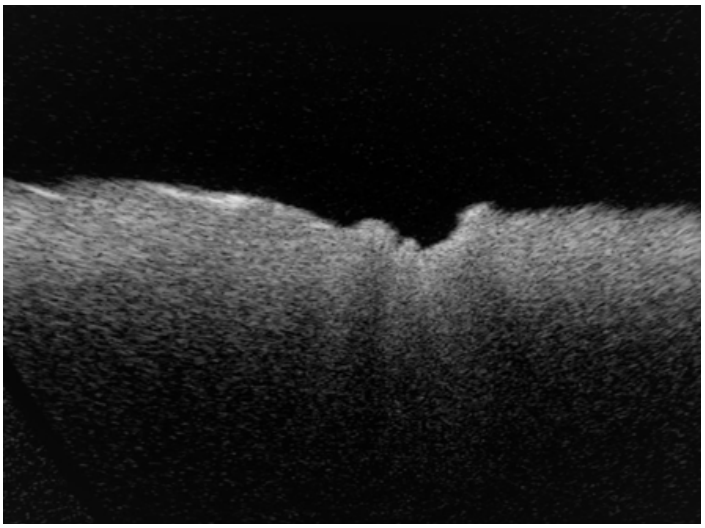

Figure 7: Crater-shaped defect of gingival epithelium after depigmentation using 940 nm laser (OCT examination)

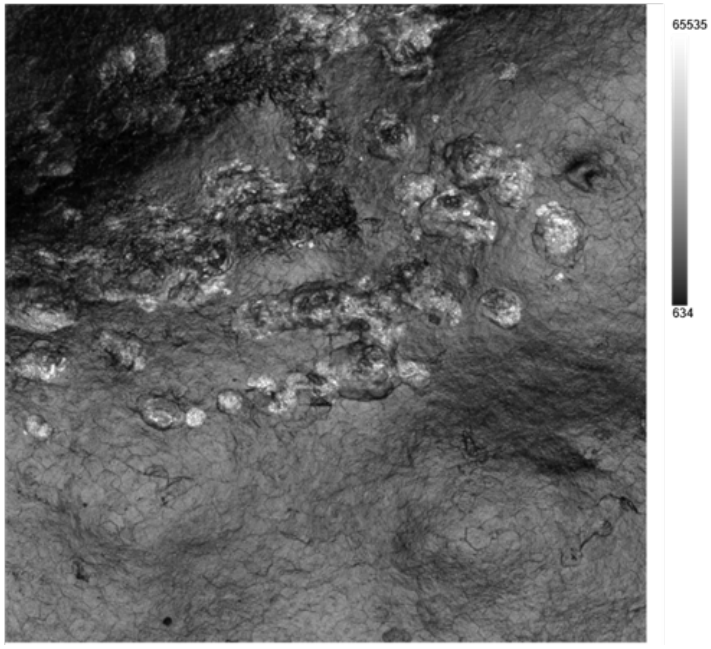

Figure 8: Confocal laser microscopy of gingival epithelium surface after 940 nm laser application

Treatment with the 2780 nm Laser

This wavelength was also associated with a specific ablative mechanism of depigmentation, but the mechanism of cell removal was different. Considering the wavelength, the laser beam had very low penetration ability and the border between the affected and non-affected tissue was sharp. As a result of this phenomenon, the OCT image is characterized by marks indicating the laser tip movement and its contact with epithelial cells, which form "white lines" corresponding to OCT manifestations of the keratinized layer of normal epithelium. This effect could be a result of fluid cell content removal and subsequent concentration of the remaining cell walls. Confocal laser microscopy showed very clear evaporation of epithelial layers of different depths corresponding to the shape of the used laser tip. Insignificant differences were detected between the used power settings because the obtained evaporated layers are thin, and progression of cell destruction was mainly a function of a laser tip movement.

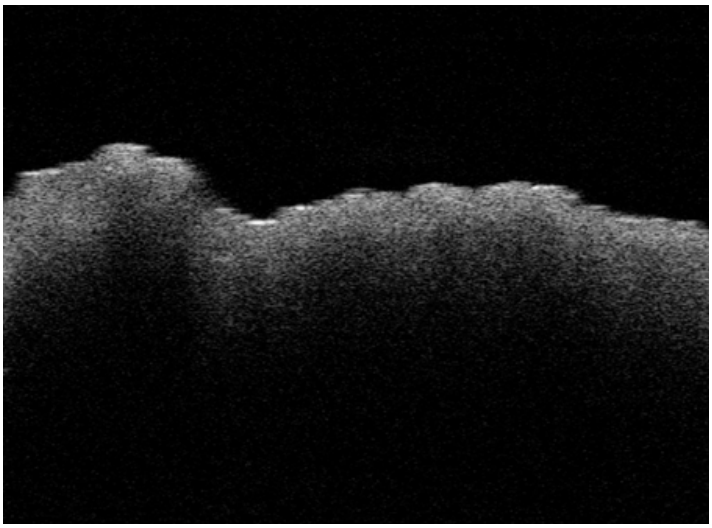

Figure 9: Irregular relief of gingival epithelium after partial removal using Er,Cr:YSGG laser with typical dense traces located on the contact area with laser tip (OCT examination)

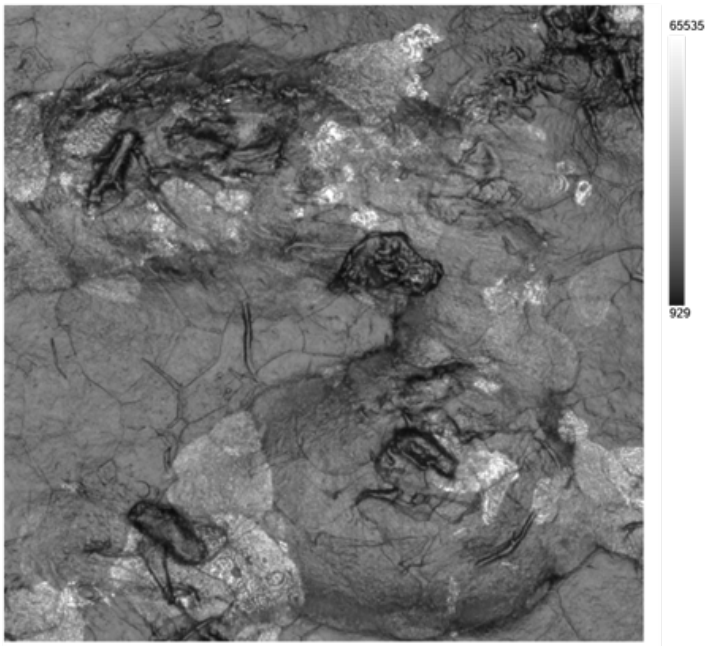

Figure 10: Details of interaction between chisel tip of Er,Cr:YSGG laser and superficial (light areas) as well as deeper epithelial layers (dark areas) in confocal laser microscopy

Table 1: Examined power levels in 445 nm laser: 320 µm tip, cw, non-contact mode

Power	Power density
200 mW	249 W/cm^2
300 mW	373 W/cm^2
400 mW	497 W/cm^2

Table 2: Examined power levels in 940 nm laser: 400 µm tip, cw, contact mode

Power	Power density
600 mW	477 W/cm^2
700 mW	557 W/cm^2
800 mW	637 W/cm^2

Table 3: Examined power levels in 2780 nm laser: MC3 chisel tip, 30 Hz, 30% air, 40% water, contact mode

Power	Power density
1,5 W	417 W/cm^2
2,0 W	550 W/cm^2
2,5 W	694 W/cm^2

Discussion

Considering the histological structure of gingival tissue, a theoretical cutting depth of slightly greater than 0.3 mm is required in depigmentation procedures. Therefore, mechanical or chemical methods of depigmentation may not completely eliminate the basal cell layer that contains melanin [15]. This is consistent with the postulation that deep de-epithelialization of shallow gingival tissue tends to result in superior outcomes.

Gingival depigmentation with lasers is currently identified as the most effective, compatible, and valid method and is the treatment of choice among clinicians [16,17]. Laser treatment is suggested for this purpose since it does not require any periodontal dressing and offers advantages such as easy handling, short treatment time, and optimal homeostasis, decontamination, and sterilization. The parameters used in laser depigmentation are based on the primary effect of melanin removal as well as the re-pigmentation tendency.

Considering the mechanisms of depigmentation, Rosa, et al. believed that re-pigmentation may be attributed either to high melanocyte activity or to incomplete melanocyte ablation during surgery (due to their close proximity to the adjacent teeth), which induces migration of melanocytes from the adjacent free gingiva to the treated areas [18].

Therefore, in order to prevent the "migration effect" from residual melanocytes, maximal ablation of peripheral melanocytes should be attempted. The laser fiber diameter is known to be critical in the efficacy of laser therapy and has been reported to influence the energy density and energy output of the laser used [19]. With larger laser fiber diameters, an increase in the power results in higher amounts of energy delivered to tissues, leading to the destruction of desired cells [20].

The present review showed an interesting association between the recurrence rate of the pigmented lesions and the number of times the lesions were exposed to laser irradiation (1 to 9 times). Except one study, all studies included in the present review involved repeated laser irradiation, and no recurrence of pigmented lesions was found over up to 12 months of follow up [21,22].

Because of the physical nature of erbium family lasers, they show biologically safe and precise effects with consecutive ablation of tissue layers. The use of an erbium laser can minimize damage to the deep tissues and prevent scarring from the application of the laser. Lee, et al. also found this laser to be more favorable than other types of lasers [23].

Er:YAG laser irradiation is known to stimulate the proliferation and secretion of gingival fibroblasts, suggesting that this type of laser may offer therapeutic benefits for tissue repair [24]. Rosa, et al. used an Er:YAG laser, which is known for its smooth application and high bactericidal effect, and concluded that it is one of the most promising lasers [18]. Tal, et al. investigated the use of the Er:YAG laser and reported speedy recovery of patients due to the narrow zone of thermal disruption [25]. These studies did not report any cases of re-pigmentation. However, when Giannelli, et al. compared the Er:YAG laser with the diode laser, they observed incomplete ablation of the gingival epithelium in the Er:YAG laser irradiation sites with some remnant deeper gingival mucosal injuries [26]. Therefore, the authors favored the use of diode lasers over Er:YAG lasers.

Tal, et al. reported that the treatment time and laser power are dependent on the epithelial thickness, the degree of pigmentation, and the affected area to be treated [25].

Hegde, et al. reported more sites showing re-pigmentation in the Er:YAG laser group in comparison with those in the CO2 laser group in the 6th month of follow up [27]. However, incomplete ablation of the gingival epithelium in Er:YAG laser irradiation sites with some remaining deeper epithelial ridges was noted. Therefore, more sessions were required, which increased the risk of damage to the lamina propria [28].

The same laser as that used in our study, the Er,Cr:YSGG laser (2780 nm), was used by Bakhshi, et al. at a frequency of 15 Hz, power of 1.75 W, 10% water and 20% air [29]. In that study, the hand piece was used in a non-contact mode with a sweeping motion and was held 1 mm away from the tissue in the defocused mode. The MZ8 tip (0.8 mm diameter) was used for the de-epithelialization procedure, and the spot size was 0.8 mm. In comparison with our settings, this adjustment was slightly more aggressive, but it was reported to be effective without evaluation of the re-pigmentation rate. Clinical re-pigmentation after Er,Cr:YSGG laser treatment was monitored by Berk, et al. over a 6-month follow up period with negative results [30]. In a study conducted by Suthprasertporn, among two patients who were treated with Er,Cr:YSGG, slight re-pigmentation was seen in one patient, a light smoker, over a follow up period of 11 months [31]. Re-pigmentation after Er,Cr:YSGG laser treatment primarily depends on complete destruction and complete removal of melanocytes, since the Er,Cr,YSGG laser does not show any selective effects but causes

general cell destruction. Thus, application of proper laser settings and effective laser tip movement are very important for effective ablation up to the lamina propria.

Bleeding is a clinical manifestation of the laser penetration into the lamina propria, but it is covered up by simultaneous coagulation and sealing of blood vessels up to a depth of 0.5 mm. This finding was consistent with those reported by Kishore et al., who observed that the extent of bleeding was directly correlated with the depth of ablation [32].

In treatment procedures using diode lasers, the use of appropriate protocols for long-term depigmentation is important. Different documented results with laser treatment provide the best evidence for these protocols. A broad spectrum of wavelengths with affinity for melanin have been used in depigmentation procedures with diode lasers, and the use of effective settings appears to be crucial to ensure immediate and long-term effects. The published results mostly used power levels of 1-2 W with continuous wave in contact mode. The parameters of the used laser tips (300-400 µm) were also relatively uniform and delivered approximately comparable laser energy density. However, the movement of the tip, which is a crucial aspect influencing the final effects of laser application, has been rarely discussed in previous studies. Giannelli, et al. indicated that the hyperpigmented gingiva had numerous melanin deposits in the basal and supra-basal epithelial cell layers before laser treatment [28]. The diode laser allowed complete, uniform removal of the squamous epithelium without noticeable changes in the lamina propria.

In comparison with Er:YAG lasers, diode lasers require shorter treatment times and provide greater tissue penetration (1-10 nm vs. 1 µm) [17]. Results of the Bakshi et al. study revealed that treatment of gingival melanin hyperpigmentation with diode laser had better efficacy in the reduction of pigmentation than Er,Cr:YSGG laser [29]. But there was no statistically significant difference between groups in terms of re-pigmentation. Soliman, et al. used a 400-µm fiber with a wavelength of 808nm and power of 1-2 W for depigmentation of the gingiva and buccal mucosa [33]. The diode laser was used in the continuous mode and was operated in the contact mode. Excellent results were achieved with pulse length of 300 ms being more longer than the estimated relaxation time for the melanosomes. In our experimental settings, a power of approximately 600 mW was sufficient and safe for melanin depigmentation, which is consistent with the findings reported by Giannelli, et al. who assessed histological changes in gingival pigmentation after Er:YAG and diode laser treatment [26]. They showed that an increase in diode laser power caused greater thermal damage and increased the time needed for complete healing. However, even higher levels of used power cannot guarantee the absence of pigmentation recurrence.

Doshi, et al. treated one patient with a 940 nm diode laser using a 400 µm tip in the 2.5 W pulsed mode with 0.5 µs pulse length and pulse interval [34]. They observed mild patchy pigmentation after 6 months and an increase in the size of the pigmented area during the one-year follow up. Similarly, Bakutra, et al. used an 810 nm semiconductor diode laser to perform a depigmentation procedure with a 300 µm tip in the contact mode (3 W power, continuous mode) [35]. The laser beam was applied using the "brush technique," as described by Tal, et al. and the tip was kept in motion throughout. After laser application, a carbonized layer of tissue was found at the impact site. At 12 months postoperatively, re-pigmentation with different grades of the Hedin index was observed at all sites [25].

The use of the 445 nm wavelength is relatively new in oral laser dentistry, and very few studies have described this approach. This laser shows excellent affinity to melanin, which is much better than the affinity of near-infrared lasers such as 810 nm lasers and is very suitable for gingival depigmentation. Thus, in comparison with the newer infrared lasers (810, 940, 980, and 1064 nm), the use of this wavelength allows the application of a much lower power density to achieve the same clinical effect. The non-ablative use of the 445 nm laser was first reported by Luk, who used this laser with 1 W of power and a 320 µm uninitiated tip in continuous wave [36]. We used the same tip in the non-contact mode, but reduced the power to 200 mW. Clinically, the pigmentation completely disappeared, and OCT examination revealed intact superficial epithelial layers and only localized destruction affecting the basal and suprabasal locations. Considering these findings, this method presents a non-traumatic and promising approach for melanin depigmentation.

Conclusions

- All examined wavelengths could effectively remove gingival melanin pigmentations. However, their mechanisms and the influence of surrounding structures were different. Considering the depigmentation effect, biology of healing, postoperative comfort, and re-pigmentation tendency, it is necessary to identify a balance between parameters of treated tissue and processes of laser tissue influence.
- OCT and confocal laser microscopy were helpful for monitoring tissue changes associated with the application of different wavelengths and laser settings.
- The Er,Cr:YSGG laser (2780 nm) is effective and safe for gingival depigmentation, especially considering its very thin effective laser trace, and a power range between 1.5 and 2.0 W with continual scanning movement of the laser tip allowed optimal depigmentation of gingival tissue.
- With the 940-nm diode laser, treatment at a power of 600 to 700 mW in the continuous mode with continual movement of the laser tip was effective without causing unwanted damage to the lower structures.
- Non-contact application of a 445 nm laser with only 200 mW power in the continuous mode appears to be a promising method for gingival depigmentation.

The authors report no conflict of interest.

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