Microstructural Evaluation of Contaminated Implant Surface Treated by Laser, Photodynamic Therapy, and Chlorhexidine 2%

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Purpose: Decontamination of the rough surfaces of dental implants is a challenge in the treatment of peri-implantitis. An acceptable cleaning technique must be able to debride and detoxify the surface without traumatizing it. This study assessed the possible implant surface alterations following decontamination with laser, photodynamic therapy (PDT), and application of chlorhexidine (CHX).

Materials and Methods: Of 16 dental implants with sandblasted, large-grit, acid-etched surfaces, Aggregatibacter actinomycetemcomitans was cultured on the surfaces of 15 implants for 48 hours. These 15 implants were divided into five groups of three and were subjected to erbium-doped yttrium aluminum garnet (Er:YAG) laser irradiation, 630 nm light-emitting diode and toluidine blue O photosensitizer, 810 nm diode laser, and indocyanine green-based photosensitizer, 2% CHX, and control group (no treatment). One implant remained intact. The morphology and element/phase identification of the implants were studied using scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDS), respectively.

Results: The SEM images and EDS maps revealed that the decontamination methods did not alter the surface quality of the implants. However, in photodynamic therapy, sodium chloride that remained from rinsing liquid can make an adhesive layer on the surface of the treated implants.

Conclusion: Er:YAG laser irradiation and PDT did not alter the surfaces of sandblasted, large-grit, acid-etched implants. INT J ORAL MAXILLOFAC IMPLANTS 2018;33:1019–1026. doi: 10.11607/jomi.6325

Keywords: EDS, implant surface, implant surface alteration, laser, SEM, surface roughness

Despite efforts to improve the quality and speed of osseointegration, bacterial infections may compromise the long-term success of dental implants. Biologic healing does not occur when the implant surface is contaminated with bacteria, toxins, and inflammatory products.1,2 In such cases, treatment must include debridement and detoxification of implant surfaces for attachment of the cells to the implant surface and bone regeneration.2 Several methods have been introduced for cleaning of the implant surfaces (mechanical and chemical). However, these methods have drawbacks such as the inability to completely eliminate the microbial biofilm and inefficient surface debridement, causing implant surface alteration, damaging the superficial titanium oxide layer, traumatizing the surrounding tissues, and depositing chemical residues.3–6 Such alterations would eventually decrease the biocompatibility of the implant surface.3,6 Since surface quality (ie, chemical, mechanical, and topographic) plays an important role in cellular behavior leading to osseointegration, peri-implantitis treatments should maintain and preserve the implant surface quality while using detoxifying and debridement procedures.7–9
Lasers are used as an adjunct or alternative to conventional treatments for cleaning the implant surface in peri-implantitis. Among all lasers, erbium-doped yttrium aluminum garnet (Er:YAG) laser has unique properties such as excellent tissue ablation, even at low intensities, and high bactericidal potential for decontamination of rough titanium surfaces. Irradiation of Er:YAG laser with suitable parameters (laser intensities of less than 140 mJ/pulse, 10 Hz, and irradiation time of less than 2 minutes) does not result in a temperature increase due to its high absorption in water; therefore, the surface remains biocompatible after irradiation. Antimicrobial photodynamic therapy (aPDT) is another alternative for decontamination of the implant surface. This method uses a nontoxic photosensitizer and light in the presence of oxygen. The advantages of aPDT are antimicrobial activity and access to porosities, niches, and recesses that are inaccessible by hand instruments.

In the treatment of peri-implantitis, decontamination of rough implant surfaces has always been a challenge. Therefore, an accurate microscopic study of the implant surface is needed to understand the probable implant surface alteration and decontamination. Although several studies have determined the antimicrobial effects of aPDT on implant surfaces, the effect of this technique on surface alteration of implant surfaces still needs to be studied. The antimicrobial effects of different treatment modalities for implant surface decontamination, ie, Er:YAG laser, aPDT (810-nm diode laser along with indocyanine green-based [ICG-based] photosensitizer and 630-nm light-emitting diode [LED] along with toluidine blue O [TBO]) and 2% chlorhexidine (CHX) on aggregatibacter actinomycetemcomitans (Aa) biofilms were investigated in the present authors’ previous study. In this study, the aforementioned treatment methods were used to investigate the effect of these methods on implant surface morphology and surface elemental composition. In this study, scanning electron microscopy (SEM) along with energy-dispersive x-ray spectroscopy (EDS) were used. These techniques provided a better understanding about the surface quality of the treated implants, and they confirmed how decontamination techniques can affect surface quality.

### MATERIALS AND METHODS

This study was conducted on 16 dental implants (Dentium, SuperLine) with 4.3 mm in diameter and 10 mm in length. Aa (ATCC 33384 obtained from Rayen Biotechnology) was cultured on the surface of 15 implants under microaerophilic conditions for 48 hours. After bacterial culture, the implants were rinsed with 3 mL of saline for 30 seconds. Then, these 15 implants were divided into five groups of three according to Table 1.

### Light Sources, Photosensitizers, and Treatment Procedures

**Er:YAG Group.** Implants in this group were subjected to Er:YAG laser (N32701, Smart 2940D plus, DEKA Laser) irradiation with 10 Hz frequency, 100 mJ/pulse, 1 W power, and very short pulse length (230 µs). Mean power density was 0.3 W/cm² under air flow of 10 mL/min and water pressure of 1 psi spray for 60 seconds. Irradiation was done using a fiber optic tip in a circular motion from the coronal to the apical direction, so that all implant threads were irradiated, as the beam was perpendicular to the implant surface. The spot size was 1 mm when the handpiece contact tip was placed 5 mm above the surface.

All of the decontamination procedures were performed by a skilled calibrated operator. To match the process, all implants were rinsed again with 3 mL of sterile saline solution for 30 seconds after the treatment.

**aPDT1 Group.** In this group, TBO (0.1 mg/mL) (FotoSan agent medium viscosity, FotoSan, CMS Dental) was used as a photosensitizer. LED was used to activate the TBO in red spectrum (wavelength of 625 to 635 nm, peak of 630 nm, FotoSan 630LED, CMS

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of implants</th>
<th>Microbial culture</th>
<th>Decontamination methods</th>
</tr>
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<tbody>
<tr>
<td>Er:YAG</td>
<td>3</td>
<td>Yes</td>
<td>Er:YAG laser irradiation</td>
</tr>
<tr>
<td>aPDT1</td>
<td>3</td>
<td>Yes</td>
<td>Toluidine blue + LED 630 nm irradiation</td>
</tr>
<tr>
<td>aPDT2</td>
<td>3</td>
<td>Yes</td>
<td>ICG-based photosensitizer + Diode laser 810 nm irradiation</td>
</tr>
<tr>
<td>CHX</td>
<td>3</td>
<td>Yes</td>
<td>Chlorhexidine 2%</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>Yes</td>
<td>Rinsed with saline solution</td>
</tr>
<tr>
<td>Intact</td>
<td>1</td>
<td>No</td>
<td>None</td>
</tr>
</tbody>
</table>

Er:YAG = erbium-doped yttrium aluminum garnet; aPDT = antimicrobial photodynamic therapy; CHX = chlorhexidine.
Dental) with maximum output intensity of 2,000 to 4,000 mW/cm², using a disposable blunt tip. Implants were soaked in 0.1% concentration of TBO for 1 minute using a specific syringe. According to the manufacturer’s instruction, implants were rinsed by 3 mL of saline solution for 30 seconds. Implant surfaces were then subjected to LED irradiation from coronal to apical in a circular motion for 60 seconds. The beam was perpendicular to the implant surface.

**aPDT2 Group.** In this group, implants were first soaked in ICG-based photosensitizer solution (EmunDo, A.R.C. Laser) for 5 minutes; then, the surface of the implants was irradiated with 810-nm diode laser (A.R.C. Laser), from coronal to apical in a circular motion for 60 seconds. The laser beam was perpendicular to the implant surface. Laser irradiation was done with the parameters recommended by the manufacturer (power of 300 mW, power density of 2.38 W/cm² in continuous mode). When the tip of the handpiece was held in a distance of 3 to 5 mm of the implant surface, the spot diameter was 4 mm.

**CHX Group.** All implants in this group were placed in 1.5-mL sterile plastic Eppendorf tubes containing 3 mL of 2% CHX (Consepsis, Ultradent) for 30 seconds. Afterward, they were rinsed with 3 mL of sterile saline solution for 30 seconds to eliminate the residual CHX.

**Control Group.** To assess the effect of bacterial culture on the implant surface, the control implants remained intact.

**Intact Group: One Implant Remained Intact for Comparison**

In order to separate the residual bacteria on the implant surfaces, the implants were immersed in 1 mL of brain heart infusion broth and ultrasonicated (Branson Ultrasonic) for 5 minutes at 50 Hz and 150 W and vortexed at maximum intensity for 1 minute (Scientific Industries).

**SEM and SEM-EDS Analyses**

All implants were sputter-coated with a thin layer of gold to avoid sample charging and microscope beam damage. The microstructure, morphology, and phase/element identification of the surface processed implants were studied using SEM along with EDS (Hitachi S-400 scanning electron microscope, equipped with an Oxford Instrument energy dispersive spectrometer). The accelerating voltage of the incidence beam was 10 kV. The treated surfaces of the implants were separately scanned two times, and several SEM images were taken. All the SEM and EDS studies were done on the third and fourth implant screw.
Fig 2  SEM micrographs and EDS analysis of (a) Er:YAG, (b) CHX, (c) aPDT1, and (d) aPDT2 groups.
RESULTS

Figure 1a shows the SEM image in different magnifications and EDS analysis of the intact implant. The SEM image reveals a rough and irregular surface with a honeycomb appearance, which is the characteristic of the sandblasted, large-grit, acid-etched implant surfaces. The EDS analysis of the sample (zone A) shows the existence of pure titanium. Figure 1b shows the SEM micrograph and EDS analysis of the control group. In comparison to Fig 1a, the low-magnification SEM image shows traces of extra substances dispersed on the implant surface. The higher-magnification image demonstrates the honeycomb appearance; however, the valleys have been filled. The EDS spectra (inset, zone B) and EDS map (right) show the existence of Ti along with traces of Na and Cl. Consequently, the valleys were filled with remaining sodium chloride (NaCl) from the rinsing solution.

Figures 2a and 2b show SEM images and EDS analyses of the Er:YAG and CHX groups, respectively. Accordingly, the treatment did not result in any chemical or morphologic changes on the implant surface. In addition, the elemental EDS map confirms that the depth of the valleys in the Er:YAG sample has been filled similar to the control group, which is due to the deposition of NaCl. However, the EDS spectra of zone B show that there is no trace of NaCl in the CHX group.

The SEM image of the aPDT1 group showed fern-like patterns on the implant surface (Fig 2c). They had covered large areas of the implant surface, and porosities were filled. The honeycomb appearance is not detectable under the pattern. The EDS map revealed that the pattern belonged to NaCl deposition. As shown in Fig 2d, in the aPDT2 group, NaCl has a dendritic structure, which has covered smaller areas of the implant surface.

For further analyses, the implants of the aPDT1 and aPDT2 groups were sonicated and rinsed with distilled water. Figure 3 shows SEM images of (a) aPDT1 and (b) aPDT2 groups after double rinsing and sonicating in distilled water. Higher-magnification images indicate that surface roughness of the implants remained unchanged.
DISCUSSION

In this study, the possible surface alterations following different treatment modalities of sandblasted, large-grit, acid-etched implant surfaces were assessed. The results showed that although in some of the treatment techniques, NaCl deposition on the surface of the implants was observed, the original roughness of the implants, ie, the surface roughness and morphology under NaCl deposition, remained unchanged. In other words, NaCl has covered the surface of the implants; however, there is neither a chemical reaction nor surface melting on the surface of the implants.

Studies on the efficacy of cleaning techniques of the implant surface should not merely focus on the microbiologic effects of treatments. Treatments may affect the chemical composition and topography of the implant surface. It has been shown that rough surfaces induce faster differentiation of stem cells to osteoblasts. Osteoblast-like cells attach faster to a rough surface, while epithelial cells and fibroblasts have higher affinity for smooth surfaces. Therefore, changes in surface topography may have a selective effect on the attachment of the cells and can subsequently affect the type of tissue that regenerates around the implant after treatment. Chemical alterations due to deposition of debris or changes in the titanium oxide layer can also impair the cell attachment by decreasing the surface biocompatibility. Thus, since the cell responses to the implant surface and the regenerated tissues around the implant are highly influenced by the surface quality of the implant, an optimal treatment should eliminate the bacteria, debride and detoxify the surface, and should preserve the original surface quality without traumatizing it.

In this study, Er:YAG laser with 2,940-µm wavelength, 10 Hz frequency, 100 mJ/pulse laser intensity, and 1 W power irradiated for 60 seconds caused no surface alteration. Schwarz et al reported that Er:YAG laser irradiation (100 mJ/pulse, 10 Hz, 60 seconds) was significantly more effective than plastic curettes in elimination of microbial biofilm from the sandblasted, large-grit, acid-etched implant surface and resulted in no surface changes. Lee et al found that when the time was increased to 90 seconds, surface roughness decreased. Shin et al also showed that melting and fusion phenomena were observed with all application times with 180 mJ/pulse irradiation.

Pulsed mode lasers, such as Er:YAG, transfer high levels of energy in a very short period of time to a small surface and lead to evaporation of calculus, granulation tissue, and microbial biofilm. For this reason, these lasers have higher antimicrobial efficacy than continuous mode lasers. However, when working with Er:YAG laser, its power must be carefully adjusted. Due to the considerable energy peaks, these lasers are capable of generating power densities over 10^6 W/cm^2, which induce plasma formation and light reflection in a small area in superficial layers of metal (0.1 to 1.0 µm). It leads to generation of a very high temperature at the laser-implant interface, which can partially melt the implant surface and decrease the biocompatibility. In conclusion, Er:YAG laser with 100 to 120 mJ/pulse intensity, 10 Hz frequency, and 60-second irradiation time can be safely used on a sandblasted, large-grit, acid-etched implant surface with no morphologic alterations.

Although the roughness of the implant surface enhances the quality and the speed of osseointegration, surface cleaning will be much more difficult when the surface is contaminated with microbial biofilm. Rough surfaces have numerous micron-size porosities, which are inaccessible by hand instruments. This problem may be resolved by using aPDT, because photosensitizers can penetrate into the porosities on the rough sandblasted, large-grit, acid-etched surfaces and bond to target bacterial cells. Irradiation of laser with a proper wavelength to target cells in the presence of oxygen generates singlet oxygen and other reactive species, which are toxic to target cells. Thus, by marking the target cells, photosensitizers overcome the accessibility limitations. Haas et al showed that aPDT killed Aa and P gingivalis lodged in niches and recesses. In addition to antimicrobial effects, aPDT detoxifies the surface.

Using low-energy light for activation of the photosensitizer is another advantage of aPDT, which decreases the risk of heat generation and surface damage. In this study, no sandblasted, large-grit, acid-etched surface changes were found in the aPDT1 and aPDT2 groups. Non-laser light sources such as LED are increasingly used for aPDT, since LED devices are more compact and portable and are more affordable than lasers.

The ICG-based photosensitizer used in this study is a newly introduced product with antimicrobial effects in the sulcus depth. Indocyanine green is a photosensitizer from the family of tricarbocyanine. Like other photosensitizers, ICG can convert the absorbed energy from the laser light to reactive oxygen radicals (phototoxicity). Additionally, it can transform laser energy to heat via photothermal actions. It is not clear which mechanism is more effective in killing the bacteria; however, according to the manufacturer, the photothermal action is the dominant mechanism, especially in the areas where the oxygen pressure is low, such as in deep pockets. On the other hand, increased temperature carries the risk of implant surface alteration. Nagahara et al showed that in aPDT with ICG-loaded nanospheres and 805-nm diode laser, the temperature raised by 4.23°C in 1 minute. The titanium and titanium oxide melting points are 1,668°C and 1,843°C, respectively. Therefore,
a temperature rise by 4.23°C does not result in melting of the titanium surface. The present results also confirm this finding (Fig 2d). Based on these results, the diode laser at 300 mW power for 60 seconds in combination with ICG-based photosensitizer did not result in surface alteration of the implants. Basically, it seems that the diode laser does not cause alterations in the implant surface regardless of its power density.26,29,33 By irradiation of continuous wave mode lasers (eg, diode), a lower maximum peak of energy is generated; thus, less energy is transferred to the surface of the implant during the irradiation time.28 Therefore, surface alteration does not occur. However, such a slow, gradual, and continuous heating increases the temperature of metal without attaining a sufficiently high temperature to evaporate debris, which leads to heat generation and temperature rise even in remote areas of the object, while it is less efficient in surface decontamination.26,29 Kreisler et al28 also concluded that after 9 seconds of 809-nm diode laser irradiation at 2.5 W or 30 seconds at 1 W, the temperature at the implant-bone interface exceeded the critical threshold of 47°C. Accordingly, for the implant surface decontamination, diode laser power must be between 1 W for 25 seconds and 2 W for 10 seconds. However, such a short duration may not be sufficient for decontamination of exposed implant surfaces.28 However, aPDT decreases the risk of thermal damage to the surrounding tissues by lowering the power of the device; consequently, the working time can be increased.

The patterns of surface melting by laser have been reported as flat melting, glossiness, cracking, ripple pattern, and slip-line formation.26,34 However, analysis of SEM images and EDS maps reveals fern-like and dendritic patterns in the aPDT1 and aPDT2 groups, respectively, due to NaCl deposition, which comes from rinsing liquid. These patterns are uniform and do not resemble previously reported patterns (Figs 2c and 2d). At first glance, these deposits resembled melt patterns; however, the EDS map revealed that they are related to NaCl deposition. In the melting process, initially, the peaks of the porosities are melted. However, as seen in Figs 2c and 2d, and 3a and 3b, the peaks remained intact, and NaCl was deposited at the depth of the niches and recesses. Figures 3a and 3b clearly show the NaCl deposition on the implant surface. These fern-like and dendritic patterns on the surface of the aPDT1 and aPDT2 groups were not observed in the other groups. In comparison to literature data,13,15,24,25,27,35 the present study used EDS analysis along with the SEM technique to precisely study the implant surface alteration after different microbial treatments. It should be considered that to accurately characterize phase evaluation of the surface changes of dental implants, the SEM technique along with EDS is required, and using only the SEM image is not totally informative.

In some of the treatment methods, NaCl is adhesive to the surface of the titanium implant. NaCl in aqua solution provides chloride ion, which is a corrosive ion. Titanium is known as a corrosion resistance material in chloride-contained solutions. Therefore, the residual NaCl has no destructive effect on the surface of the implant. To remove the residual NaCl, it is suggested to rinse the implants multiple times with distilled water. Thus, rinsing with distilled water is recommended to avoid the formation of NaCl deposition and decrease the risk of diagnostic errors.

In this study, the biofilm of Aa was used. Certainly, Aa is a less-common bacteria in periodontitis and peri-implantitis than P gingivalis; however, this bacteria is strongly associated with aggressive periodontitis, severe periodontitis, and peri-implantitis, and is one of the bacteria frequently associated with implant failure.36,37 In addition, some studies17,30,38,39 confirm that the ability of laser light to kill periodontal pathogens is species-dependent, and Aa is more resistant against laser light and aPDT than P intermedia, P gingivalis, and S sanguis. Black-pigmented species have internal pigments such as protohaemin and protoporphyrin that can absorb red light and therefore enhance the killing efficacy of laser and aPDT.

This study has evaluated the effect of the mentioned treatments on surface alteration; thus, future cellular and animal studies are recommended to assess the biocompatibility and efficacy of these decontamination techniques. It should be noted that the results of this study were obtained from an in vitro study without the limitations encountered in a clinic (ie, isolation and limited accessibility). Therefore, to generalize the results to in vivo conditions, evidence-based clinical trials regarding the effect of these treatments on tissue regeneration and peri-implant lesions should be done and compared with conventional methods.

**CONCLUSIONS**

Within the limitations of this study, the results showed that irradiation of Er:YAG laser, diode laser, and LED with the applied parameters, in combination with a photosensitizer, did not result in alteration, such as melting, glossiness, cracking, ripple pattern, and slip-line formation, on the sandblasted, large-grit, acid-etched implant surface. However, analysis of EDS maps revealed that in the aPDT1 and aPDT2 groups, antimicrobial treatments can result in an adhesive deposition of NaCl with different patterns on the surface of the implants. Therefore, the decontamination techniques can be safely used in the clinical setting. However, the results of in vitro studies must be generalized to the in vivo setting with caution.
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