Photomedicine and Laser Surgery
Volume 28, Number 2, 2010

Pp. 273–280
DOI: 10.1089/pho.2009.2496

Effectiveness of 980-mm Diode and 1064-nm Extra-Long-Pulse Neodymium-Doped Yttrium Aluminum Garnet Lasers in Implant Disinfection

Fábio Gonçalves, D.D.S., M.Sc.,[1] Artemio Luiz Zanetti, D.D.S., Ph.D.,[1] Raquel Virgínia Zanetti, D.D.S., Ph.D.,[1] Francesco Savério Martelli, D.D.S.,[2] Mario Júlio Avila-Campos, Ph.D.,[3] Luiz Fernando Tomazinho, Ph.D.,[3] and José Mauro Granjeiro, D.D.S., Ph.D.[4]

Abstract

Objective: To evaluate the potential of 980-nm gallium aluminum arsenide (GaAlAs) and 1064-nm neodymium-doped yttrium aluminum garnet (Nd:YAG) lasers to reduce bacteria after irradiation of implant surfaces contaminated with *Enterococcus faecalis* and *Porphyromonas gingivalis* and on irradiated implant surface morphology. ***Background:*** Despite the frequency of implant success, some implant loss is related to peri-implantitis because of difficulty in eliminating the biofilm. ***Methods:*** Implants (3.75×13 mm) with machined surfaces, surfaces sand blasted with titanium oxide (TiO_2), and sand-blasted and acid-etched surfaces were exposed to *P. gingivalis* and *E. faecalis* cultures and irradiated with 980-nm GaAlAs or 1064-nm Nd:YAG lasers. After laser treatments, the number of remaining colony-forming units and implant surface morphology were analyzed using scanning electron microscopy (SEM). ***Results:*** The Nd:YAG laser was able to promote a total contamination reduction on all implants irradiated. The results with the GaAlAs laser showed 100% bacteria reduction on the implants irradiated with 3 W. Irradiation with 2.5 W and 3 W achieved 100% of bacteria reduction on *P. gingivalis*–contaminated implants. Decontamination was not complete for the sand-blasted TiO_2 (78.6%) and acid-etched surfaces (49.4%) contaminated with *E. faecalis* and irradiated with 2.5 W. SEM showed no implant surface changes. ***Conclusion:*** The wavelengths used in this research provided bacteria reduction without damaging implant surfaces. New clinical research should be encouraged for the use of this technology in the treatment of peri-implantitis.

Introduction

Implantology has been the dental specialty that has evolved the most in the last 3 decades. Since the advent of osseointegration, the predictability of the results of prostheses over implants has led a growing number of dentists to practice that activity.

Innovations in surgical techniques, as well as in the shape and surface of implants and in prosthetic abutments, have brought better results to implantology. However, amid the numerous positive results, we find a few doubts, mainly related to the implant maintenance. Notwithstanding the excellent results obtained in implant rehabilitation, the emergence of an inflammatory pathology that affects the peri-implant tissues called peri-implantitis, which can cause bone loss and the implant's failure, has been observed.

That pathology has been defined as an inflammatory process affecting the tissues that surround the implant, leading to loss of the supportive bone, and the bacterial biofilm being its major etiologic agent.

The process of bacterial colonization of the implant surface is complex and involves different stages and bacteria species,[1] yet its pathogenic mechanism is similar to periodontitis.[2]

Just like periodontal disease, peri-implantitis is multifactorial, and many therapies are suggested for its treatment, among them the mechanical procedures of scaling and removal of the peri-implant biofilm, associated or not with local or systemic antimicrobial therapies. Previous work[2,3] has found that the results obtained are not so positive owing to the difficulty in eliminating the biofilm, which contributes to maintaining the pathology, blocking local tissue repair.

[1]Implantology Center, University City of Sao Paulo, Sao Paulo, Brazil.
[2]Institute for Training and Research in Microinvasive Dentistry, Florence, Italy.
[3]Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.
[4]Department of Cell and Molecular Biology, Fluminense Federal University, Rio de Janerio, Brazil.

The main difficulty in the treatment of peri-implantitis is in obtaining effective decontamination of the implant surface owing to the difficulty of removing the biofilm and to the existence of implants on rough surfaces. These surfaces, although highly beneficial for the initial process of osseointegration, promote a larger accumulation of peri-implant biofilm. The literature describes local chemical (antiseptic solutions, antibiotics),[4,5] systemic,[6,7] or physical (scaling, ultrasound, plastic curettes)[8] methods or a combination of these.[9]

Insufficient bacteria elimination results in a decrease of the treatment's success rate, which ranges between 20% and 40%.[5] Decontamination combined with regenerative techniques is fundamental for the remission of peri-implantitis.[10]

The use of lasers, in many wavelengths, has been described in dentistry,[11] mostly for its interaction with tissues and its therapeutic and antimicrobial effects, being indicated in restorative and surgical procedures, particularly in endodontics and periodontics. The use of laser for decontaminating periodontal pockets has been shown to be effective[12] and has encouraged research for determining or clarifying its effectiveness in the treatment of peri-implantitis. The microbiota of infectious periodontal processes is similar to that observed as the main cause of peri-implantitis, but important differences also exist, requiring research to study them and evaluate the clinic consequences.

Many wavelengths, energetic parameters, and types of equipment have been used and described in the literature aiming at determining an effective and secure protocol in the treatment of peri-implantitis that could provide more-satisfactory results.[13–20]

This research aimed at evaluating the level of reduction in bacteria of *Enterococcus faecalis* and *Porphyromonas gingivalis* contaminating three different implant surfaces after the use of a light beam of 980-nm diode and 1064-nm neodymium-doped yttrium aluminum garnet (Nd:YAG) lasers with different power, as well as assessing possible damage to implant surfaces using scanning electron microscopy (SEM).

Material and Methods

Materials

Titanium implants (n = 144) with profile and external dimensions of 3.75 mm diameter and 13 mm height were used. They were especially manufactured and machined in Branemark standard (Conexão Sistemas de Prótese, São Paulo, Brazil), with a format developed especially for this experiment, without an orifice for the adaptation of prosthetic components and with an extension of the top section for its adaptation and stabilization during irradiation (Fig. 1). The implants were divided into three groups according to their surface properties: machined, sand blasted with titanium oxide (TiO_2), and sand blasted and submitted to sulfuric acid etching.

Cells

We used a pure culture of reference stock of *P. gingivalis* American Type Culture Collection (ATCC) 33277 and *E. faecalis* ATCC 29212 prepared in sterile brain heart infusion (BHI) (Laboratório DIFCO Ltda., São Paulo, Brazil) plus hemin (5 μg/mL) and menadione (1 μg/mL). For counting the colony-forming units (cfus), we used 5 mL of a peptonized water solution (1.25%) containing sodium chloride (2.50%).

FIG. 1. Specially designed implant presenting elongated apical region for adaptation on irradiation device.

The solid medium used for evaluating the number of cfus was the trypticase soy agar (TSA, Laboratório DIFCO Ltda.), supplemented with hemin (5 μg/mL) and menadione (1 μg/mL) and enriched with 10 mL of defibrinated lamb blood. The Petri dishes with blood-agar were divided into five groups and identified with different colors. Each dish was limited with four fields for the differentiation of the

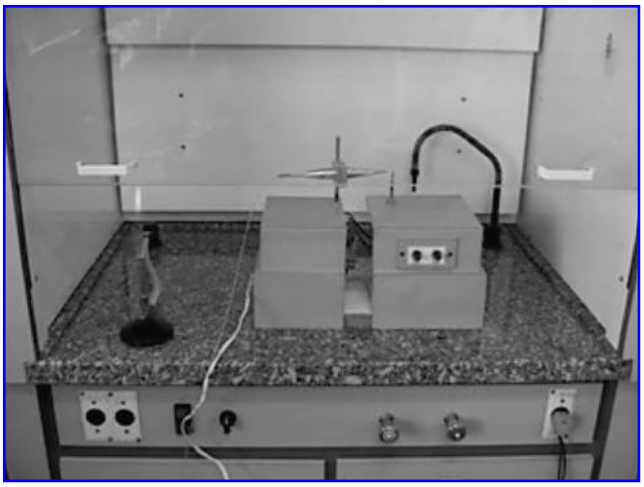

FIG. 2. Device for standardized laser irradiation of implants (patent pending n. 0031871).

Table 1. Mean Reduction of *E. Faecalis* and *P. Gingivalis* Colony Forming Units (CFU) of Contaminated Implants with 980-nm Diode Laser

	CFU Reduction (%)[a]			
	2.5 W		*3.0 W*	
Surface	P. gingivalis	E. faecalis	P. gingivalis	E. faecalis
Machined	100	100	100	100
Sand blasted	100	79	100	100
Sand blasted and acid etched	100	50	100	100

[a]Number of cfu on nontreated implant (considered as 100%). *P. gingivalis*: 5×10^8 ($\pm0.4\times10^8$); *E. faecalis*: 5×10^8 ($\pm0.6\times10^8$) cfu/mL.

dilutions of the samples that were afterwards harvested in aliquots of 10 μL.

The pure *P. gingivalis* and *E. faecalis* cultures were standardized according to the medium turbidity for the McFarland scale of 0.5 ($\sim10^8$ cells/mL). Then the sterile proof bodies were immersed in the culture and homogenized in vortex for 30 s, aiming at removing dispersed bacteria from the culture medium not adhering to the implant surface (biofilm), thus preventing excessive contamination on the implant surface. The culture medium was then aspirated, and the proof bodies were removed and tested. In all stages of the experiment, we controlled the culture purity through harvesting strains of supplemented blood-agar and Gram stain. After incubation, the purity was again checked for the morphocolonial characteristics of each species.

After irradiation, each implant was removed from the device uisng tweezers, which were sterilized and introduced in test tubes containing BHI broth. The test tubes containing implants previously contaminated with *P. gingivalis* were incubated under anaerobic conditions at 37°C for 3 days, whereas those contaminated with *E. faecalis* were incubated in anaerobiosis at 37°C for 18 h. At the end of the incubation periods, we checked in which test tubes there occurred bacteria growth (to reach approximately 5×10^7 cfu), which were separated to perform the series dilution, with the purpose of analyzing and counting the number of cfu to determine the reduction of the microbial population of the irradiated implants. The methodology used for the microbiologic analysis, as well as in the cultures, followed the guidelines of the Anaerobic Lab of the Microbiology Department of the Institute of Biomedical Sciences, University of Sao Paulo (ICB-USP).

Laser equipment and experimental drawing

Two types of laser equipment were used. The first was a surgical diode (980-nm gallium aluminum arsenide (GaAlAs)) laser type (model Smarty–A 900, DEKA M.E.L.A. s.r.l, Firenze, Italy), a laser system electronically controlled through a maximum 10-W microprocessor, frequency up to 150 Hz, pulse length of 2 ms to 2 s, connectable to 200-, 300-, and 600-μm fiber optics, and with a 3-mW laser guide. The second was a 1064-nm Nd:YAG laser (model Smartfile, DEKA M.E.L.A. s.r.l), with a maximum power of 10 W, frequency up to 150 Hz, and a pulse length of 1 ms. As a negative control, contaminated implants (n = 3/implant group/bacteria, 18 implants total) were autoclaved. Contaminated implants neither autoclaved nor irradiated were the positive control (n = 3/implant group/bacteria, 18 implants total).

A device was developed for maintaining a constant distance between the laser's fiber optic extremity and the implant surface (in process for patenting (protocol No. 0031871), presented by the Anaerobic Lab of the Microbiology Department of ICB-USP) (Fig. 2). That device kept the implants in continuous rotating movement around their own axes, whereas the handle of the laser equipment had a vertical movement of 15 mm amplitude, allowing irradiation to occur throughout the implant surface.

The 980-nm diode laser was set in a continuous emission mode for 5 min, whereas the 1064-nm Nd:YAG laser was used in a pulsating mode, both keeping the fiber optics of 300 μm at a 3-mm distance, perpendicular to the irradiated surface and with a diameter of focused beam of 0.3 mm. The irradiations were performed continuously for 5 min at 2.5 W (1143 J/cm^2) or 3 W (1371 J/cm^2). Irradiated implants (n = 3/

Table 2. Mean Reduction of *E. Faecalis* and *P. Gingivalis* Colony Forming Units (CFU) of Contaminated Implants with 1064-nm Neodymium-Doped Yttrium Aluminum Garnet Laser

	CFU Reduction (%)[a]			
	2.5 W		*3.0 W*	
Surface	P. gingivalis	E. faecalis	P. gingivalis	E. faecalis
Machined	100	100	100	100
Sand blasted	100	100	100	100
Sand blasted and acid etched	100	97	100	100

[a]Number of cfu on nontreated implant (considered as 100%). *P. gingivalis*: 5×10^8 ($\pm0.4\times10^8$); *E. faecalis*: 5×10^8 ($\pm0.6\times10^8$) cfu/mL.

surface/power/type of laser/bacteria, 72 implants) immediately underwent microbiological analysis.

Surface morphology

For analysis of possible alterations on the implant surfaces after the laser irradiation, we used 18 uncontaminated implants (n = 3/power/type of laser, 36 implants) submitted to irradiation using two different sets of equipment. The energetic parameters were varied, as well as the contaminated implants irradiation, that is: 2.5 W and 3 W and control, continuous irradiation for 5 min and the three types of implant surfaces studied.

We obtained three microphotographs of each irradiated implant and a control, with enlargements of 200×, 500×, 1000×, referring to the regions of the implant threads. For that analysis, we used a scanning electron microscope (VEGA TS 5136 MM, Tescan, Brno, Czech Republic), totally computer controlled, working in high, medium, and low vacuum, with enlargements of up to 1,000× and with a reading speed of 600 ns to 10 ms per pixel.

Results

When using 3W, regardless of the type of irradiated implant surface, the contaminating bacteria, or the laser wavelength used, there was a complete reduction of the contaminating microorganisms (Tables 1 and 2). At 2.5 W with the 1064-nm Nd:YAG laser, bacteria reduction was 97.2% for the implants with the surface treated with acid etching (Table 2); with the diode laser, bacteria reduction was complete for *P. gingivalis*, regardless of the surface, whereas reduction of *E. faecalis* was 100%, for machined, 79% for sand blasted, and 50% for sand blasted and acid etched (Table 1).

The SEM analysis compared the nonirradiated implants (Fig. 3) with the 980-nm diode (Fig. 4) and 1064-nm Nd:YAG (Fig. 5) irradiated implants; no evident change occurred for 2.5 W or 3 W.

Discussion

In view of the results collected in the literature revision, we can say that peri-implant disease is similar to periodontal disease, the former usually being faster and more aggressive. Although many treatments have been proposed for the remission of both pathologies, there is not a defined protocol for the treatment of peri-implantitis. In addition, its prognostic in relation to periodontal disease is worse, because patients suffering from past periodontal disease have more chances to develop peri-implant disease.[1,21]

We found, in this study, that at 3W, both lasers decontaminated all surfaces no matter the type of bacteria tested, whereas at 2.5 W, the rough implants were partially decontaminated. It has previously been found that the Er:YAG laser does not decontaminate rough implants as well as smooth or machined implant surfaces.[22] According to the methodology used, it was necessary to irradiate the implants for 5 min to cover the entire implant surface. For this reason, for each experiment a high value for the total energy (J/cm^2) was used. Clinically, full implant irradiation is not necessary due to implant failure/loss, because it is no longer attached to bone.

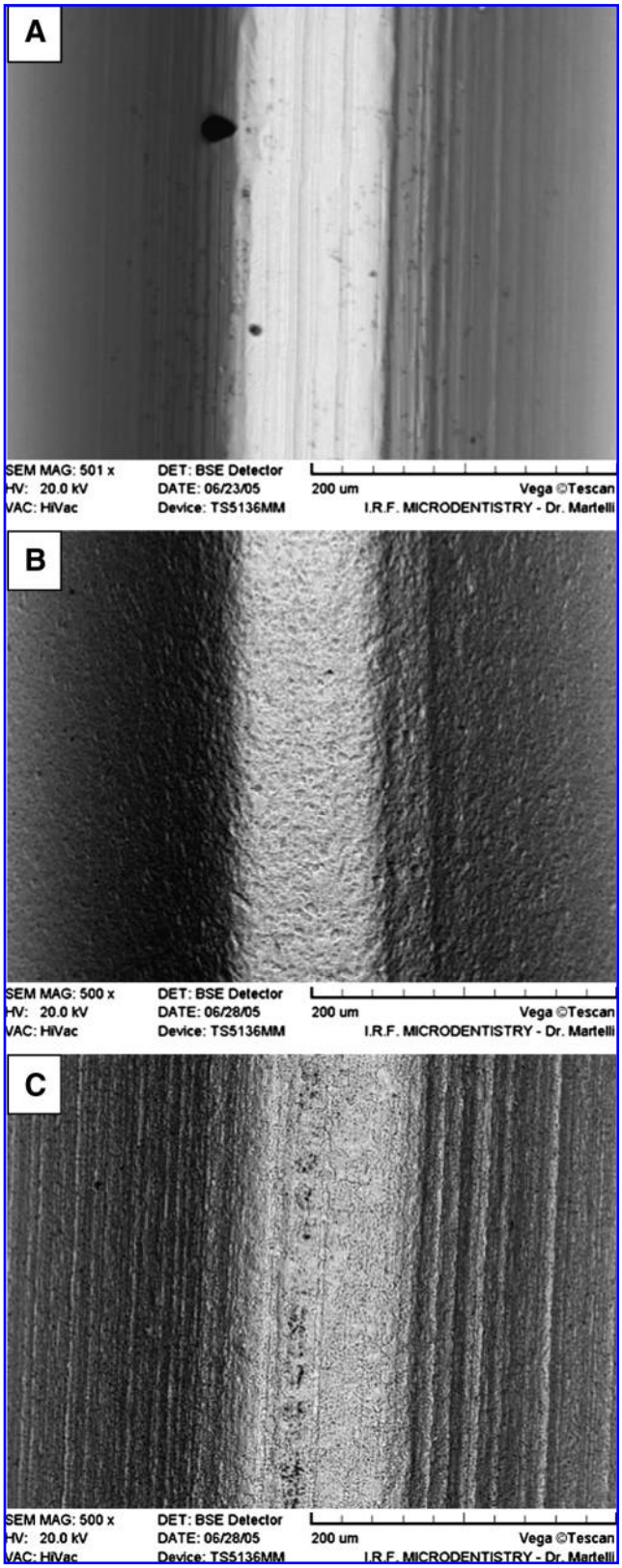

FIG. 3. Scanning electron microscopy micrographs revealing the surface morphology of nonirradiated implants. (**A**) machined, (**B**) sand blasted with titanium oxide (TiO_2), and (**C**) sand blasted and acid etched.

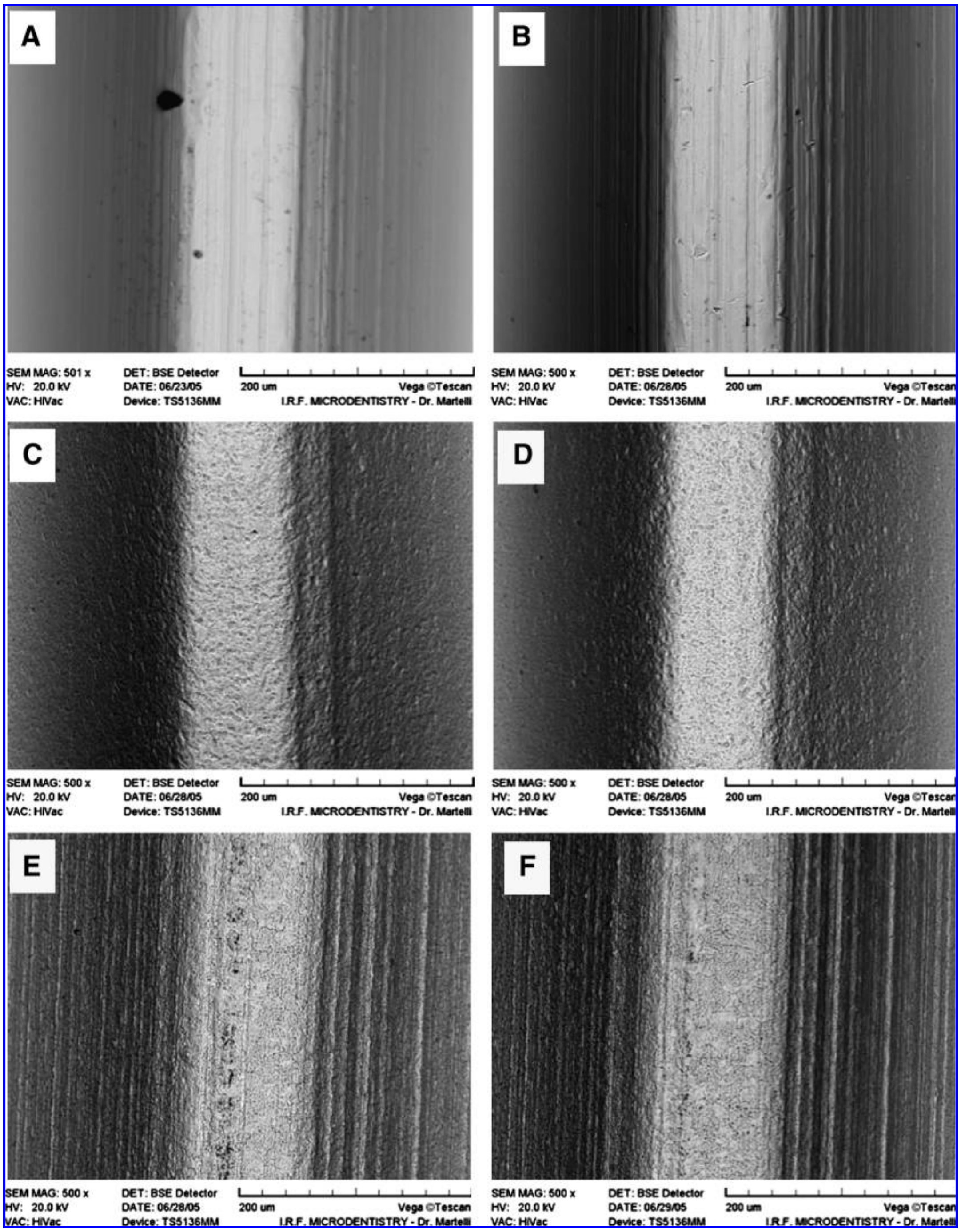

FIG. 4. SEM micrographs revealing the surface morphology of the 980-nm diode irradiated implants. Machined (**A**, **B**), sand blasted with TiO_2 (**C**, **D**), and sand-blasted and acid-etched surfaces (**E**, **F**). Laser power: 2.5 W/1143 J/cm^2 (**A**, **C**, **E**) and 3.0 W/1371 J/cm^2 (**B**, **D**, **F**).

The use of different types of lasers for the decontamination of periodontal pockets, bone surfaces, and root channels is a new field in peri-implantitis treatment. The reduction of microorganisms under the laser action, although confirmed by multiple studies, has some characteristics when used for the treatment of peri-implant disease, because in many cases the laser action may affect the implants' titanium surface.[23] The Nd:YAG laser is not indicated to peri-implantitis treatment due to important alterations on the titanium surface by laser.[24,25] In the present study, the use of the 980-nm diode and the 1064-nm Nd:YAG lasers at 2.5 W and 3W was not enough to produce any alteration on the implants' surface, which were smooth or sand blasted using $TiO_2$and submitted to acid etching surface.

Even considering the thermal and superficial damage of the laser action on the implants, the prime objective is their decontamination for the regression of peri-implant disease. Parker et al. observed the reduction of bacteria using the diode

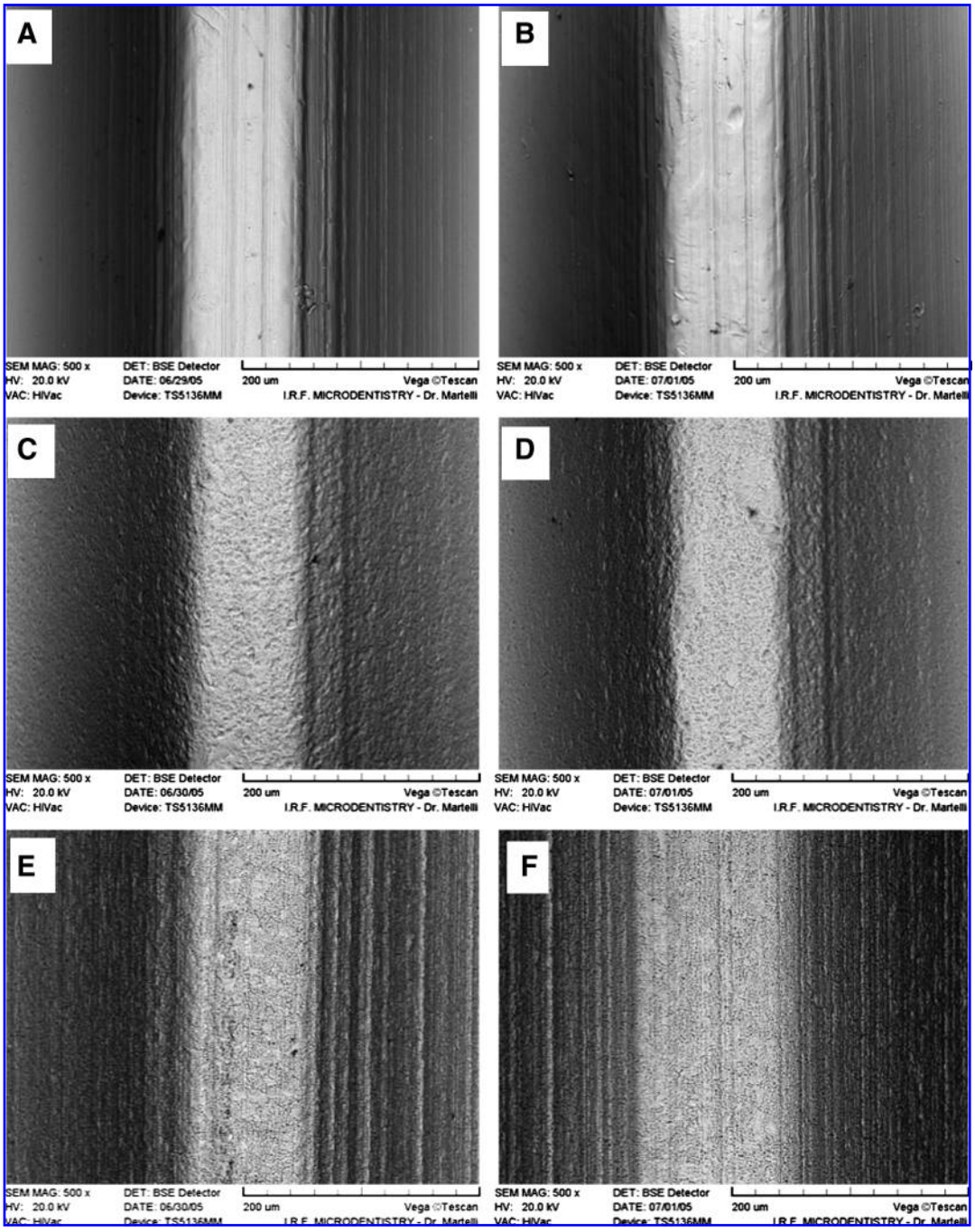

FIG. 5. SEM micrographs revealing the surface morphology of the 1064-nm neodymium-doped yttrium aluminum garnet irradiated implants. Machined (**A**, **B**), sand blasted with TiO_2 (**C**, **D**), and sand-blasted and acid-etched surfaces (**E**, **F**). Laser power: 2.5 W/1143 J/cm^2 (**A**, **C**, **E**) and 3.0 W/1371 J/cm^2 (**B**, **D**, **F**).

laser,[11] finding it quite effective. Castro et al.[26] demonstrated in their in vitro study that the 980-nm diode laser did not change the titanium surface of dental implants, regardless of the energetic parameter used during the irradiation. Another benefit of the 980-nm diode and the 1064-nm Nd:YAG laser in the treatment of peri-implantitis is the possibility of its transmission through thin fiber optics, whose diameter may vary from 200 to 600 μm. That allows them to be used in peri-implantitis without the need to open slashes through peri-implant furrows in initial processes like mucositis.

Because 980-nm the diode laser was capable of decontaminating the implants for the two contaminating agents proposed at 3 W, its use should be encouraged. *E. faecalis* optional anaerobic is resistant, whereas *P. gingivalis* is strict anaerobic, not so resistant, but quite pathogenic and capable of causing great destruction in the peri-implant tissue.

The 1064-nm extra-long-pulse Nd:YAG laser eliminated the bacteria in all irradiated implants at 3 W. At 2.5 W, the bacteria were eliminated on all implants, except the one with the surface conditioned to the acid etching, whose reduction was 97.2%. During the assays, using the Nd:YAG laser, no implant surface was affected. Those facts are mostly due to the anti-bacterial power of the 1064-nm Nd:YAG laser, evidenced in endodontics[27] and periodontal treatments[9,28] because of the extra long pulse of the 1064-nm Nd:YAG, which has a duration of 1 ms, whereas the normal pulse used in all research described in the literature up to now is 100 μm. When extending the pulse, it became almost continuous like the diode, and we decreased the pick power of the 1064-nm Nd:YAG laser, this one being less absorbed by the titanium, not causing alterations on its surface. The reason for the poorer performance of the diode laser cannot be concluded with this methodology. Other research assessing thermal damages or other energetic parameters should be encouraged.

Conclusion

The 980-nm diode and the 1064-nm Nd:YAG lasers were effective in decontamination of *P. gingivalis* and *E. faecalis* without promoting surface alteration on the implants.

Acknowledgments

The authors would like to thank the staff of the Anaerobic Laboratory of the Microbiology Department at ICB-USP.

Author Disclosure Statement

Fábio Gonçalves, Artemio Luiz Zanetti, Raquel Virgínia Zanetti, Francesco Savério Martelli, Mario Julio Avila-Campos, Luiz Fernando Tomazinho, and José Mauro Granjeiro state that competing financial interests exist in this work.

References

1. Zitzmann N.U., Berglundh T., Ericsson I. et al. (2004). Spontaneous progression of experimentally induced periimplantitis. J. Clin. Periodontol. 31, 845–849.
2. Souza K., Shibli J.A., and Marcantonio J.T.E. (2001). Clinical considerations about the treatment of periimplantitis diseases. Rev. Bras. Cir. Traum. 8, 144–148.
3. Martelli F.S. (2004). New Perspectives for the Treatment of Peri-Implant Diseases (in Portuguese). São Paulo: Artes Médicas.
4. Renvert S., Lessem J., Dahlén G., Renvert H., and Lindahl C. (2008). Mechanical and repeated antimicrobial therapy using a local drug delivery system in the treatment of periimplantitis: a randomized clinical trial. J. Periodontol. 79, 836–844.
5. Persson G.R., Salvi G.E., Heitz-Mayfield L.J., and Lang N.P. (2006). Antimicrobial therapy using a local drug delivery system (Arestin) in the treatment of periimplantitis. I: Microbiological outcomes. Clin. Oral Implants Res. 17, 386–393.
6. Heitz-Mayfield L.J., Lang N.P. (2004). Antimicrobial treatment of peri-implant diseases. Int. J. Oral Maxillofac. Implants. 19, 128–39.
7. Di Carlo F., Quaranta A., Di Alberti L., Ronconi L.F., Quaranta M., and Piattelli A. (2008). Influence of amine fluoride/stannous fluoride mouthwashes with and without chlorhexidine on secretion of proinflammatory molecules by peri-implant crevicular fluid cells. Minerva Stomatol. 57, 215–225.
8. Guentsch A., and Preshaw P.M. (2008). The use of a linear oscillating device in periodontal treatment: a review. J. Clin. Periodontol. 35, 514–524.
9. Mummolo S., Marchetti E., Di Martino S., Scorzetti L., and Marzo G. (2008). Aggressive periodontitis: laser Nd:YAG treatment versus conventional surgical therapy. Eur. J. Paediatr. Dent. 9, 88–92.
10. Nowzari H., and Slots J. (1994). Microorganisms in polytetrafluoroethylene barrier membranes for guided tissue regeneration. J. Clin. Periodontol. 21, 203–210.
11. Parker S. (2007). Surgical laser use implantology and endodontics. Br. Dent. J. 14, 202, 377–386.
12. Grassi R.F., Pappalardo S., Frateiacci A., et al. (2004). Antibacterial effect of Nd:YAG laser in periodontal pockets decontamination: a in vivo study. Minerva Stomatol. 53, 355–359.
13. Deppe H., Horch H.H., and Neff A. (2007). Conventional versus CO2 laser-assisted treatment pf peri-implant defects with concomitant use of pure phase beta-tricalcium phosphate: a 5-year clinical report. Int. J. Oral Maxillofacial Implants. 22, 79–86.
14. Deppe H., Horch H.H., Schrodl V., Haczek C., and Miethke T. (2007). Effect of 308-nm excimer laser light on periimplantitis-associated bacteria: an in vitro investigation. Lasers Med. Sci. 22, 223–227.
15. Schwarz F., Sculean A., Rothamel D., Schwenzer K., Georg T., and Becker J. (2005). Clinical evaluation of an Er:YAG laser for nonsurgical treatment of periimplantitis: a pilot study. Clin. Oral Implants Res. 16, 44–52.
16. Schwarz F., Jepsen S., Herten M., Sager M., Rothamel D., and Becker J. (2006). Influence of different treatment approaches on non-submerged healing of ligature induced periimplantitis lesions: an experimental study in dogs. J. Clin. Periodontol. 33, 584–595.
17. Schwarz F., Bieling K., Bonsmann M., Latz T., and Becker J. (2006). Nonsurgical treatment of moderate and advanced periimplantitis lesions: a controlled clinical study. Clin. Oral Investig. 10, 279–288.
18. Persson L.G., Mouhvi J., Berglundh T., Sennerby L., and Lindhe J. (2004). Carbon dioxide laser and hydrogen peroxide conditioning in the treatment of periimplantitis: an experimental study in dogs. Clin. Implant Dent. Relat. Res.6, 230–238.
19. Giannini R., Vassalli M., Chellini F., Polidori L., Dei R., and Giannelli M. (2006). Neodymium:yttrium aluminum garnet laser irradiation with low pulse energy: a potential tool for the treatment of peri-implant disease. Clin. Oral Implants Res. 17, 638–643.
20. Hayek R.R., Araújo N.S., Gioso M.A., et al. (2005). Comparative study between the effects of photodynamic therapy and conventional therapy on microbial reduction in ligature-induced periimplantitis in dogs. J. Periodontol. 76, 1275–1281.
21. Suh J.J., Simon Z., Jeon Y.S. et al. (2003). The use of implantoplasty and guided bone regeneration in the treatment of periimplantitis: two case reports. Implant. Dent. 12, 277–282.

22. Kreisler M., Kohnen W., Marinello C., et al. (2002). Bactericidal effect of the Er:YAG laser on dental implant surfaces: an in vitro study. J. Periodontol. 73: 1292–1298.
23. Park C.Y., Kim S.G., Kim M.D., Eom T.G., Yoon J.H., and Ahn S.G. (2005). Surface properties of endosseous dental implants after NdYAG and CO2 laser treatment at various energies. J. Oral Maxillofac. Surg. 63, 1522–1527.
24. Romanos G.E., Everts H., Nentwig G., et al. (2000). Alterations of the implant surface after CO2- or Nd:YAG-laser irradiation: A SEM examination. J. Oral Laser Applications. 1, 29–33.
25. Romanos G.E., Everts H., and Nentwig G.H. (2000). Effects of diode and Nd:YAG laser irradiation on titanium discs: a scanning electron microscope examination. J. Periodontol. 71, 810–815.
26. Castro G.L., Gallas M., Núñes I.R., Borrajo J.L., Alvarez J.C., and Varela L.G. (2007). Scanning electron microscopic analysis of diode laser-treated titanium implant surfaces. Photomed. Laser Surg. 25, 124–128.
27. Bergmans L., Moisiadis P., Teughels W., Van Meerbeek B., Quirynen M., and Lambrechts P. (2006). Bactericidal effect of Nd:YAG laser irradiation on some endodontic pathogens ex vivo. Int. Endod. J. 39, 547–557.
28. Ben Hatit Y., Blum R., Severin C., Maquin M., and Jabro M.H. (1996). The effects of a pulsed Nd:YAG laser on subgingival bacterial flora and on cementum: an in vivo study. J. Clin. Laser Med. Surg. 14, 137–143.

Address correspondence to:
Fábio Gonçalves, D.D.S., M.Sc.
Rua Vergueiro 2045 cj 109
São Paulo, SP, CEP 04101–000
Brazil

E-mail: fabio@topconsult.com.br

José Mauro Granjeiro, D.D.S., Ph.D.
Departamento de Biologia Celular e Molecular, IB, UFF
Outeiro de Sao Joao Batista, s/n,
Campus do Valonguinho, Centro
Niterói, RJ, CEP 24020–150
Brazil

E-mail: jmgranjeiro@vm.uff.br

This article has been cited by:

1. Samo Pirnat, Matjaz Lukac, Alojz Ihan. 2010. Thermal tolerance of E. faecalis to pulsed heating in the millisecond range. *Lasers in Medical Science* . [CrossRef]