Comparative Study Between the Effects of Photodynamic Therapy and Conventional Therapy on Microbial Reduction in Ligature-Induced Peri-Implantitis in Dogs

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Background: Progressive peri-implant bone losses, which are accompanied by inflammatory lesions in the soft tissues, are referred to as peri-implantitis. The aim of this study was to compare the effects of photodynamic therapy (PDT) and conventional technique on microbial reduction in ligature-induced peri-implantitis in dogs.

Methods: Eighteen third premolars from nine Labrador retriever dogs were extracted and the implants were submerged. After osseointegration, peri-implantitis was induced. After 4 months, ligature was removed and natural bacterial plaque was allowed to form for another 4 months. The animals were then randomly divided into two groups. In the conventional group, they were treated using mucoperiosteal flaps for scaling the implant surface and chlorexidine (conventional) irrigation. In the PDT group, only mucoperiosteal scaling was carried out before photodynamic therapy. Inside the peri-implant pocket, a paste-based azulene photosensitizer was placed and then a GaAlAs low-power laser (λ = 660 nm, P = 40 mW, E = 7.2 J for 3 minutes) was used. Microbiological samples were obtained before and immediately after treatment. Before treatment, one implant was removed and analyzed by scanning electron microscopy to validate the contamination.

Results: The results of this study showed that *Prevotella sp., Fusobacterium sp.*, and *S. Beta-haemolyticus* were significantly reduced for both groups. After treatment, no significant differences were observed between the groups.

Conclusion: These findings suggest that photodynamic therapy is a non-invasive method that could be used to reduce microorganisms in peri-implantitis. *J Periodontol 2005;76:1275-1281.*

KEY WORDS

Animal studies; bacteria/growth; peri-implant diseases/prevention and control; peri-implant diseases/microbiology; phototherapy.

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Early studies documented the excellent long-term prognosis of osseointegration.[1] Several etiologic factors are associated with dental implant failures, such as poor surgical management, failure to achieve osseointegration, premature loading, biomechanical overload, and, mainly, peri-implant infection.[2]

Peri-implantitis is an infectious illness that affects peri-implant tissues such as gingiva and supporting bone. It is a local and relatively superficial infection, caused by well-known specific microflora colonization on the implant surface.[3-5] It can lead to alveolar bone destruction and, if left untreated, can cause implant loss. It is also characterized by acute episodes of peri-implant tissue destruction, alternated with periods of relative dormancy. Lesions in peri-implantitis are characterized by inflammation of gingiva, apical migration of the junctional epithelium, and exposure of the implant surface to the oral environment resulting in formation of peri-implant pockets.

It is unknown to what extent bacterial and non-bacterial residues have to be removed from an implant surface to obtain a predictable, stable clinical result after treatment. Decontamination by mechanical, chemical, and physical methods have been used. Surgical intervention has also been considered.[6,7] Cleaning rough implant surfaces is very difficult since bacteria are protected in microirregularities or undercuts of the surface.[3,8]

Systemic and local antibiotics have also been shown to have a positive effect on clinical and microbiological parameters of peri-implantitis;[9,10] however, growing bacterial resistance to antibiotics makes this an unattractive option.

Laser technology has been introduced in medicine and dentistry as a means of both diagnosing and treating several diseases.[11-14]

The use of high power lasers such as Nd:YAG and diode lasers for bacterial reduction has been described previously and has opened up a new possibility in periodontal treatment[11,12] by eliminating bacteria from tissues through heating. However, the thermal effects may cause tissue damage, e.g., bone reabsorption and pulpar tissue lesion, if the laser parameters are not correctly controlled.

Photodynamic therapy (PDT), which involves the use of low power lasers with appropriate wavelength to kill cells or microorganisms previously treated with a photosensitizer drug, is an athermal alternative approach. The excited photosensitizer reacts with the substrate, mostly oxygen or water, to produce highly reactive oxygen species, as free radicals and/or singlet oxygen. These compounds cause injury and death of microorganisms.[13,14] The selective action of PDT, which does affect normal cells, is one of the most important characteristics of this therapy.[15]

Photodynamic therapy has been studied as a means of eradicating periodontopathogenic bacteria, and photosensitizers have been tested in vivo and in vitro in combination with low-power lasers to determine their bactericidal effect.[16-20] Dobson and Wilson demonstrated that toluidine blue O (TBO) and methylene blue (MB) enabled detectable killing of *Streptococcus sanguis, Porphyromonas gingivalis, Fusobacterium nucleatum*, and *Actinobacillus actinomycetemcomitans* after exposure to He-Ne light.[16] Pfizner et al. demonstrated that it is possible to inactivate *P. gingivalis, F. nucleatum, and Capnocytophaga gingivalis* completely using chlorine 6 and BLC 1010 as photosensitizers and a diode laser at λ = 662 nm, while *A. actinomycetemcomitans* and *E. corrodens* responded only minimally to treatment.[18] Dörtbudak et al. reported a significant reduction of *A. actinomycetemcomitans, P. gingivalis*, and *Prevotella intermedia* following photodynamic therapy with a diode laser at λ = 690 nm and TBO, although complete elimination of bacteria was not achieved; the technique was not compared to any other antimicrobial treatment.[20]

The purpose of this study was to compare conventional peri-implantitis treatment, consisting of mucoperiosteal flap and irrigation with chlorexidine, and photodynamic therapy on the viability of microorganisms during the treatment of ligature-induced peri-implantitis in dogs.

MATERIALS AND METHODS

Animals and Implants

Nine treated Labrador retrievers (48 months old with an average body mass of 18 kg) with ligature-induced peri-implantitis around 18 dental implants were treated in this study. The animals were kept in a kennel under care of a veterinarian during the experimental period, and the surgical protocol followed routine procedures. All animals received humane care in compliance with the Ethical Principles of Animal Experimentation formulated by the Brazilian College for Animal Experimentation, and in accordance with guidelines approved by the Council of the American Psychological Society for the use of animal experiments.

Third mandibular premolars were extracted under general inhalatory anesthesia to create space for dental implants and immediately after, porous surface implants‖ were submerged in each quadrant of the mandible.

Three months after fixture installation, healing abutment connections were installed according to the manufacturer's instructions. At the same time, ligatures were placed around the dental implants to induce peri-implantitis through plaque accumulation. At 120 days, when approximately 25% to 30% of the initial bone support was lost, the ligatures were removed. For the next 120 days, natural bacterial plaque was allowed to accumulate and class 2 peri-implantitis (moderate horizontal bone loss with isolated vertical defects) was induced.[7] The peri-implantitis progression was observed by clinical status and radiography.

One implant was randomly chosen and removed from an animal before treatment and glutaraldehyde fixed, alcohol series dehydrated, critical-point dried, coated with gold under vacuum, and evaluated qualitatively using a scanning electron microscope¶ to verify peri-implantitis and validate the implant contamination.

Treatment

The nine animals were randomly divided into two groups, and peri-implant microbial samples were taken using paper points. In the conventional group, dogs were treated with traditional techniques.[7,21] A crestal incision was made through the mucosa. Buccal and lingual full-thickness flaps were elevated as mucoperiosteal flaps for scaling the implant surface, and the granulation tissue present in the bone craters around the dental implants was then curetted and irrigated with 0.12% chlorexidine solution (Fig. 1).

In the PDT group, only implant surface scaling was carried out before photodynamic therapy. Inside the peri-implant pocket, a polycyclic aromatic hydrocarbon (PAH) photosensitizer in a paste base delivery was

‖ Conexão System, São Paulo, Brazil.
¶ Leo Electron Microscopy Ltd., Cambridge, U.K.

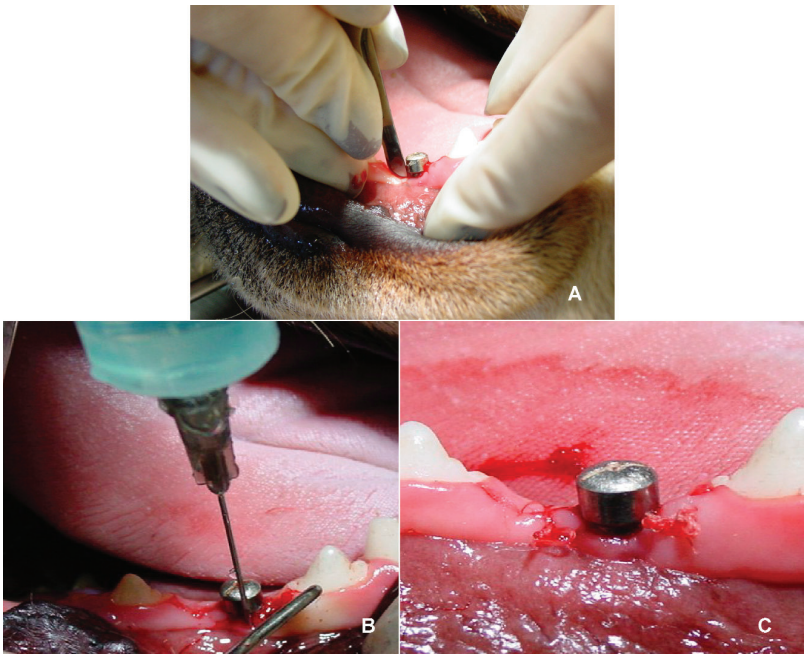

Figure 1.
Conventional treatment. ***A)*** *A crestal incision was made through the mucosa and buccal and lingual full-thickness mucoperiosteal flaps were elevated.* ***B)*** *Irrigation with 0.12% chlorexidine solution.* ***C)*** *Peri-implant tissue after the suture.*

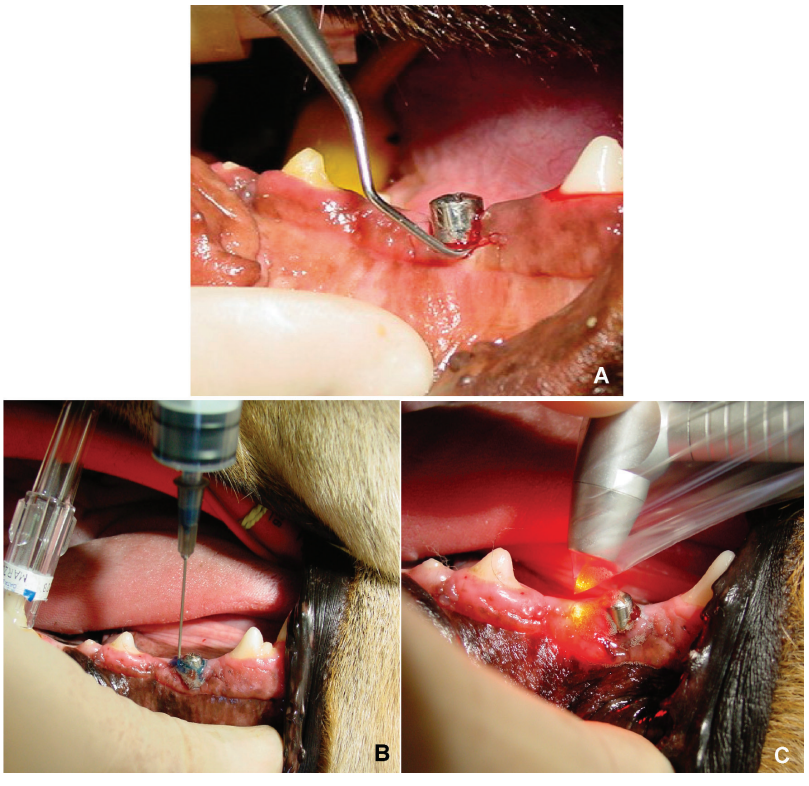

Figure 2.
Photodynamic therapy. ***A)*** *Scaling of implant surface.* ***B)*** *Azulene in a paste base delivery (0.01% w/w) placed into the peri-implant defect.* ***C)*** *Transmucosal scanning of implant surface using a GaAlAs diode laser.*

placed into the peri-implant defect as far as the bony border with a thin needle. The paste was left in place for 5 minutes and then the implant surface was irradiated with a GaAlAs diode laser# at $\lambda = 660$ nm, P = 40 mW, E = 7.2 J for 3 minutes.

The PAH photosensitizer was a commercial solution of azulene 25% (w/v). The paste base was composed of 10% urea peroxide, 15% detergent (tween 80), and 75% vehicle (carbowax). Four hundred (400) µl of azulene solution was used per gram of paste resulting in a 0.01% (w/w) final concentration of azulene.

The diode laser was focused in contact with the mesial, distal, buccal, and lingual surfaces by a scanning method on each surface for an exposure time of 180 seconds (Fig. 2). After this procedure, saline solution was used to remove the photosensitizer from the peri-implantar pocket. Another microbial sample was then obtained from each implant.

Microbial Samples

After treatment, the paper points were removed and placed into 3 ml vials containing VMGAIII anaerobic transport medium according to Shibli et al.[21] All samples were collected by the same operator and coded by an assistant to mask identification. The microbiological analysis began within 24 hours. The samples were centrifuged for 60 seconds and were serially diluted 10-fold in peptonated water to between 10^{-1} and 10^{-6} for quantitative evaluation of colony forming units (CFU)/ml and to obtain isolated colonies for qualitative identification. Aliquots of 0.1 ml of the dilutions were plated onto enriched tryptic soy agar (ETSA) and tryptic soy serum bacitracin–vancomycin agar (TSBV) in a standard manner.

ETSA plates were incubated in anaerobic jars containing a mixed gas atmosphere (85% N_2, 10% H_2, 5% CO_2) at 37°C for 7 to 10 days. TSBV agar plates were incubated in a 5% CO_2 atmosphere for 5 days at 37°C.

The bacterial species were identified from anaerobic cultures based on Gram stain, aerotolerance, colony morphology, esculin hydrolysis, nitrate reduction, indole production, [alpha]- glucosidase and N-benzoyl-DL-arginine-2-naphthylamide (BANA) hydrolysis, oxidase, and catalase activities.

Total viable counts (TVC) and cultivable microbiota detection, including *P. gingivalis, Prevotella sp., Fusobacterium sp.,* and

M.M. Optics, S. Carlos, São Paulo, Brazil.

Streptococcus beta-haemolyticus, were performed based on colony morphology and positive catalase tests.

Statistical Analysis

The TVC were transformed into CFU/ml using predetermined conversion factors to account for dilution and the size of the evaluated surface on the plate. Data were then analyzed for each dental implant.

Differences between groups and bacterial species were assessed by Student *t* test. Results were considered significant when $P < 0.05$.

RESULTS

Only one implant failed to integrate and was removed before peri-implantitis induction. The other 17 implants were osseointegrated and clinically successful. The implants showed typical signs of class 2 peri-implantitis such as inflammation, bleeding on probing and palpation, pronounced bone loss, implant mobility, and probing depth of about 5 mm.

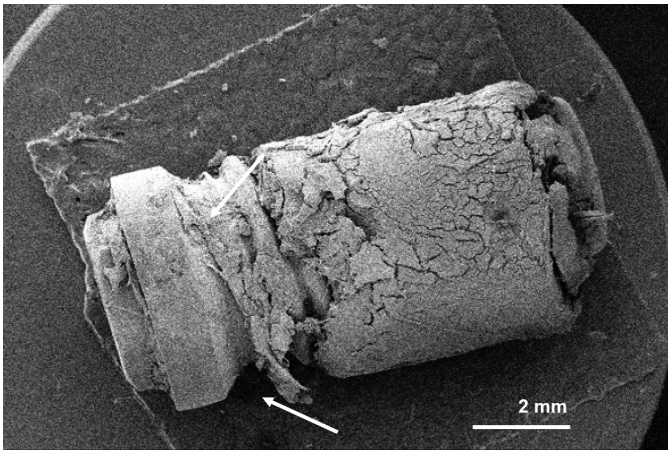

Figure 3.
Electron micrograph of the implant; arrows point to the bone loss typical of the class 2 peri-implant disease.

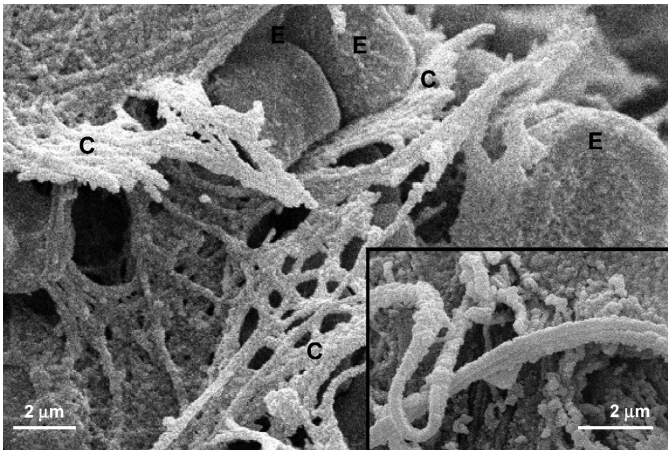

Figure 4.
Electron-micrograph of the implant surface showing bacterial cells (C) supporting peri-implantitis. E = erythrocytes. Insert shows a higher magnification of the contaminated implant.

Moderate horizontal bone loss with isolated vertical defects was observed around the implant neck, which is another sign of class 2 peri-implantitis (Fig. 3). Figure 4 shows the implant surface affected by peri-implant disease. Bacterial cells can be noticed.

P. gingivalis and *A. actinomycetemcomitans* were not detected in any peri-implantitis microbial sample. Figure 5 illustrates the mean bacterial count of *Prevotella sp., Fusobacterium sp.*, and *Streptococcus beta-haemolyticus* before and after treatment for both groups. Before treatment, viability of *Prevotella sp., Fusobacterium sp.*, and *S. beta-haemolyticus* was equal for both groups ($P > 0.05$). Both treatments reduced bacteria significantly ($P < 0.05$), with no statistically significant difference between the conventional and the PDT groups ($P > 0.05$).

Table 1 shows the percentage of bacterial reduction for each group.

DISCUSSION

The purpose of this study was to evaluate the bacterial reduction of peri-implantitis in dogs following photodynamic therapy and conventional therapy consisting of flap surgery and chlorexidine 0.12% irrigation. Significant decreases on total counts of *Prevotella sp., S. beta-haemolyticus*, and *Fusobacterium sp.* were observed for both therapies, with no statistically significant differences.

Superficial lesions caused by well-known microorganisms characterize oral infections. The standard treatment consists of reducing bacteria in the contaminated tissue by mechanical removal by ultrasound, scaling of implant surface, and local or systemic administration of antimicrobial agents such as chlorexidine 0.12%.[4,6] Sometimes, a surgical intervention is needed in order to improve access to the peri-implant pocket.[6,22,23] In this study, the induced peri-implantitis was identified as class 2, and the following signs of peri-implant disease were observed: inflammation, bleeding on probing and palpation, pronounced bone loss, implant mobility, and probing depth of about 5 mm.[7,24] Therefore, mucoperiosteal flaps were elevated to clean the implant surface in the conventional group. This invasive method can cause discomfort to the patient. For the PDT group, mucoperiosteal flaps were not elevated since photodynamic therapy is a non-invasive approach.

Photodynamic therapy for a wide range of bacteria involved in caries, periodontal diseases, and root canal infections has been demonstrated using red light in conjunction with a number of photosensitizers.[25] Haas et al., for example, evaluated the effectiveness of photodynamic therapy in different implant surfaces by

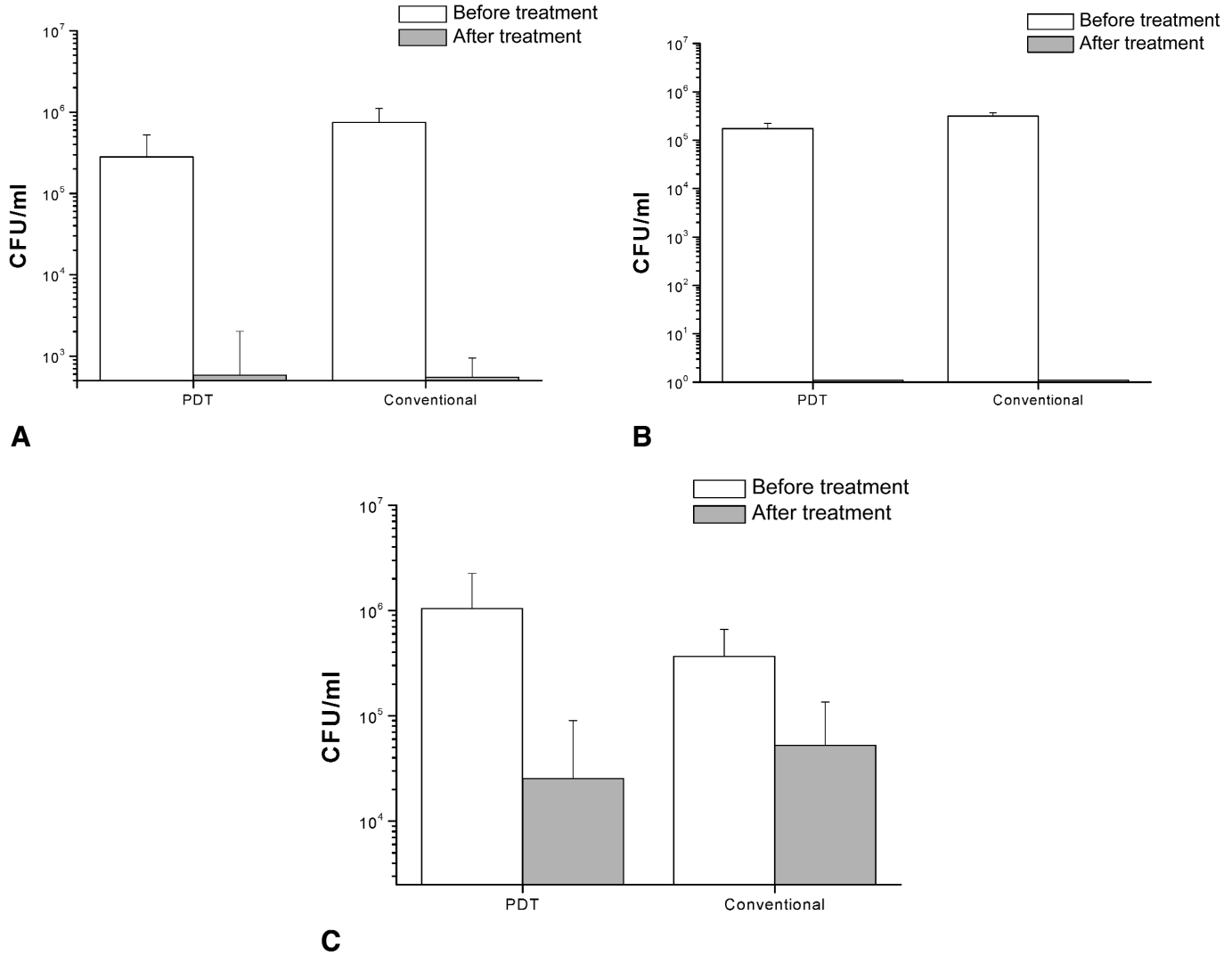

Figure 5.
A) *Mean value and standard deviation of the effect of photodynamic therapy and conventional treatment on the viability of* Prevotella sp. *in peri-implant microbial samples.* ***B)*** *Mean value and standard deviation of the effect of photodynamic therapy and conventional treatment on the viability of* Fusobacterium sp. *in peri-implant microbial samples.* ***C)*** *Mean and standard deviation of the effect of photodynamic therapy and conventional treatment on the viability of* Streptococcus beta-haemolyticus *in peri-implant microbial samples.*

Table 1.

Percentage of Bacterial Reduction by PDT and Conventional Treatment

Bacteria	PDT	Conventional
Prevotella sp.	99.8%	100%
Fusobacterium sp.	100%	100%
Streptococcus beta-haemolyticus	97.6%	85.7%

microbiologic examinations.[19] They concluded that although TBO as a photosensitizer plus laser was effective in reducing bacteria, it did not completely eliminate bacteria from the implant surface. In a more recent study, Dörtbudak et al. examined the effects of photodynamic therapy for decontamination of implant surfaces in the treatment of peri-implantitis.[20] PDT resulted in a significant bacterial reduction, although complete elimination of bacteria was not achieved.

In this work, complete eradication of *Fusobacterium sp.* was obtained after PDT using azulene as a photosensitizer. Azulene is a fused-ring, planar, polycyclic aromatic hydrocarbon (PAH); chemically, it corresponds to cyclopentacycloheptene. Azulene is an essential oil derived from the German chamomile plant *Matricaria chamomile* used in face and body creams, sunburn remedies, burn ointments, and bath salts.[26] Azulene and its derivatives have been found to possess anti-allergic,

anti-inflammatory, and anti-ulcerative properties.[27] Some reports indicate that PAH phototoxicity is predominately caused by photodynamic mechanisms requiring oxygen.[28,29] It is known that oxygen radicals; i.e., hydroxyl, superoxide, or singlet oxygen, may damage cell membranes via lipid peroxidation and may damage DNA.[30-34]

A photosensitizer in liquid solution could stain the implant surface or the surrounding tissues, causing esthetic concerns to clinicians and patients, since the dye presents a dark blue color even at 0.01% (w/v).[35] Delivering the photosensitizer in a paste base allows easy removal from the target tissue through irrigation with saline solution, without any esthetic damage. It is worth noting that in a previous study the azulene in a paste base delivery without light was not able to kill bacteria (unpublished observations). A 1% concentration of azulene is commonly used in cosmetics. In this work, the 25% azulene solution in a paste base delivery resulted in a final concentration of 0.01% (w/w). In this concentration, the azulene photosensitizer is not toxic. Moreover, there is no evidence in the literature that microorganism pathogenicity could increase after PDT.[25] Burns et al. reported that toxicity of TBO, a dye frequently used in PDT, in concentrations higher than 1%, was able to kill oral bacteria in a dark incubation for 5 minutes.[30]

The safety of PDT in clinical trials is based on results of Soukos et al., which demonstrated that the light dose required to kill *S. sanguis* was much lower than that necessary to reduce fibroblast and keratinocyte viability.[15]

The possible advantages of PDT over conventional antibiotic therapy include topical treatment where only affected sites requiring antimicrobial treatment receive the dye and illumination and, unlike antibiotics, do not disrupt microflora in unaffected sites. Also, there is no evidence of resistance development in the target bacteria after PDT.[31,32]

Many factors may interfere in the effectiveness of laser irradiation, including the capacity for light absorption by the photosensitized microorganism, wavelength of the laser, physiological state of the bacteria, emission from the laser, time of laser exposure, pH of the medium, staining of the area to be irradiated, water content, thermal conductivity, and the organic matrix.[33]

In a study in dogs, Shibli et al. investigated the effects of photodynamic therapy on peri-implantitis and reported that PDT was able to reduce bacterial counts. *Prevotella sp., Fusobacterium sp.*, and *S. betahaemolyticus* were not 100% destroyed in all samples, although complete elimination of those pathogens was achieved in some samples.[21] In that work, particularly no more than 50% of *Prevotella sp.* reduction was reached. The photosensitized inactivation of pathogenic microorganisms is a complex phenomenon and depends on many parameters such as the dye, the dye concentration, the type of microorganism, the exposure to light, etc.[34]

Comparing those studies with our findings, it could be concluded that although *Prevotella sp.* was not completely eradicated following PDT, it was reduced by 99.8%. Thus, biofilm present on dental implant surfaces in dogs was susceptible to photodynamic treatment under our study conditions.

Seal et al. reported that the use of TBO at a range of concentrations (12.5, 25, 50, 100 µg/ml) could give a bluish tinge to teeth,[35] which was removed following EDTA irrigation.[35] No bluish staining was observed in this study, probably because of the use of the paste base.

The results in this study indicate that photodynamic therapy is an effective non-invasive method for treating peri-implantitis compared to conventional therapy with elevated mucoperiosteal mucosa flaps for scaling the implant surface. The use of azulene delivered in a paste as photosensitizer seems to be effective against peri-implantitis pathogenic microorganisms and did not stain the implant surface and/or surrounding tissues. The encouraging results of this study indicate that photodynamic therapy for peri-implantitis warrants further investigations as a potential alternative to antibiotic therapy.

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