Treatment of Ligature-Induced Peri-Implantitis by Lethal Photosensitization and Guided Bone Regeneration: A Preliminary Histologic Study in Dogs

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Background: The purpose of this pilot study was to evaluate the healing potential and reosseointegration in ligature-induced peri-implantitis defects adjacent to various dental implant surfaces following lethal photosensitization.

Methods: A total of 36 dental implants with 4 different surface coatings (9 commercially pure titanium surface [CPTi]; 9 titanium plasma-sprayed [TPS]; 9 hydroxyapatite [HA]; and 9 acid-etched [AE]) were inserted in 6 male mongrel dogs 3 months after extraction of mandibular premolars. After a 2-month period of ligature-induced peri-implantitis and 12 months of natural peri-implantitis progression, only 19 dental implants remained. The dogs underwent surgical debridement of the remaining dental implant sites and lethal photosensitization by combination of toluidine blue O (100 µg/ml) and irradiation with diode laser. All exposed dental implant surfaces and bone craters were meticulously cleaned by mechanical means, submitted to photodynamic therapy, and guided bone regeneration (GBR) using expanded polytetrafluoroethylene (ePTFE) membranes. Five months later, biopsies of the implant sites were dissected and prepared for ground sectioning and analysis.

Results: The percentage of bone fill was HA: 48.28 ± 15.00; TPS: 39.54 ± 12.34; AE: 26.88 ± 22.16; and CPTi: 26.70 ± 16.50. The percentage of reosseointegration was TPS: 25.25 ± 11.96; CPTi: 24.91 ± 17.78; AE: 17.30 ± 15.41; and HA: 15.83 ± 9.64.

Conclusion: These data suggest that lethal photosensitization may have potential in the treatment of peri-implantitis. J Periodontol 2003;74:338-345.

KEY WORDS
Animal studies; dental implants; guided bone regeneration; osseointegration; peri-implant diseases/therapy; photochemotherapy; photosensitizing agents.

Several animal experiments have shown that bacterial biofilm accumulation around dental implants promoted by ligature placement can result in peri-implant tissue breakdown or peri-implantitis.1-4 Although there are difficulties in attempting reosseointegration on dental implant surfaces after contamination by periodontal pathogens such as Actinobacillus actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Bacteroides forsythus, and Fusobacterium nucleatum, several therapeutic strategies have been used to treat peri-implantitis,2,5-8 including decontamination with mechanical,3,4,8 chemical,2,5 and physical6,9 methods.

The physical method utilizes a low-power laser following application of a photosensitizing substance. Toluidine blue O (TBO) (see reference 10 for review) has been utilized in both periodontal11-16 and peri-implant diseases.6,17 The mechanism by which TBO kills microorganisms such as P. gingivalis, P. intermedia, A. actinomycetemcomitans, and F. nucleatum has not yet been established, but it is believed that lethal photosensitization of these microorganisms may involve changes in the membranes and/or plasma membrane proteins and DNA damage mediated by singlet oxygen.15,18,19

The objective of this pilot study was to report the results of a prospective study on lethal photosensitization on ligature-
induced peri-implantitis in dogs with different dental implant surfaces.

**MATERIALS AND METHODS**

*Animals*

The experiment outline is shown in Figure 1. Six adult, systemically healthy, male mongrel dogs, 2 years of age, with an average weight of 18 kg were used. Animal selection, management, and surgical protocol followed routines approved for this study by the Dental School of Araraquara Institutional Animal Care and Use Committee.

All surgical and clinical procedures as well as the laser irradiation were performed under general anesthesia accomplished by 0.05 mg/kg of subcutaneous preanesthesia sedation (atropine sulphate) and intravenous injection of chlorpromazine and thiopental. Oral prophylaxis was performed within 2 weeks of tooth extraction. Mandibular premolars were then extracted, creating an edentulous ridge. Both the mandibular quadrants and the alveoli were allowed to heal for 3 months. The upper premolars were also extracted to avoid occlusal trauma interference. During the healing period, bacterial biofilm was controlled by daily scrubbing with 0.12% chlorhexidine and monthly scaling and root planing until cotton ligature placement.

*Implant Design and Surfaces*

Thirty-six dental implants with 4 different surfaces from 3 implant systems were used as follows: 9 commercially pure titanium implants (CPTi); 9 titanium plasma-sprayed (TPS); 9 hydroxyapatite (HA); and 9 hybrid surfaces: machined titanium in the first 3 screws and acid-etched in the other screws (AE). All implants were 10 mm long with a diameter of 3.75 mm (except TPS, which had a 4.1 mm diameter).

*Implant Surgery*

The dental implants were placed after the full-thickness flap under aseptic surgical conditions. The recipient sites were prepared for each implant surface, according to the manufacturer instructions. The implants were randomly distributed so that each dental implant surface was placed at least once in each animal. The implants were positioned at the bone level and a cover screw was screwed onto the implant, including the TPS dental implant surface based on a technique modification indicated by the manufacturer. The flaps were sutured with single interrupted sutures, submerging all implants.

Antibiotics were given once a week for 2 weeks to avoid postsurgical infection. Acetaminophen was given for pain control medication. The sutures were removed after 10 days.

*Experimental Peri-Implantitis*

Three months after implant placement, healing abutment connections were installed, according to manufacturer instructions. After 2 months of a plaque control program and healing of the soft tissue, cotton floss ligatures were placed around the dental implants and sutured in the peri-implant mucosa, not only to facilitate plaque accumulation, but also to hold the ligatures in position. Additional ligatures were placed at 20-day intervals for 60 days to accelerate peri-implant bone loss. At 60 days after first placement, when approximately 40% of the initial bone support was lost, ligatures were removed.

A 12-month plaque control program was initiated by daily scrubbing with 0.12% chlorhexidine and scaling the abutment surface once a month. At the end of this period, natural peri-implantitis progression was observed and only 19 dental implants (6 TPS; 5 CPTi; 5 AE; and 3 HA) were viable. The other 17 were mobile due to significant peri-implant bone loss and were excluded from our sample.

All dogs were subjected to surgical debridement of the dental implant surface and bone craters; lethal photosensitization; and guided bone regeneration (GBR) of the implant sites.

*Lethal Photosensitization and Guided Bone Regeneration*

A crestal incision was made through the mucosa, and buccal and lingual full-thickness flaps were elevated (Fig. 2A). The abutments were removed, and the granulation tissue present in bone craters around the dental implants was curetted with a plastic curet (Fig. 2B).
Each animal received anti-inflammatory medication (2 mg betamethasone††† twice a day) and appropriate analgesia (acetaminophen) for 3 days following surgery to reduce postoperative swelling and pain. Sutures were removed 2 weeks after surgery. Oral prophylaxis was performed with 0.12% chlorhexidine daily for 5 months. Sites were observed daily for gingival health, maintenance of suture line closure, material exposure, or infection.

A fluorochrome### (25 mg/kg body weight) was injected 19 weeks after lethal photosensitization. Five months after surgery, the animals were sacrificed by induction of deep anesthesia followed by intravenous sodium pentobarbital euthanasia.

**Histological Procedures**

The mandibles were removed and block biopsies of each implant site were dissected. The biopsies were

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### Notes

- Sigma Ltd., Poole, UK.
- IR 500-Laser Beam, São Paulo, Brazil.
- Sitema INP, Implantes Nacionais e de Proteses Comercio Ltda, SP, Brazil.
- Gore-Tex sutures, W.L. Gore & Associates, Inc.
- Celestone, Schering-Plough S/A, Rio de Janeiro, RJ, Brazil.
- Oxytetracycline, Pfizer do Brasil, São Paulo, SP, Brazil.
fixed in 4% neutral formalin for 48 hours and then prepared for ground sectioning according to previously described methods. The specimens were cut into a mesio-distal plane using a cutting-grinding unit. From each implant site, one central section was prepared and reduced to a final thickness of 50 to 70 µm by microgrinding and polishing. Before staining, each section was evaluated regarding the location of the fluorochrome marker. The analysis was carried out with a microscope equipped with an image system. In the unstained sections, fluorescence light and a filter cube compatible to the fluorochrome were used to check the osseointegration in the one-third apical area of the implant not affected by peri-implant infection, and to assess the bone remodeling 24 months after insertion.

The sections were then stained with toluidine blue to assess the histometric parameters (Fig. 3): 1 = distance from the bottom of original defect, identified by the difference in coloration after staining (a) to the most coronal point of the newly formed bone with intimate contact to the implant surface (b = reosseointegration); 2 = area of (a) to the most apical border of the newly formed bone; c = to implant shoulder; d = bone fill; 3 = percentage of osseointegration (mineralized bone contact with the implant surface); and 4 = bone area within the limits of the implant threads at the portion of the implant, apical of the peri-implant defect where peri-implantitis did not occur. The data were obtained in pixels and transformed into percentages to avoid the influence of the different macrostructure among the dental implants.

RESULTS
Clinical Observations
Nineteen implants successfully integrated and survived the subsequent treatment periods including mechanical debridement, lethal photosensitization, and GBR. Clinically, the 3 HA dental implant surfaces appeared to be resorbed (Fig. 4A). Heavy calculus deposits were also observed in some dental implants (Figs. 2A and 2B).
4B). None of the membranes placed to treat the peri-implantitis defects had to be removed.

**Histological Examinations and Measurements**

The peri-implant soft and hard tissues generally appeared healthy. The alveolar bone was apical to the connective tissue, and the implants were embedded to a noticeable variable height. The old bone was mostly lamellar and compact, and numerous osteocytes were evident in their lacunae (Fig. 5A). The newly formed bone exhibited different stages of maturation and remodeling. When observed under fluorescent light, the presence of bone remodeling adjacent to dental implant screws was present at 19 months (Fig. 5B).

The defects created by the 2 months of ligature-induced plaque accumulation and 12 months of supragingival plaque control amounted histometrically to 5.86 ± 2.24 mm (TPS); 5.52 ± 0.73 mm (HA); 4.68 ± 1.39 mm (AE); and 3.26 ± 1.58 mm (CPTi).

The highest proportion of mineralized bone contact with the dental implant surface was seen with HA (75.69% ± 12.94), followed by TPS (58.95% ± 2.43), AE (62.40% ± 9.62), and CPTi (52.73% ± 4.47). The mean and standard deviation of the bone area within the limits of the implant threads showed the highest percentage in HA (79.29 ± 5.35), followed by TPS (75.87 ± 16.32), AE (51.61 ± 12.65), and CPTi (48.40 ± 11.39). Figures 6A and 6B characterize the percentage of bone fill, which amounted to 48.28 ± 15.00; 39.54 ± 12.34; 26.88 ± 22.16; and 26.70 ± 16.50 for HA, TPS, AE, and CPTi, respectively. In some specimens, the lateral aspect of the coronal part of the dental implant, i.e., the previously contaminated portion, was covered by a dense connective tissue capsule that separated the newly formed bone from the dental implant surface (Fig. 7).

The percentage of reosseointegration (Figs. 8A and 8B) was 25.25 ± 11.96 for the TPS surface, 24.91 ± 17.78 for the CPTi surface, 17.30 ± 15.41 for the AE surface, and 15.83 ± 9.64 for the HA surface. However, in one AE specimen, there was no observation of new bone in contact with the previously contaminated implant surface (Fig. 9). Data for acid surface, as well as for the other surfaces, demonstrate that all implants remained in place. Therefore, we considered all 19 sites, even though one specimen did not demonstrate reosseointegration.

**DISCUSSION**

The difficulties in obtaining reosseointegration after treatment of peri-implantitis have been documented in several animal studies.2,3,21-23 Most of these studies utilized systemic antibiotics associated with air-powder abrasive8,24,25 or mechanical debridement.4,23,26 To our knowledge, this pilot study was the first to histometrically evaluate the treatment of induced peri-implantitis using lethal photosensitization associated with guided bone regeneration.

Recently, several studies have demonstrated the bactericidal effect of high-power lasers on contaminated dental implant surfaces.27-29 This energy is specifically absorbed by water molecules, which causes the water-rich tissue to be preferentially vaporized. In bacterial cytoplasm, this effect causes cell lysis and variable degrees of damage to the dental implant surface.

In addition, other studies6,8,17 have shown the effectiveness of lethal photosensitization in decreasing the viable count of periodontal pathogens in peri-implan-
titis lesions without damage to the dental implant surface.

The histometric analysis depicts new bone formation in variable degrees in all dental implant surfaces tested. Although the percentage of bone fill observed in studies such as Wetzel et al.\textsuperscript{23} and Persson et al.\textsuperscript{26} was higher, our results ranged from 48.28\% for HA surface to 26.70\% for the CPTi surface, in agreement with Persson et al.,\textsuperscript{3} although their data utilized only CPTi surfaces.

Reosseointegration was achieved with all dental implant surfaces principally at the base of the angular bony defect, in agreement with Persson et al.,\textsuperscript{7} Jovanovic et al.,\textsuperscript{24} and Singh et al.\textsuperscript{30} The highest percentage observed in our investigation was 25.25 ± 11.96 (TPS) and the lowest 15.83 ± 9.64 (HA), in the same range as Hanisch et al.\textsuperscript{22} and Wetzel et al.\textsuperscript{23} Despite controversy on the amount of reosseointegration,\textsuperscript{3,4,7,8,24-26} these different results can be attributed to experimental designs and variables such as ligature-induced peri-implantitis period, microstructure utilized, cleansing methods of contaminated implant surface and their efficiency, bony defect shape, and combination of graft materials and GBR. The different dental implant surfaces, their chemical compositions (CPTi, HA, TPS, AE), and their different surface-free energies did seem to be relevant for the amount of histometrical variables.

On the other hand, Persson et al.\textsuperscript{26} found 83.7 ± 8.6\% reosseointegration in sandblasted, large-grit, acid-etched surface (SLA) and 21.8 ± 16.7\% for turned surface. The authors speculated that the SLA surface could provide a better condition for coagulum stability, facilitating the bone regeneration process. In addition, it has also been suggested that single monolayers from the environment or bulk material can invalidate or make reosseointegration more difficult.\textsuperscript{31,32} However, these results are not totally understood. The other important observation realized in this study was the dissolution of HA coating after peri-implant infection. Factors such as thickness of coating and crystallinity of the coating may be altered due to periodontal pathogen exposure.\textsuperscript{33} Consequently, this factor could complicate peri-implantitis treatment. It can be speculated that the poorest results would be achieved using dental implants with HA coatings. However, these results should be analyzed with caution due to the small sample size used in our study.
A dense connective tissue capsule that separated the newly formed bone from the dental implant surface was observed in some specimens, which agrees with Persson et al.3,4 and Wetzel et al.23 In these studies, the treatment utilized systemic antibiotics and chemical means: delmopinol or chlorhexidine. These substances formed a dense, stable film 7 to 10 nm thick on the oxide layer of the dental implant surface.32 According to Persson et al.,7 this film may prevent bone fill and reosseointegration. In our study, the utilization of lethal photosensitization presented similar results. However, we could not conclude that either TBO alone or TBO plus diode laser would form the same film.

In addition, the use of lethal photosensitization to kill periodontal pathogens offers some advantages over the use of conventional antimicrobials. It avoids development of resistance among target organisms to the photochemically generated free radicals thought to be responsible for bacterial killing and, unlike antimicrobials and antibiotics, there is no need to maintain high concentrations of the TBO in the peri-implant defects for long periods.

In conclusion, data from the present pilot study suggest that the treatment of chronic peri-implantitis by means of lethal photosensitization may obtain significant bone fill associated with reosseointegration. However, these results should be considered with caution and further investigations must be conducted.

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