The Effect of Antimicrobial Photodynamic Therapy in the Osseointegration of Immediately Placed Implants in Sites with Ligature-Induced Periodontitis in Dogs

This study evaluated the effect of the antimicrobial photodynamic therapy (aPDT) on the osseointegration of immediate implants in a healing situation with greater challenges. The mandibular premolars of eight beagle dogs were submitted to ligature-induced periodontal disease. After 3 months, teeth were extracted and immediate implants were placed in the sockets previously decontaminated by mechanical debridement (MD) or MD+aPDT. Following 12 weeks, the dogs were euthanized and the specimens were analyzed. Histologic and histomorphometric analyses demonstrated significantly better results for the immediate implants decontaminated by debridement associated with aPDT. The sites treated with MD+aPDT led to osseointegration of the immediate implants without evidence of inflammation; conversely, evidence of peri-implantitis was observed where aPDT was not used. Int J Periodontics Restorative Dent 2020;40:917–923. doi: 10.11607/prd.4507

Traditionally, during the development of modern implantology, efforts were concentrated on the phenomenon of osseointegration. According to the Bränemark protocol, a healing period from 6 to 12 months would be recommended prior to implant placement. In addition to extending the treatment period, it has been observed that a horizontal bone loss of 29% to 63% and a vertical bone loss of 11% to 22% takes place during this period.

In the past two decades, the placement of immediate implants to reduce treatment time, surgical interventions, and morbidity was being performed quite frequently and had success rates compatible to those found for implants placed into healed, mature bone. Nevertheless, the presence of some clinical conditions, such as active periodontal disease, may contraindicate the technique due to the higher risk of complications in the healing process. However, several clinical and preclinical studies have shown that postextraction sockets associated with periodontal disease may be immediately implanted following tooth extraction, provided that meticulous socket debridement and adequate pre- and postoperative antibiotic therapy are performed. It is important to highlight that the widespread use of antibiotics has led to antimicrobial resistance, which
has become a global public health problem. Antimicrobial photodynamic therapy (aPDT) has been introduced to potentially overcome some of these limitations and may become a new method for antimicrobial treatment of pathogens involved in periodontal disease, with the possibility of oral pathogen suppression with minimal damage to the host systemic health. Therefore, aPDT has been suggested as a new and promising approach in the treatment of both periodontal and peri-implant disease.

To the present authors’ knowledge, there are no preclinical studies with immediate placement of dental implants in periodontally infected sites without the adjunctive use of systemic pre- and postoperative antibiotic therapy. Therefore, the aim of this study was to evaluate the effect of the aPDT in the osseointegration of immediate implant placement in a more impaired healing situation, such as ligature-induced periodontitis.

Materials and Methods

The study protocol was approved by the Animal Experimental Ethics Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo, Brazil (protocol no. 2013.1.992.58.0). Eight dogs, around 18 to 24 months of age, with normal mandibles, no generalized occlusal trauma, no viral or fungal mouth lesions, good overall health, and no systemic compromises attested by veterinary examination, were used.

First Surgical Phase: Periodontal Disease Induction

Following anesthesia, surgery was carried out by quadrants in each animal and periodontitis was induced according to the technique described by Schliephake and Kracht. The experimental periodontitis was induced for 8 weeks and confirmed by clinical and radiographic examinations.

Second Surgical Phase: Tooth Extraction and Implant Placement

Following periodontal disease induction, the animals were anesthetized and mucoperiosteal flaps were raised on both sides of the mandible in the region from the first to fourth mandibular premolars. The teeth were sectioned in a buccolingual direction at the furcation so that the roots could be removed without damaging the bone walls. After random tooth extraction, one hemimandible was decontaminated solely with alveoli debridement, thorough curettage, and rinsing with sterile saline solution to remove all soft tissue tags, and there was a slight decortication to expose the marrow cavities (control group [CG]). The other hemimandible was submitted to the same procedure, with additional aPDT treatment (test group [TG]).

The respective distal sockets of the second (P2), third (P3), and fourth (P4) premolars on both quadrants of the mandible were selected for immediate placement of implants (blueSKY, bredent; 3.5 mm wide and 10 mm long). All implants were airborne-particle abraded and acid etched, and they were positioned at the bone crest and slightly dislocated to the lingual aspect, leaving a small gap between the implant and the buccal bone. Differences in socket anatomy were not a concern, as the randomization procedure would account for any differences. During the procedure, transfers were placed for silicone impression, and wounds were sutured with bioabsorbable sutures (4-0 Vicryl, Ethicon).

Antimicrobial Photodynamic Therapy

For the TG, a dye/laser system was applied before implant placement. The system consists of a handheld battery-operated diode laser (TheraLite Laser, HELBO Photodynamic Therapy, bredent). The photosensitizer was applied to the socket for 5 minutes followed by irrigation with distilled water to remove the photosensitizer excess. Then, a soft laser with a 660-nm wavelength, power of 100 mW/cm², and fluency of 212.23 J/cm² was irradiated through an 8.5-cm–long flexible fiber-optic tip curved at an angle of 60 degrees with a spot size 0.06 cm in diameter. Light was applied to the entire length and circumference of the alveoli, totaling 1 minute of treatment, and an additional 1 minute of light was applied both to the buccal and lingual inner surfaces of the soft tissues in order to decontaminate the flaps. Phenothiazine chloride solution (10 mg/mL; HELBO Blue Photosensitizer, HELBO Photody-
Namic Therapy, bredent) was used as the photosensitizer. Seven days later, the sutures were removed, prosthetic connections were adapted, and temporary metallic prostheses were placed.

**Euthanasia**

The animals were euthanized 12 weeks after the second surgical procedure. After that, the hemimandibles were collected, dissected, and fixed in 4% phosphate-buffered formalin (pH 7) until histomorphometric analysis.

**Histomorphometric Analysis**

From each experimental implant, one buccolingual section (representing the central area) was prepared and reduced to a thickness of about 25 µm by microgrinding and polishing. The sections were stained using toluidine blue and alizarin red, and the images were analyzed by the same blinded and calibrated examiner (C.M.R.M.). The morphometric measurements were performed as described in Fig 1.

**Statistical Analysis**

The primary outcome measure of this study was the level of peri-implant bone (buccal bone resorption) following immediate implant placement in previously infected alveoli, and the secondary outcomes included bone quantity in the same implants (bone-to-implant contact and bone density). Quantitative data are presented as mean ± SD. Each dog was considered as the experimental unit (N = 7). The mean differences between the groups were analyzed through Wilcoxon matched pairs test. For all statistical analysis, a significance level of 5% was adopted.

**Results**

**Clinical Findings**

Healing was uneventful in all surgical phases. However, only seven out of the eight dogs completed the present study. One animal presented problems with the fixed anesthesia after the second surgical phase and subsequently died. Of the implants in the seven remaining dogs, 38 out of 42 were available for the histologic analysis; 4 implants failed to osseointegrate. The treatment distribution of the 38 buccolingually sectioned implants evaluated histologically was as follows: 20 implants in TG and 18 implants in CG.
Fig 2  Histologic images (Alizarin red stain) of the test sites. (a) The implant is well integrated into the surrounding bone tissue with the buccal plate (B) positioned coronally to the lingual bone plate (L) (x2.5 magnification). (b) Presence of the two types of bone tissue: parent lamellar bone (PLB), representing the “old bone,” and newly formed bone (NB), separated by the dotted yellow line (x10 magnification). (c) Under polarized light, the area occupied by the different bone types (PLB and NB, separated by the dotted yellow line) is highlighted by the orientation of the collagen fibers (x10 magnification).

Fig 3  Histologic images (Alizarin red stain) of the control sites. (a) Compromised integration of the implant (x2.5 magnification). (b) Presence of the two types of bone tissue: parent lamellar bone (PLB), representing the “old bone,” and newly formed bone (NB), paved with osteoclasts (arrows) (x10 magnification). Inflammatory infiltrate is represented by the asterisk. (c) Higher magnification (x20) of the red box in (b), detailing the PLB paved with osteoclasts. (d) Higher magnification (x20) of the yellow box in (b), detailing the presence of an osteoclastic cell within the Howship lacunae on the surface of the NB.
Histologic Observations

Microscopic analysis demonstrated that the TG implants were well integrated into the surrounding bone tissue (Fig 2), while the CG implants demonstrated compromised integration to the bone tissue (Figs 3a and 3b) and the presence of an inflammatory infiltrate (Fig 3b). The vertical buccal bone resorption level varied between the groups and was systematically more pronounced in the CG implants (Fig 3a) than in TG implants (Fig 2a).

In general, both groups showed the presence of the parent lamellar bone (PLB), representing the “old bone,” and newly formed bone (NB) (Figs 2b, 2c, 3b, and 3c). The NB was present mainly in the bone area adjacent to the implant surface. In the TG, the NB sometimes exhibited a layer of osteoblasts on the external surfaces of the most recently formed bone, paving the osteoid matrix and representing the active bone formation process (Fig 2b). In the TG, the presence of Howship lacunae and osteoclasts, representing the bone resorption process, were observed in both NB and PLB (Figs 3b to 3d).

Histomorphometric Results

Data regarding the histomorphometric analysis are summarized in Table 1. In the present histomorphometric analysis, statistically significant differences were observed for almost all the intergroup comparisons, favoring the TG. The exception was the parameter bone density, in which the TG (70.57% ± 8.49%) had a numerically better value than the CG (58.37% ± 9.55%).

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TG = test group; CG = control group.
*P ≤ .05 (Wilcoxon matched pairs test).

Discussion

Immediate implant placement is a widely used procedure, and it presents survival rates similar to those observed for implants placed in healed, mature bone17,18 with high degrees of success.3,19 However, frequent tooth loss is associated with chronic diseases,20 which conventionally contraindicates immediate implant placement.3,6

One of the main requirements to ensure implant success is the maintenance of the peri-implant tissues in such a way that neither the osseointegration nor esthetic aspects are compromised. The histomorphometric analysis of the present study showed significantly less bone loss in the TG (2.03 ± 1.76 mm) compared to the CG (5.84 ± 1.44 mm). Suaid et al24 also in a histomorphometric study in dogs, reported that equicrestal implants presented a buccal bone resorption of 1.67 ± 0.82 mm after a healing period of 12 weeks. Different from
the present study, the immediate implants were placed in disease-free sites associated with a rigid pre- and postoperative systemic antibiotic therapy. Moreover, a flapless approach was used, and the worse results observed in the present study may be assigned to this different approach. Supporting this hypothesis, a comparative study by Novaes et al25 that evaluated buccal bone remodeling after immediate implantation demonstrated vertical bone resorptions of 2.14 mm in implants placed with a mucoperiosteal flap elevation and of 0.95 mm in implants placed with a flapless approach. In a histomorphometric study on bone healing in four different implant systems, de Santics et al26 showed a mean vertical bone loss of 2.5 mm in the buccal plate of immediate implants placed with a mucoperiosteal flap approach. The buccal bone resorption observed in the TG of the present study was quite similar to that reported by Novaes et al’s25 group of immediate implants placed with a flap approach. However, de Santics et al26 showed higher resorption values compared to the present study, which may be related to the 6-week healing period vs the present study’s 12-week healing period.

The success of a dental implant is affected by both bone-to-implant contact (BIC) and marginal bone loss. The percentage of BIC, which is considered a critical variable to long-term implant success, is directly influenced by the bone density.29 The percentage of BIC observed in the present study highlights a significantly better result in the TG, which is in accordance with other immediate implants studies.28 In the bone healing process, the bone density is dependent on the blood clot stabilization around the implant surface, and this condition can be negatively affected by the presence of contamination. In the present study, although the TG did not show a significantly better result in relation to CG, it presented numerically higher values (demonstrating coherence) and had better BIC values. This suggests that this type of decontamination protocol can influence the adjacent bone healing.

Several clinical studies suggest that immediate implant placement at sites associated with periodontal infection increases the failure risk of marginal bone loss and/or implant failure.8 However, the immediate implants in the present study were placed in periodontally infected sites, the TG implants had a survival rate of 95.24% (1 of 21 implants failed), comparable with those of immediate implants placed in disease-free sites.17,18 In contrast, the CG implants demonstrated a lower survival rate of 85% (3 of 21 implants failed), with most of the remaining implants showing signs of peri-implantitis.

In this first study in a new line of research that may take years to consolidate, the authors proposed a simple surgical technique without many variables in order to avoid doubts concerning the interpretation of results and findings. Still, the present study has some limitations, such as the reduced number of animals, which is limited by the Animal Experimental Ethics Committee and costs; however, many studies use this number of animals or fewer.29,30 Also, as in all animal studies, direct translation of results to humans should be done with caution.

Conclusions

The TG sites treated with aPDT led to osseointegration of the immediate implants placed into a more challenging healing situation (induced periodontitis) and presented healing without evidence of inflammation. On the other hand, where aPDT was not used, evidence of peri-implantitis was observed on the immediate implants.

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References


