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ORIGINAL ARTICLE

Microbiologic effect of two topical anti-infective treatments on ligature-induced peri-implantitis: A pilot study in dogs

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Abstract

Background: The aim of this split-mouth design pilot study in dogs was to assess microbiologic effects of two topical anti-infective treatment protocols on dental implants subjected to ligature-induced peri-implantitis, without use of systemic antibiotics.

Methods: Eight adult Beagle dogs each received four dental implants in contralateral, edentulated, mandibular jaw quadrants. After 8 weeks, silk ligatures were installed, to be removed after another 8 weeks. After 6 additional weeks, induced peri-implantitis lesions were subjected to either antimicrobial photodynamic therapy (aPDT) or a topical tetracycline (TTC) hydrochloride (50 mg/mL) solution. Microbiologic samples were collected from the deepest proximal peri-implantitis site in each jaw quadrant before and after treatment. The samples were analyzed using DNA-DNA hybridization checkerboard technique.

Results: Peri-implantitis induction successfully produced lesions with microbiologic characteristics similar to those found in humans. Overall results showed effective bacterial count reductions for both protocols. aPDT demonstrated major reductions of the red complex, but no statistical differences between groups were observed when adjusted for multiple comparisons.

Conclusion: aPDT and TTC successfully decontaminated infected implant surfaces. Implant decontamination with aPDT appears to be a viable alternative to TTC in the management of peri-implantitis infection.

KEYWORDS
antimicrobial photodynamic therapy, checkerboard, peri-implantitis, tetracycline

1 | INTRODUCTION

Peri-implantitis is an inflammatory disease caused by oral bacteria that promulgates bone loss at osseointegrated dental implants,[1] with an estimated prevalence of 22%.[2] Using DNA-DNA checkerboard,[3] peri-implantitis–associated microbiota have been shown to be similar to microbiota in chronic periodontitis, and teeth have been found to be microbial reservoirs for peri-implant infections.[4] Moreover, patients with residual pockets after periodontal therapy appear to exhibit elevated risk of peri-implant disease, increasing the strength of the relationship between peri-implantitis and periodontitis.[5]

Several protocols have been proposed to manage peri-implant infections, most of which have their basis in periodontal therapy.[6–10] Once a peri-implant infection is established,

it is challenging to decontaminate and clean the affected implant surface,[11] which is vital for re-establishing implant bone anchorage and osseointegration.[12] Clinical and preclinical studies report the use of systemic antibiotics, including amoxicillin and metronidazole as standalone or in conjunction with biologics, with fair outcomes.[7,13,14] The use of topical antibiotics, gel compositions, or topical solutions to decontaminate and clean infected implant surfaces has been proposed to circumvent the use of systemic antibiotics, however little is known regarding the microbiologic effects of such protocols.[8,15,16] For example, topical tetracycline (TTC), commonly used to decontaminate periodontally involved root surfaces,[15] has also been used to decontaminate dental implant surfaces.[8]

In order to avoid the use of antibiotics and reduce the risk for microbial resistance,[16] several alternatives, including antimicrobial photodynamic therapy (aPDT), have been proposed.[17] aPDT targets the bacterial intra- and extracellular structures via a photosensitizer that absorbs light energy, usually from a red laser source, to produce singlet oxygen and, in turn, bacterial cell protein denaturation and death.[18] Clinical studies have shown promise in the use of aPDT in periodontal defects,[19,20] particularly in sites with deep pockets.[21] Preclinical studies have shown aPDT advances healing in the treatment of peri-implantitis with guided bone regeneration (GBR).[22] Yet, outcomes after GBR remain unpredictable with respect to bone gain and defect resolution.[23] To date, no microbiologic assessments have been made in the treatment of induced peri-implantitis using DNA-DNA checkerboard hybridization. Such data could be valuable for understanding treatment stability. The aim of this split-mouth design pilot study in dogs was to assess the microbiologic effects of two topical anti-infective treatments of implant surfaces subject to ligature-induced peri-implantitis.

2 | MATERIALS AND METHODS

This study followed the protocol approved by the Animal Experimental Ethics Committee, University of São Paulo, Ribeirão Preto, Brazil (protocol number 06.1.458.53.5). The study was divided into two phases: 1) preparation for and induction of ligature-induced peri-implantitis and 2) treatment of induced peri-implantitis defects using different anti-infective therapies. The general outline of the study is shown in Figure 1.

2.1 | Animals

Eight male, purpose-bred Beagle dogs, aged 18–24 months, approximate weight of 15 kg, obtained from a licensed vendor, were used. The animals were individually housed and had *ad libitum* access to water and a standard laboratory dog food diet throughout the study. The number of animals was determined based on previous studies addressing the treatment of periimplantitis.[12,24]

2.2 | Materials and devices

Titanium dental implants* and healing abutments† were used. For the aPDT, a handheld, battery-operated, diode laser‡ was used. The device was set to a wavelength of 660 nm and irradiance of 212 mW/cm^2. The laser was irradiated through an 8.5-cm-long flexible fiber optic tip curved at a 60° angle with a ø0.06 cm spot size. A 10-mg/mL phenothiazine chloride solution§ was used as photosensitizer.

2.3 | Surgical procedures

Food was withheld the night before the surgeries. For all surgical procedures, the animals were pre-anesthetized using 10% zolazepam (0.10 mL/kg intramuscular [IM]) and acepromazine (0.2-0.3 mg/kg IM). The animals were then maintained on gas anesthesia (isoflurane 1%-2% to effect). Depth of anesthesia was monitored by lack of response to toe pinch, lack of corneal reflex, as well as continuous monitoring of depth of respiration, respiratory rate, and heart rate; all anesthesia and related procedures were performed and monitored by veterinary staff. Routine dental infiltration anesthesia (mepivacaine 2% norepinephrine 1:100,000) was used at the surgical sites. An opioid (tramadol 3 mg/kg twice daily) and a non-steroidal anti-inflammatory agent (ketoprofen 1 mg/kg IM once daily) were used for postoperative pain control.

Flapless bilateral surgical extractions of the mandibular premolar and first molar teeth were performed. Atraumatic extractions were secured by bi-sectioning the teeth using a high-speed hand-piece and elevators. Interrupted sutures¶ were placed for wound closure and primary intention healing. Sutures were removed at 2 weeks, and the extraction sites were allowed to heal for an additional 6 weeks.

For implant placement, mid-crestal incisions were carried out and the buccal and lingual mucoperiosteal flap raised to expose the edentulated alveolar ridge. Osteotomies were prepared following the manufacturer's protocol; four dental implants were placed in each mandibular jaw quadrant. Flaps were sutured using non-resorbable sutures# to allow transmucosal healing. Sutures were removed at 2 weeks and the surgical sites allowed to heal for an additional 6 weeks.

* BoneLevel TML SLA, ø3.3x10 mm, Straumann, Basel, Switzerland

† ø4.1x3.5 mm, Straumann, Basel, Switzerland

‡ HELBO mini-laser 2075 F dent, HELBO Photodynamic Systems, Grieskirchen, Austria

§ HELBO Blue Photosensitizer, HELBO Photodynamic Systems, Grieskirchen, Austria

¶ | 5.0 Vicryl Ethicon, Johnson & Johnson, São Jose dos Campos, Brazil

5-0 Mononylon Ethicon, São Jose dos Campos, Brazil

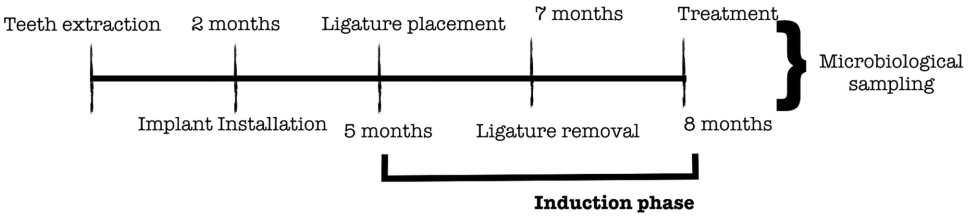

FIGURE 1 Outline of study

2.4 | Peri-implantitis induction

For peri-implantitis induction, silk ligatures* were placed around the healing abutments over 8 weeks. Ligatures were checked weekly and missing ligatures immediately replaced. Ligatures were then removed and peri-implant lesions were allowed to progress undisturbed for an additional 6 weeks. The standard dog food diet was wetted to a slurry during peri-implantitis induction to support dental plaque accumulation.

2.5 | Implant surface decontamination

A mucoperiosteal flap was elevated without releasing incisions. The peri-implantitis defects were debrided of granulation tissue and the dental implants instrumented using plastic curette.† Exposed dental implants were then equally allocated to one of the following treatments:

- TTC: Implant surfaces were rubbed with cotton pellets soak-loaded with a tetracycline hydrochloride (HCl) (50 mg/mL) solution for 3 minutes, cotton pellets substituted every 30 seconds. The surgical sites were then thoroughly rinsed using sterile saline.
- aPDT: Peri-implant defects were filled to capacity with the photosensitizer and the solution was left in place for 5 minutes. The surgical sites were then thoroughly rinsed using sterile saline to remove the solution and avoid optical shielding. Next, the implant surface was divided into six areas and the laser was applied for 30 seconds over each area, for a total dose of 44 J/cm^2.

2.6 | Microbiologic analysis

Biofilm samples were collected from the deepest proximal site for each dental implant and pooled. Sampling was performed before and after TTC or aPDT treatments, with all efforts to maintain sites isolated to prevent contamination by swabbing with sterile micro-brushes‡ and transferred to microcentrifuge tubes with a 100 μL 10-mM Tris-HCl and 1-mM EDTA, pH 7.6 solution.

After collection and conditioning, 100 μL of a 0.5-mM NaOH solution was added to the centrifuge tube. The samples were then dispersed using a vortex mixer, and counts of 40 species were performed for each sample using checkerboard DNA-DNA hybridization.[25] Signals were evaluated visually by an experienced examiner by comparing standards at 10^5 and 10^6 bacterial cells for the test species on the same membrane. The scores ranged from 0 to 5 as follows: 0, not detected; 1, $< 10^5$ cells; 2, 10^5 cells; 3, 10^5–10^6; 4, 10^6; 5, $> 10^6$.

2.7 | Statistical analysis

The proportion of each species in relation to total DNA count for the 40 bacterial species evaluated were computed for each animal then averaged within experimental groups. Bacterial species were also grouped into complexes,[26] intra- and inter-group comparisons made for pre- and post-treatment counts, and reductions achieved.

Non-parametric statistical methods were used for the statistical analysis. Median, 25th, and 75th percentiles were calculated and reported for bacteria counts before and after treatment. Pre- and post-treatment (delta) count reductions were compared between groups. Mann-Whitney two-sample tests were used to compare between groups, and Wilcoxon matched-pair signed-ranks tests were used for within-group comparisons. The animal was the unit of analysis and the P value was set to 5%. A comparison was considered statistically significant if the calculated P value was < 0.00125, to account for multiple comparisons (0.05/40 bacterial species).

3 | RESULTS

Overall results showed that ligature-induced peri-implantitis introduced a microbiota characterized by a predominance of bacteria of the red (12% versus 16%) and orange (40.18% versus 40.89%) complexes for the TTC and aPDT groups, respectively. Low levels of *Actinobacillus actinomycetemcomitans* ($< 1\%$) and low proportions of yellow, blue, and purple complexes were observed for both groups at baseline (Figures 2 and 3). No significant differences were observed between treatments for any bacterial species at baseline, showing consistent induced infection for both groups (Figure 2).

* 3-0 Silk Ethicon, Johnson & Johnson, São Jose dos Campos, Brazil

† Implacare-IMPHDL6, Hu-Friedy, Chicago, IL

‡ KG Sorensen brush extra fine, KG Sorensen Cotia, SP Brazil

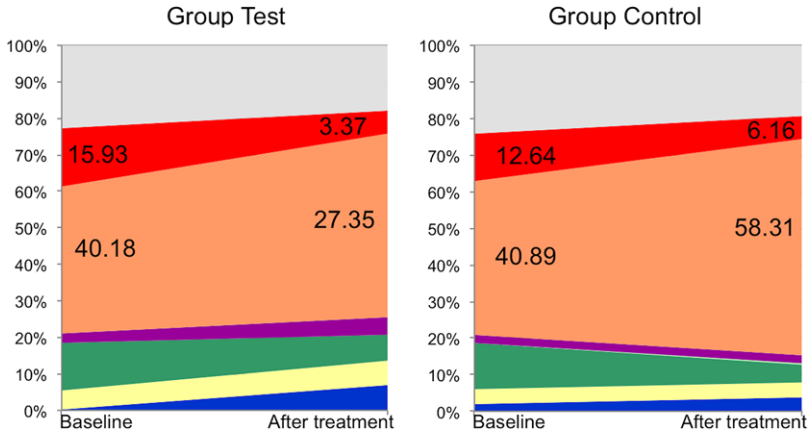

FIGURE 2 Proportions of bacteria in each group after disease induction and after treatment Note a tendency to reduced levels after treatment favoring aPDT

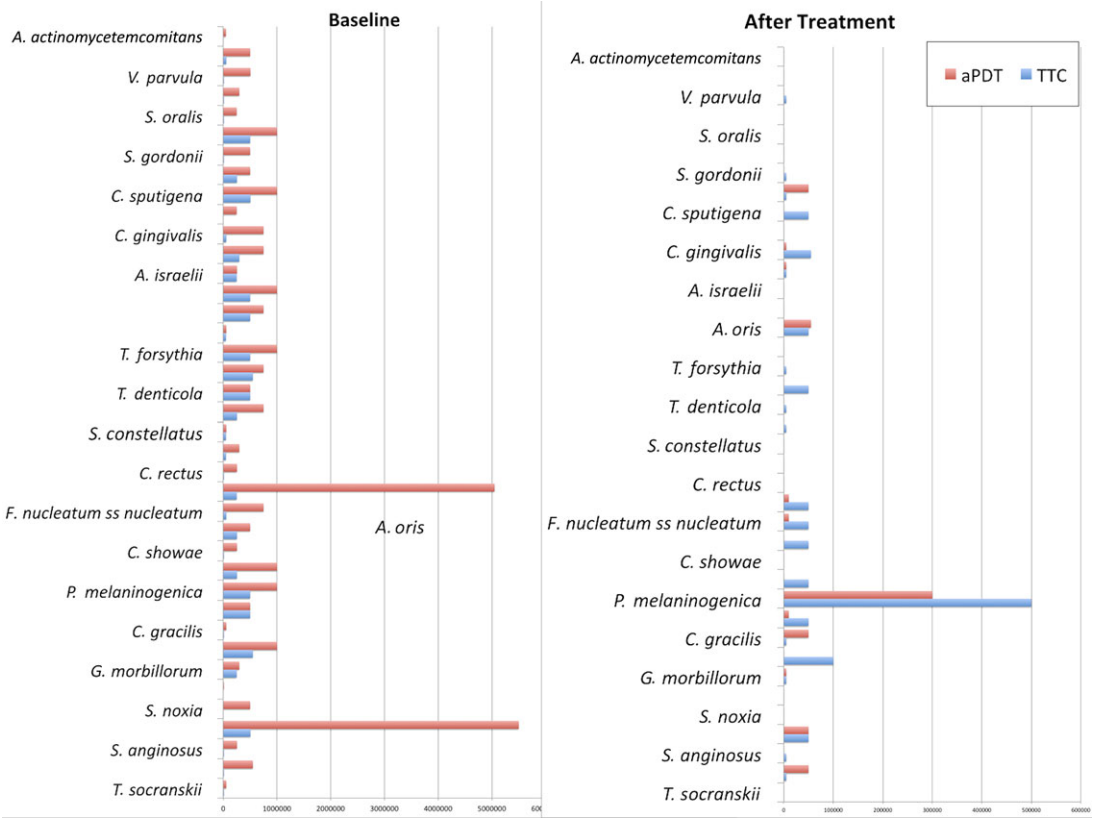

FIGURE 3 Bacterial counts before and after treatment for both groups Despite the major reductions achieved with aPDT treatment, no differences could be detected

After treatment, major reductions in bacterial counts for all bacterial species were observed (Table 1 and Figure 2). Whereas the aPDT group generally exhibited lower red complex bacterial counts than did the TTC group (> 1% versus 6%), no significant differences were observed between groups following treatment (Figure 4). Similarly, no significant differences were observed in the amount of bacterial reduction between groups, despite overall counts indicating a numerically greater reduction favoring the aPDT group. The proportions of orange complex showed a post-treatment increase tendency for the TTC group, whereas the aPDT group showed lower proportions, together with a reduction tendency.

4 | DISCUSSION

This microbiologic pilot study was carried out to evaluate antimicrobial effects of two anti-infective protocols in the surgical treatment of ligature-induced peri-implantitis using a dog model, without the use of systemic antibiotics.

TABLE 1 Reduction in subgingival bacterial species after treatments. Median, 25th, and 75th percentiles of bacteria counts × 1000

	TTC				aPDT				
Bacterial species	**Median**	**25%**	**75%**	***P* value before versus after[a]**	**Median**	**25%**	**75%**	***P* value before versus after[a]**	***P* value TTC versus TFA[a]**
T. socranskii	5	0	90	0.33	55	0	100	0.05	0.16
E. saburreum	5	0	500	0.45	450	0	1000	0.19	0.28
S. anginosus	10	0	400	0.34	250	0	500	0.05	0.43
N. mucosa	500	0	9500	0.52	5400	0	10000	0.09	0.29
S. noxia	0	0	490	0.57	500	0	500	0.046	0.33
P. acnes	0	0	10	0.56	5	0	10	0.08	0.32
G. morbillorum	250	0	990	0.19	295	0	500	0.09	0.75
L. buccalis	50	-100	900	0.53	995	100	10000	0.035	0.46
C. gracilis	5	0	90	0.52	50	-90	400	0.34	0.53
P. intermedia	450	0	900	0.17	495	0	990	0.09	0.83
P. melaninogenica	5	0	500	0.52	500	10	900	0.07	0.43
F. nucleatum ss polymorphism	255	0	9000	0.21	1000	10	9900	0.035	0.19
C. showae	5	0	10	0.45	255	0	990	0.05	0.66
F. periodonticum	200	0	500	0.39	495	0	1000	0.05	0.2
F. nucleatum ss nucleatum	55	0	900	0.29	740	10	1000	0.06	0.12
F. nucleatum (sp vicentii)	0	0	500	0.73	4995	10	9990	0.06	0.21
C. rectus	10	0	490	0.34	255	0	500	0.05	0.49
P. micra	50	0	1000	0.38	300	0	1000	0.05	0.49
S. constellatus	50	0	500	0.38	55	0	500	0.05	0.67
E. nodatum	0	0	490	0.57	750	0	1000	0.05	0.2
T. denticola	500	0	500	0.09	500	0	990	0.05	0.49
P. gingivalis	5	0	9900	0.28	750	0	1000	0.05	0.67
T. forsythia	495	0	1000	0.33	1000	0	1000	0.05	0.67
A. naeslundii I	0	0	100	0.57	55	0	1000	0.05	0.16
A. oris	500	0	900	0.14	650	-10	1000	0.12	0.83
A. gerencseriae	500	0	500	0.29	1000	0	1000	0.046	0.28
A. israelii	250	0	500	0.51	255	0	500	0.05	0.9
C. ochracea	290	0	1000	0.2	740	0	1000	0.05	0.73
C. gingivalis	0	-10	400	0.99	740	0	1000	0.09	0.09
E. corrodens	0	0	490	0.57	250	0	500	0.08	0.57
C. sputigena	500	0	9500	0.2	1000	0	10000	0.05	0.25
S. mitis	200	0	500	0.28	400	0	500	0.09	0.39
S. gordonii	0	0	400	0.57	500	0	990	0.05	0.09
S. sanguis	500	0	500	0.29	1000	0	1000	0.046	0.28
S. oralis	5	0	100	0.45	250	0	500	0.08	0.43
S. intermedius	10	0	490	0.34	300	0	500	0.05	0.23
V. parvula	0	0	500	0.65	500	0	1000	0.05	0.09
A. odontolyticus	55	0	900	0.34	500	0	1000	0.05	0.53
A. actinomycetem-comitans	0	0	100	0.56	50	0	100	0.08	0.32

aPDT, antimicrobial photodynamic therapy; TTC, topical tetracycline.

[a]Wilcoxon signed-rank test.

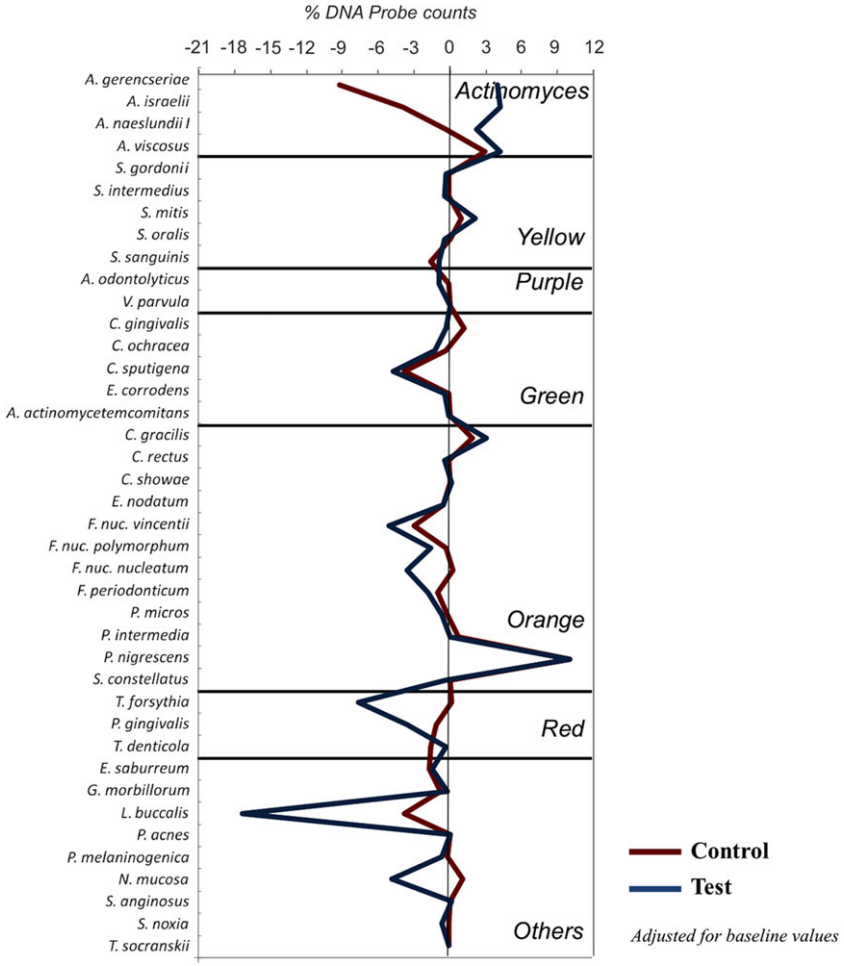

FIGURE 4 Proportional reduction of DNA probes after treatment adjusted to baseline values

Observed pre-treatment proportions of bacteria from the red (12%-16%) and orange (approximately 40%) complexes in the present study were compatible with the bacterial composition reported in humans (17.2% and 46% for red and orange, respectively).[3] The overall outcomes following implant surface decontamination showed a tendency toward greater reduction of bacterial counts and proportions favoring aPDT, but no statistically significant differences were discerned.

aPDT appeared more effective at reducing the red complex microbiota compared with TTC, with final proportions approaching 0%. Despite the lack of statistically significant differences, a common finding in animal studies due to small sample sizes, this observation may still influence long-term significance in clinical settings considering the pathogenic role of bacteria composing the red complex,[26] with *Porphyromonas gingivalis* playing an important role in disbiotic inflammation. Certainly, this extrapolation of our results deserves longer-term study to clarify the significance of perceived differences.

Differences between treatments in reduction of orange complex microbiota might point to a greater efficacy of aPDT versus tetracycline in suppressing this microbial complex, in particular, the *Fusobacterium* genus, especially considering that subgingival *Fusobacterium nucleatum* appears to facilitate co-aggregation of periodontal pathogens, including *A. actinomycetemcomitans*, *P. gingivalis,* and *Tannerella forsythia*.[27] The efficacy of aPDT in reducing *F. nucleatum* has been also been shown in periodontitis sites in humans.[28] In addition, the present study showed that differences were obtained relative not only to pathogenic bacteria but to health-compatible microbial species, including blue complex *Actinomyces* genus.

A few preclinical studies have evaluated microbiologic outcomes of implant surface decontamination, usually using selective culture.[29,30] The DNA-DNA checkerboard hybridization technology manages a larger number of species than do traditional culture and polymerase chain reaction (PCR) methods to elucidate the effects and consequences of an intervention. Despite the fact that the DNA-DNA hybridization used in the present study was based on probes designed for human strains, it detected bacteria from dog microbiota. Previous studies using DNA-DNA hybridization to characterize oral microbiota in dogs also successfully

detected bacteria using human strain probes,[31] possibly by cross-reaction.[32]

Peri-implantitis induction in the present study showed a microbial composition similar to that in clinical settings using DNA-DNA hybridization checkerboard. When analyzed using a 16S gene clone library and real-time PCR, it can be seen that the peri-implantitis microbiome shares some of the classic periodontopathogens, mainly red complex bacteria, as well as some of the synergistic associations that differentiate the two diseases, with a more complex microbiota at dental implants.[33,34] Observed efficacy toward the red complex is in accordance with previous in vitro studies evaluating photo damage of aPDT on periodontopathogens.[35–38] There is a growing interest in novel strategies to replace commonly used antibiotics to avoid development of multidrug-resistant bacterial strains, among other favorable outcomes.[16,39] aPDT may represent a valuable strategy to replace the use of antibiotics in peri-implantitis management and should be considered in further studies; the protocol for this pilot study may still be optimized before clinical introduction.[38,40]

5 | CONCLUSION

In this study, aPDT and TTC successfully decontaminated infected implant surfaces. Implant decontamination with aPDT appears to be a viable substitute for TTC in the management of peri-implantitis defects.

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