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HUMAN RANDOMIZED CONTROLLED TRIAL

# Multiple sessions of antimicrobial photodynamic therapy associated with surgical periodontal treatment in patients with chronic periodontitis

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**Abstract**

**Background:** This double-blind, randomized, controlled clinical trial assessed the efficacy of multiple sessions of antimicrobial photodynamic therapy (aPDT) as an adjunct to surgical periodontal treatment (ST) in patients with severe chronic periodontitis (SCP).

**Methods:** Sixteen patients with SCP were treated with aPDT+ST (test group, TG) or ST only (control group, CG), in a split-mouth design. aPDT was applied at 0, 2, 7, and 14 postoperative days only in TG. All patients were followed up for 90 days after surgery. The following clinical and microbiological parameters were assessed: clinical attachment level (CAL), probing depth (PD), gingival recession (GR), bleeding on probing (BOP), plaque index (PI), and count of 40 subgingival microbial species (checkerboard DNA-DNA hybridization). Data were collected at baseline (preintervention), at 60 days (30 days after the end of non-surgical therapy), and at 150 days (90 days after surgery).

**Results:** A significant reduction in PD was observed at 150 days for the TG, when compared with the CG ($P < 0.05$). CAL gain was significantly higher in the TG at 60 and 150 days ($P < 0.05$). Changes in the subgingival microbiota were similar between the groups ($P > 0.05$), but the TG revealed a larger number of bacteria associated with periodontal disease at the end of the experiment compared with the CG ($P < 0.05$).

**Conclusion:** Multiple sessions of aPDT as an adjunct to surgical periodontal treatment significantly improved clinical parameters at 90 postoperative days.

**KEYWORDS**
antimicrobial, chronic periodontitis, photosensitizing agents, surgical treatment

## 1 | INTRODUCTION

Periodontitis is a multifactorial disease that affects tooth supporting tissues. Several predisposing factors (genetic, environmental, and systemic) are involved in its pathogenicity and may alter their expression and the patient's susceptibility to its development.[1] Scaling and root planing (SRP) is the gold standard treatment for periodontitis.[2] However, SRP instrumentation techniques are not always effective in eliminating microbial and mineralized deposits in more severe cases of the

  

disease. Conventional treatment of severe chronic periodontitis (SCP) is largely based on non-surgical SRP; adjunctive antimicrobial therapies and surgical interventions are indicated only for specific cases.[3] New adjunctive therapies have been investigated so as to offer better outcomes with mechanical treatment, such as antibiotic and laser therapies, including antimicrobial photodynamic therapy (aPDT).[4,5]

The mechanism of action of aPDT consists in the combination of a light source at a wavelength that is adequate for the therapeutic window with a photosensitizing agent that becomes excited when activated, going from a state of singlet oxygen to a state of doublet or triplet oxygen.[6] This change in state causes the transfer of electrons that react with molecular oxygen and produce reactive oxygen species that are cytotoxic to the target cells, leading to cell necrosis and microbial death.[7] The use of aPDT as an adjunct to conventional periodontal therapy has been suggested as an alternative for the elimination of periodontopathogens from the subgingival region, as its efficacy in killing microorganisms attached to the dental biofilm has been corroborated by in vitro[8] and in vivo studies.[9,10]

In the knowledge that periodontal pockets are not totally effective in completely eliminating all calculus and bacteria with non-surgical therapy,[11] additional procedures are necessary to obtain appropriate clinical outcomes. Surgical access procedures for root decontamination and the use of adjunctive therapies such as aPDT should be considered for these cases. Some studies of aPDT associated with non-surgical therapy for periodontal treatment are controversial.[12,13] However, only one study in the literature presents the association of a single session of aPDT with a surgical periodontal treatment.[14] Thus far, multiple sessions of aPDT have not been evaluated as an adjunctive therapy for surgical periodontal treatment. Therefore, the aim of the present study was to evaluate the clinical effects and the subgingival microbiota after multiple sessions of aPDT associated with surgical treatment of SCP.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample size calculation

This study was designed as a double-blind, randomized, controlled, and split-mouth clinical trial. The patients were considered the study unit, and the changes in probing pocket depth (PD) were the primary outcome variable. The sample size was estimated to provide 80% power, to recognize a significant difference of 1 mm ($\delta$) between groups, with a 95% confidence interval ($\alpha = 0.05$), and intragroup standard deviation of 1 mm (Müller Campanile et al.[15]). Considering changes in mean probing pocket depth as the primary outcome variable and $(Z\alpha + Z\beta)^2 = 7.84$ ($Z\alpha = 1.96$ for two-tailed 0.05 hypothesis test; $Z\beta = 0.842$ for power = 0.8), sample size was calculated using the following formula: $n = \{2[(SD)^2/(d)^2]\} \times (Z\alpha + Z\beta)^2$. Therefore, the sample should include at least 16 patients.

### 2.2 | Study population

After approval by the Human Research Ethics Committee of the Ribeirão Preto (FORP-USP) Dental School (Process no. 58437816.3.0000.5419) and registration in ClinicalTrials.gov (NCT03498404), the volunteers were selected from the Periodontal Clinic at FORP-USP. For inclusion criteria patients should have a good systemic health, presence of at least two proximal sites with PD > 5 mm after initial SRP in two contralateral posterior sextants, bleeding on probing (BOP) in these sites, aged >30 years, and presence of at least 15 teeth, excluding third molars and teeth requiring extraction. The exclusion criteria were: teeth with class III furcation lesions, positive history of antibiotic therapy in the past 6 months, basic periodontal treatment in the past 6 months, systemic involvement that could interfere with disease progression or with the response to treatment, extensive prosthetic involvement, need for antibiotic prophylaxis for dental procedures, use of anti-inflammatory drugs for a long period of time, smoking, and pregnancy. Seventy-eight subjects were initially selected for the study, of which, 53 did not meet all the inclusion criteria. Nine patients were excluded from the study after basic periodontal treatment due to the improvement of clinical parameter (PD < 5 mm after SRP). Finally, sixteen patients (9 males and 7 females, aged 30 to 75 years; mean age = 50.25 ± 9.30 years) diagnosed with severe chronic periodontitis[16] were included. Upon signing the free and informed consent form, volunteers were told about the objectives, benefits, risks, and discomfort that could be associated with the dental procedures.

### 2.3 | Examiner calibration

The Kappa coefficient ≥0.85 was used for the examiner (MBLR) calibration. Ten patients, different from the patients selected for this study, with at least five teeth with PD and CAL ≥ 5 mm on proximal sites were selected for calibration. Each patient was examined twice by a universal North Carolina-15 periodontal probe,* at a 48-hour interval between the first and second assessments.

### 2.4 | Experimental design

The TG teeth received SRP combined with aPDT at 0, 2, 7, and 14 postoperative days.[17] The teeth in the CG received SRP associated with a "sham procedure," in which aPDT was simulated using saline solution rather than a photosensitizing

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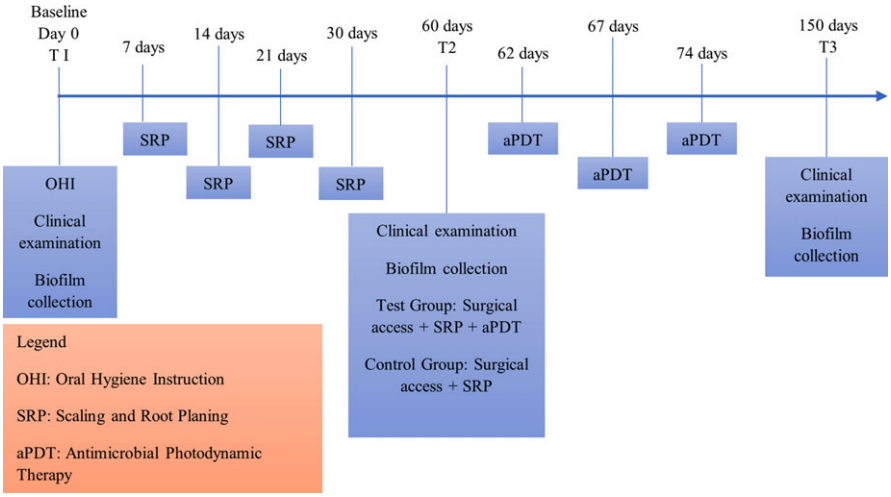


**FIGURE 1** Experimental design

dye; the laser point was positioned but not activated, thus masking the patients to the study protocol. Biofilm formation was controlled on a weekly basis during the first 30 postoperative days and on a monthly basis up to postoperative day 90. Periodontal and microbiological clinical parameters were assessed at baseline (T1 = preintervention), after basic therapy (T2 = reassessment, 60 days after baseline), and 90 days after the surgical procedure (T3, 150 days after baseline). The experimental design is shown in Fig. 1.

## 2.5 | Initial periodontal therapy and clinical procedures

Before the study, the selected volunteers received supragingival scaling, oral hygiene instructions, and had plaque retentive areas removed. Full-mouth periapical radiographs were taken for all subjects. Two contralateral single-rooted or multirooted posterior teeth presenting interproximal periodontal sites with PD ≥ 5 mm were selected for clinical and microbiological analyses. A specialist in periodontics (UBC) then performed quadrant-wise SRP for 4 weeks, using both hand instruments* and ultrasonic device, for 30 days before surgery. The selected teeth were assigned to the test group (TG) or control group (CG) using a random number table.

A researcher (SHLM) who was masked for the clinical procedures and/or sample collections was in charge of allocating the selected teeth to the appropriate groups. Sealed, nontransparent, properly identified envelopes with the allocation groups were opened only at the end of the surgical procedure and root decontamination on both treated sides. Periodontal clinical assessments and subgingival biofilm collection were performed by a single trained and calibrated researcher (MBLR), masked to the experimental groups.

* Gracey Curets, Hu-Friedy

## 2.6 | Surgical periodontal treatment

Surgical procedures were performed 4 weeks after last session of scaling and root planing (T2 = 60 days after baseline) on both test and control groups (Fig. 2). Those patients with PD ≥ 5 mm and BOP underwent surgery for root decontamination. All surgical procedures were performed by a single researcher (UBC) with expertise in periodontics. After infiltrative local anesthesia, an intrasulcular incision with 15C scalpel blade was performed, encompassing the site with PD ≥ 5 mm and both (mesial and distal) adjacent teeth, preserving gingival papillae. A mucoperiosteal flap was raised and after that, scaling and root decontamination were performed using Gracey curets and ultrasonic devices. After SRP was concluded on both sides, the envelope with information about the randomization process was opened, and aPDT was then performed in the TG. A sham procedure was performed on the contralateral side in the CG. After the procedures were completed in each group, the flaps were replaced and sutured with 5–0 nylon sutures. Paracetamol 750 mg, 6/6 hours for 2 days was prescribed for pain. Sutures were removed 14 days after surgery.

## 2.7 | Clinical assessment

The following periodontal clinical parameters were assessed at four sites (mesiobuccal, mesiolingual, distobuccal, and distolingual) of each selected tooth: probing depth (PD, in mm); clinical attachment level (CAL, in mm); gingival recession (GR, in mm); bleeding on probing (BOP), dichotomously evaluated–regarded as positive when bleeding occurred up to 20 seconds after the insertion of a graduated probe for PD measurement; plaque index (PI), assessed dichotomously[18] and calculated as the percentage of tooth surfaces with plaque/biofilm (score 1 indicated sites with plaque and score 0 indicated absence of plaque).

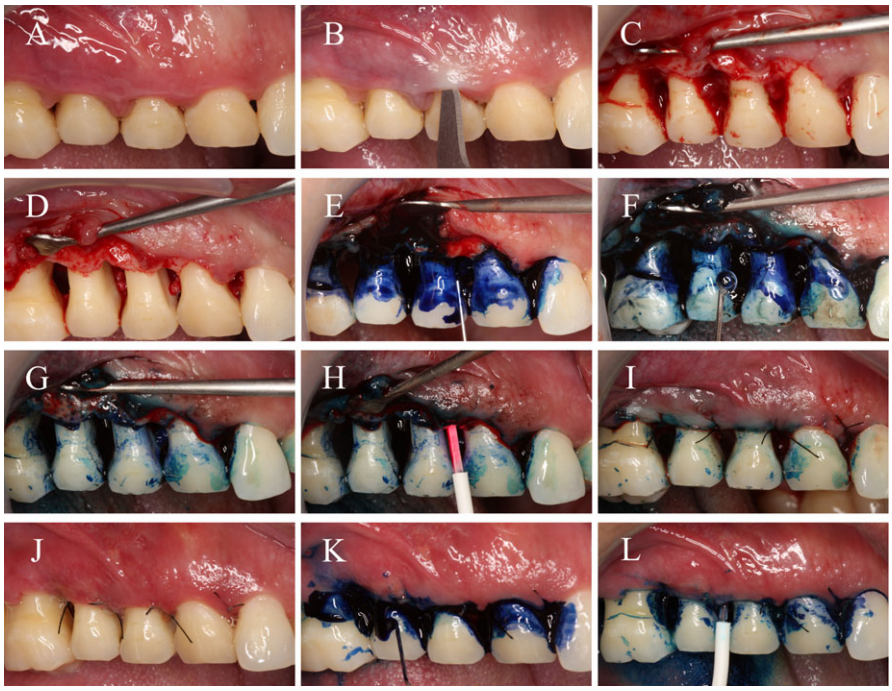


**FIGURE 2** Surgical therapy description–test group: (A) Initial case. Upper first premolar; (B) Intrasulcular incision with no.°15c blade; (C) Total flap elevated exposing calculus and granulation tissue; (D) decontaminated area and the bone defect; (E) Application of phenothiazine hydrochloride photosensitizer; (F) Irrigation with saline solution; (G) Removal of excess photosensitizer; (H) Activation of the diode laser on the dental surface (60 seconds per site); (I) Single sutures with 5-0 mononylon. (J) Postoperative of 14 days; (K) Application of the photosensitizer; (L) Activation of the diode laser on the dental surface (10 seconds per site)

## 2.8 | Microbiological monitoring

The selected teeth were isolated using sterile, air-dried cotton rolls to avoid contamination of the site with salivary flow. The supragingival plaque was carefully removed with a sterile Gracey curet. Thereafter, another sterile Gracey curet was used for collection of subgingival microbiota from the mesial and distal regions, going coronally from the deepest portion of the pocket toward each of the selected dental elements. Immediately after the collection, the sample obtained from each site was stored in a sterile Eppendorf tube containing 150 $\mu$L of buffer [10 mM Tris-HCL, 1 mM EDTA, pH 7.6]. One hundred microliters of sodium hydroxide (NaOH) at 0.5 M was added and stored at 80°C. Checkerboard DNA-DNA hybridization was used for the count of 40 subgingival bacterial species in each sample.[19–21]

## 2.9 | Antimicrobial photodynamic therapy (aPDT) protocol

### 2.9.1 | Intraoperative protocol

The selected area was irrigated with saline solution and dried with sterile gauze to prevent bleeding. Thereafter, 10 mg/mL of phenothiazine chloride* was applied and left in contact with the root surface for 5 minutes.[10] Subsequently, the area was irrigated with saline solution and irradiated with laser diode at 660 nm, with maximum power of 60 mW/cm$^2$ and energy density of 0.6 J/cm$^2$,† during 60 seconds per site. Treatment was performed on six sites (mesiobuccal, buccal, distobuccal, mesiopalatal/mesiolingual, palatal/lingual, and distopalatal/distolingual) per tooth, totaling 6 minutes of laser exposure. An additional 60 seconds was used for teeth presenting furcation defects, applying laser light inside the furcation area.

## 2.10 | Postoperative protocol

The aPDT protocol was maintained in the TG at 2, 7, and 14 postoperative days, as described by Ramos et al.[17]: laser light was applied for 10 seconds per site on six sites per tooth, totaling 1 minute of exposure. Teeth with furcation involvement were exposed for another 10 seconds.

## 2.11 | Statistical analysis

The mean and standard deviation of PD, CAL, and GR were calculated for the different groups and experimental periods. Absolute and relative frequencies were calculated for BOP and PI. Within-group differences in the three experimental

* Helbo Blue, Helbo Photodynamic Systems, Grieskirchen, Austria

† Helbo TheraLite Laser, Bredent Medical, Germany

periods were assessed by repeated-measures analysis of variance (ANOVA) and Bonferroni post-hoc test. Intergroup differences were determined by repeated-measures ANOVA and Student paired *t* test. The between groups variations in PD, CAL, and GR (at 60 and 150 days, compared with baseline) were assessed by Student paired *t* test. Statistical significance was set as $P < 0.05$.

The mean bacterial count ($\times 10^5$) was calculated for each pair of sites in the same teeth. The mean count of each species was then obtained for the different groups and experimental periods. The data were grouped into complexes, as described by Socransky et al.[22] The within-group differences in the mean counts of each bacterial species were assessed by the Bonferroni test. The analyses were fitted for multiple comparisons, as described by Socransky et al.[23] The between-group analysis of variations in the mean count of each bacterial species at 60 and 150 days compared with baseline was made using Student paired *t* test. Within-group and between-group differences in the mean relative frequencies for bacterial complexes were assessed by Bonferroni test and Student *t* test, respectively.

## 3 | RESULTS

All patients included in the sample completed the study, totaling 128 analyzed sites of 20 molars (10 TG and 10 CG) and 12 premolars (six TG and six CG). No discomfort or adverse effect was reported during the experimental period. Postoperative healing was uneventful in all cases after surgical periodontal treatment.

The clinical parameters evaluated in the CG and TG are shown in Table 1. At T1 and T2, there were no statistically significant differences between groups. At T3, PD was significantly lower for the TG when compared with the CG ($P < 0.05$). By analyzing within-group differences, PD significantly decreased when T1 values were compared with T3 and T2 versus T3 for both groups. Conversely, an increase in

**TABLE 1** Between-group and within-group comparisons of mean ± SDs of clinical parameters at T1 (baseline), T2 (60 days), and T3 (150 days)

| | Experimental Groups | | | Between-Group Comparisons Student's *t* test | | |
|---|---|---|---|---|---|---|
| Variable | Time | Control | Test | Mean differences | 95% CI | *P* value |
| PD (mm) | T1 | 6.56 ± 0.20 | 6.43 ± 0.21 | 0.13 | −0.42 0.67 | 0.6680 |
| | T2 | 5.87 ± 0.22 | 6.21 ± 0.21 | −0.34 | −0.18 0.87 | 0.1558 |
| | T3 | 4.06 ± 0.23[a,b] | 3.31 ± 0.18[a,b] | 0.75 | −1.05–0.44 | 0.0007 |
| CAL (mm) | T1 | 6.90 ± 0.36 | 7.00 ± 0.27 | −0.10 | −0.77 0.96 | >0.9999 |
| | T2 | 6.25 ± 0.54 | 6.90 ± 0.32 | −0.65 | −0.67 1.98 | 0.3221 |
| | T3 | 5.59 ± 0.54 | 5.03 ± 0.36[a,b] | 0.56 | −1.95 0.83 | 0.3365 |
| GR (mm) | T1 | 0.50 ± 0.22 | 0.56 ± 0.22 | −0.06 | −0.53 0.66 | >0.9999 |
| | T2 | 0.71 ± 0.26 | 0.71 ± 0.23 | 0.00 | −0.60 0.60 | 0.7461 |
| | T3 | 1.84 ± 0.37[a,b] | 1.71 ± 0.29[a,b] | 0.13 | −1.15 0.90 | 0.7937 |
| BOP (%) | T1 | 84.38 ± 30.10 | 68.75 ± 44.25 | 15.63 | −36.76 5.50 | 0.2500 |
| | T2 | 78.13 ± 31.46 | 65.63 ± 39.66 | 12.50 | 35.32 10.32 | 0.3984 |
| | T3 | 18.75 ± 30.96[a,b] | 9.37 ± 27.20[a,b] | 9.38 | −23.87 5.116 | 0.9100 |
| PI (%) | T1 | 71.88 ± 44.60 | 62.50 ± 42.82 | 9.38 | −25.92 9.249 | 0.5313 |
| | T2 | 31.25 ± 40.31[a] | 34.38 ± 39.66 | −3.13 | −18.92 24.47 | >0.9999 |
| | T3 | 21.88 ± 36.37[a] | 15.63 ± 35.21[a] | 6.25 | −13.60 2.485 | 0.5000 |
| PD = 5 mm | T1 | 04 | 08 | | | |
| | T2 | 15 | 13 | | | |
| | T3 | 05 | 01 | | | |
| PD = 6 mm | T1 | 16 | 10 | | | |
| | T2 | 11 | 05 | | | |
| | T3 | 04 | 01 | | | |
| PD ≥ 7 mm | T1 | 12 | 14 | | | |
| | T2 | 06 | 13 | | | |
| | T3 | 00 | 00 | | | |

CI = confidence interval; a, significant difference when compared to the value at T1 in the same group; b, significant difference when compared with the value at T2 in the same group (repeated-measures ANOVA, Bonferroni post-hoc test, $P < 0.05$).

**TABLE 2** Comparison of variations ± SDs of clinical parameters between- and within-groups at T1 (baseline), T2 (60 days), and T3 (150 days)

| | | Δ0 to 60 | % | Δ0 to 150 | % | Δ60 to 150 | % |
|---|---|---|---|---|---|---|---|
| Between-Group Comparisons | | | | | | | |
| PD (mm) | | | | | | | |
| | Control | 0.69 ± 1.09 | −10.48 ± 14.97 | −2.5 ± 1.40 a | −38.83 ± 17.59 | −1.81 ± 1.03 a | −30.85 ± 15.69 |
| | Test | −0.22 ± 0.68 | −3.52 ± 9.78 | −3.13 ± 1.06 a | −76.92 ± 12.01 | −2.91 ± 0.92 a | −87.74 ± 11.05 |
| | *P value* | 0.15 | | 0.16 | | 0.0035 | |
| CAL (mm) | | | | | | | |
| | Control | −0.66 ± 1.93 | −9.50 ± 33.95 | −1.31 ± 1.75 | −18.75 ± 27.45 | −0.66 ± 1.47 | −10.50 ± 23.94 |
| | Test | −0.09 ± 0.99 | −1.36 ± 15.59 | −1.97 ± 1.07 a | −35.20 ± 16.02 | −1.88 ± 1.04 a | −37.27 ± 15.12 |
| | *P value* | 0.30 | | 0.21 | | 0.0111 | |
| GR (mm) | | | | | | | |
| | Control | 0.22 ± 0.52 | 43.75 ± 43.66 | 1.34 ± 1.26 a | 238.89 ± 56.84 | 1.13 ± 1.20 | 156.52 ± 51.59 |
| | Test | 0.16 ± 0.51 | 21.74 ± 50.25 | 1.16 ± 1.08 a | 62.71 ± 54.68 | 1.00 ± 1.03 a | 58.18 ± 53.14 |
| | *P value* | 0.0030 | | 0.6542 | | 0.7548 | |
| BOP (%) | | | | | | | |
| | Control | −6.25 ± 35.19 | | −65.63 ± 48.06 a | | −59.38 ± 47.06 a | |
| | Test | −18.75 ± 45.51 | | −59.38 ± 53.00 a | | −56.25 ± 39.94 | |
| | *P value* | 0.3874 | | 0.7256 | | 0.8385 | |
| PI (%) | | | | | | | |
| | Control | −40.63 ± 48.06 | | −50.00 ± 48.06 | | −9.38 ± 48.80 | −13.03 |
| | Test | −28.13 ± 45.51 | | −46.88 ± 42.26 | | −18.75 ± 36.84 | −30.00 |
| | *P value* | 0.4566 | | 0.8476 | | 0.5745 | |

% = delta percentage; Δ 0 to 60, comparison of absolute delta between T1 and T2; Δ 0 to 150, comparison of absolute delta between T1 and T3; Δ 60 to 150, comparison of absolute delta between T2 and T3; a, Significant within-group difference when compared with Δ 0 to 60 (repeated-measures ANOVA, Bonferroni post-hoc test, $P < 0.05$).

GR when T1 values were compared with T3 and T2 in both groups, while no significant differences between groups in PI and BOP were observed, both groups showed a statistically significant reduction in these parameters in the within-group comparisons. However, in the TG there was a remarkable reduction in CAL between T1 and T3 and between T2 and T3.

Table 2 shows the mean variations in clinical parameters for the experimental periods, in absolute and percentage values for each group. PD demonstrated a significant decrease in the within-group comparisons between 0 to 60 days and 0 to 150 days and between 0 to 60 days and 60 to 150 days for the CG and TG, respectively. A statistically significant difference was between groups in the 60 to 150 days range. Another interesting finding was the significantly larger increase in CAL in the TG when compared with the CG for the 60 to 150 days variation, which can also be seen in the within-group analysis for the TG between 0 to 60 and 0 to 150 days and between 0 to 60 and 60 to 150 days. BOP showed statistical difference only in the within-group analysis: a significant difference was found in the TG between 0 to 60 and 0 to 150 days, and in the CG between 0 to 60 and 0 to 150 days and between 0 to 60 and 60 to 150 days. There were no statistically significant differences in PI both for within-group and between-group analyses.

In general, the volunteers treated in the present study had a high count of orange and red complex periodontopathogens at baseline. Figure 3 shows the mean total count ($\times 10^5$) of the 40 subgingival species throughout the experimental period in the CG and TG, as well as the statistically significant differences observed in the mean counts of each group at 60 and 150 days, when compared with baseline values. The experimental groups had similar results in the microbiological analysis, without any significant differences between them. In the within-group analysis, red complex bacteria did not show significant difference for any of the groups in the different experimental periods. The TG revealed a significant reduction in the count of *Fusobacterium nucleatum* (orange complex) at 60 and 150 days compared with baseline. Both groups showed a significant increase in the count of orange complex bacterium *Campylobacter gracillis* at 150 days and in the count of blue complex bacterium *Actinomyces naeslundii* when compared with baseline. Moreover, the counts of other bacteria also increased: *Streptococcus sanguinis* (yellow complex), *Actinomyces odontolyticus* (purple complex), and *Streptococcus anginosus* (nonoral bacterial complex).

Experimental groups showed a significant increase in the count of *C. gracillis* (orange complex), *A. naeslundii* (blue complex), *S. sanguinis* (yellow complex), and

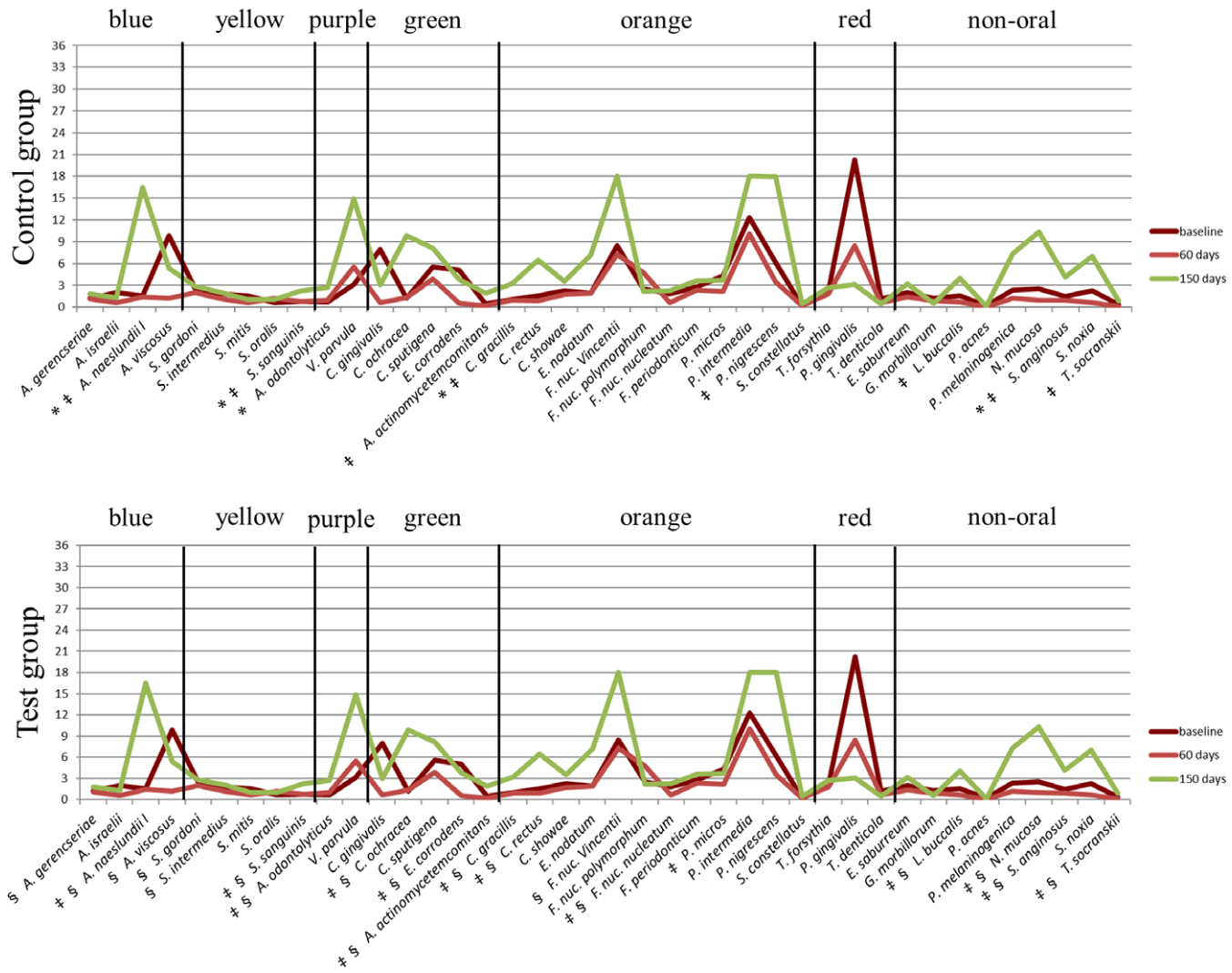


**FIGURE 3** Mean count ($\times 10^5$) of 40 bacterial species found in subgingival biofilm samples at baseline, at 60, and 150 postoperative days in both groups, as well as the results for the within-group comparisons. Significant differences when compared with baseline: *Control group–150 days; †Test group–150 days. Significant differences when compared with 60 days (T2): ‡Control group–150 days; §Test group–150 days. (Repeated-measures ANOVA, Bonferroni post-hoc test, $P < 0.05$)

*Aggregatibacter Actinomycetemcomitans* and other three species–*Leptotrichia buccalis, S. anginosus,* and *Treponema socranskii*–at 150 days when compared with 60 days. In addition, the CG had a significant increase in the count of *Prevotella nigrescens* (orange complex), whereas the TG exhibited an increase in the count of *Actinomyces gerencseriae* and *Actinomyces viscosus* (blue complex).

The mean relative frequency of microbiological complexes is displayed in Figure 4. No statistical differences were observed in bacterial complexes between the groups in the different experimental periods. The within-group analysis revealed a significant increase in orange complex bacteria at 60 days when compared with baseline for the CG and TG. Moreover, the nonoral bacterial complex showed a significant decrease at 60 days compared with baseline and an increase between 60 and 150 days, but these differences were statistically significant only for the CG.

## 4 | DISCUSSION

The present double-blind, randomized, controlled, split-mouth clinical trial assessed the efficacy of multiple sessions of aPDT as an adjunct to surgical periodontal treatment of severe chronic periodontitis, by analyzing clinical and microbiological parameters. Results indicate some beneficial clinical effects of multiple sessions of aPDT as decreasing PD and a greatest increase in CAL for TG. Despite of no significant differences in subgingival bacterial complexes between the experimental groups at 150 days, there were decreases, albeit non-significant, in the percentage of red complex bacteria during the follow-up period for both groups.

Previous experiments that associated aPDT with conventional non-surgical treatment yielded controversial results and had some discrepancies in their protocols.[9,24–26]

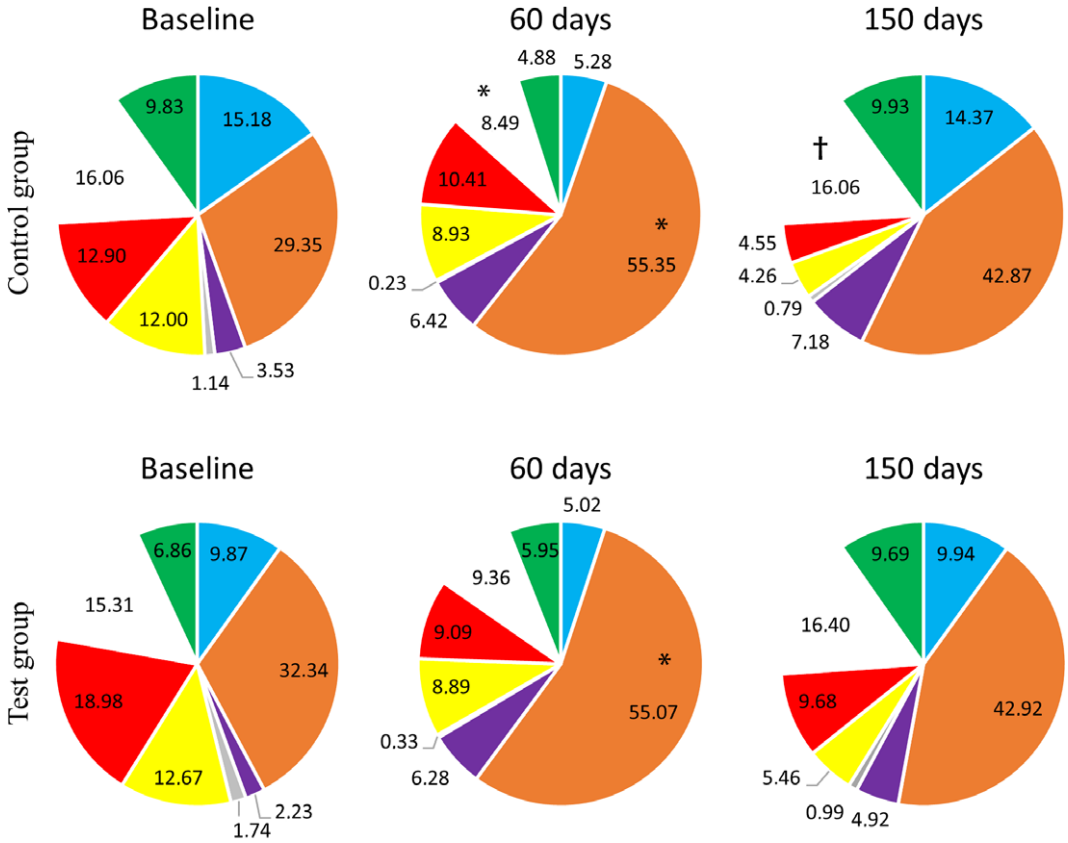


**FIGURE 4** Relative frequencies of the means in percentage of bacterial complexes in different experimental periods. *Significant difference when compared with baseline values; †Significant difference when compared with 60 days (Repeated-measures ANOVA, Bonferroni post-hoc test, $P < 0.05$). The colors represent different microbial complexes (Socransky et al.)[22] and the white represent non-oral bacterias.

However, when aPDT was used as an adjunct to non-surgical periodontal treatment, there were less remarkable reductions than those observed in studies in which surgical techniques are combined.[4,27] A possible explanation for the larger reductions in the present study, comparatively to those which described non-surgical treatment, is that surgical debridement itself contributes to reducing PD.[28,29] Moreover, surgical access can enhance the penetration of photosensitizing agents into epithelial and connective tissues, thus eliminating periodontopathogens found therein.[30] Also, there could be a biomodulatory effect triggered by low-power laser on local cells during tissue healing, improving tissue repair, and reducing periodontal inflammation.[31]

Even though there are important studies on the association of SRP procedures with multiple sessions of aPDT,[9,12,17] there is a paucity of studies that associate this therapeutic method with the surgical treatment of periodontal disease. Martins et al.[14] associated a single intraoperative session of aPDT with the surgical treatment of SCP and observed a decrease in PD at 90 postoperative days when compared with baseline (with an average of 2.30 mm in the CG and 2.93 mm in the TG). Similar reductions were reported by Giannelli et al.,[32] who removed the inner epithelium of the periodontal pocket before aPDT using high-power laser, obtaining reductions of 0.9 mm ($4.9 \pm 0.1$ to $4.0 \pm 0.1$) for PD in the CG and of 3.0 mm ($5.1 \pm 0.1$ to $2.1 \pm 0.1$) in the TG, when comparing baseline to 350 days post-treatment.

According to the American Academy of Periodontology,[33] current evidence indicates that laser therapies, such as aPDT, in isolation or as an adjunct, are unpredictable and inconsistent in their ability to reduce subgingival microbial loads beyond that achieved by SRP alone. Studies demonstrating significant reductions in periodontopathogens,[34,35] as well as studies showing no significant changes in the subgingival microbiota,[13,36] are described in the literature.

In a recent review, Mombelli[37] asserted that treated sites are prone to be recolonized with a similar microbiota to that which had been detected before therapy, and that the level and speed of recolonization depend on the treatment protocol, on the patterns of distribution of periodontal microorganisms in other areas of the oral cavity, and on the patient's oral hygiene. Conversely, Magnusson et al.[38] demonstrated possible subgingival recolonization after SRP within 4 to 8 weeks.

Periodontal sites with a high count of red complex bacteria are at a greater risk for attachment loss[39] and also show more severity and progression of periodontal disease.[40] Regardless of the increase of red complex in this study, the aPDT led to a significant increase in the count of

*A. gerencseriae* and *A. viscosus*, which are associated with periodontal health. These positive changes in the rates of host-compatible bacterial species, observed only in the TG, play an important role in the efficacy of periodontal treatment.[41]

Although there is a cause and effect relationship between the buildup of microbial deposits and gingival inflammation,[42] environmental factors,[43] as well as host-specific factors that modulate inflammatory response to microbial colonization,[44] may play a key role in the development of periodontal disease. Despite the hypothesis of the subgingival microbiota recolonization at the end of the study, significant clinical improvement of PD and CAL in the TG was maintained at 90 postoperative days. A possible explanation for this finding is the potential beneficial effect of low-power laser on tissue healing, which inhibits the expression of proinflammatory cytokines, reducing local inflammatory reactions.[45]

Among the limitations of the present study, probably the 90-day interval for reassessment of the therapeutic effects is too long for confirmation of microbiological results and should therefore be reconsidered.[46] A suggestion for future studies is to consider additional 30 and/or 60 post-operative days, as well as periodontal supportive care. Furthermore, the experimental design might have influenced the results: placement of a cannula for application of the photosensitizing agent and insertion of an optical fiber for laser irradiation at subgingival sites in early postoperative periods (2, 7, and 14 days) might have had a deleterious effect during the critical period of epithelial neoformation and of postoperative periodontal repair.[47] Another point to be considered is that the split-mouth study model may have influenced the recolonization in other sites due to the possible presence of remaining pockets that can serve as bacterial reservoirs. Finally, the study evaluated patients with chronic periodontal disease, but without systemic diseases. There are a few studies that have evaluated the potential of this therapeutic method in patients at risk for periodontal disease, such as diabetes,[48,49] smoking[50] and aging. More relevant studies should be performed in this population profile, since they represent a large number of the world population.

## 5 | CONCLUSIONS

The present study assessed the association of multiple sessions of aPDT as an adjunct to the surgical treatment of severe chronic periodontitis. Significant improvements were observed in clinical parameters, with a reduction in PD and gain in CAL as well as a numerical decrease in the percentage of red complex bacteria at 90 postoperative days. The increase in the count of some bacterial species, suggests the onset of recolonization of the subgingival microbiota.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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