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Clinical and microbiological effects of multiple applications of antibacterial photodynamic therapy in periodontal maintenance patients. A randomized controlled clinical study

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ABSTRACT

Background: At present, very limited data are available on the clinical and microbiological outcomes obtained following repeated application of aPDT following one single mechanical debridement.
Objective: To evaluate clinically and microbiologically the outcomes following one single session of subgingival mechanical debridement (scaling and root planing; e.g. SRP) followed by 1x immediate application of aPDT and 2 x subsequent use of aPDT without SRP.
Materials and methods: Forty patients diagnosed with generalized chronic periodontitis that were enrolled in periodontal maintenance (supportive periodontal therapy) program, were randomly assigned to one of the two treatments: 1. SRP by means of ultrasonic and hand instruments followed by one single session of SRP followed by 1x immediate application of aPDT and 2 x subsequent applications of aPDT without SRP (test) or 2. SRP alone (control). The following clinical parameters were recorded at baseline, at 3 and 6 months: Full-Mouth Plaque Scores (FMPS), Full-Mouth Bleeding Scores (BOP), Probing Pocket Depth (PPD), Clinical Attachment Level (CAL) and Gingival Recession (RC). Additionally, microbiological samples were evaluated at baseline and six months after treatment. The primary outcome variable was BOP.
Results: Both treatments improved statistically significantly ($p < 0.05$) the FMPS, PPD and CAL values, while no statistically significant changes occurred in terms of RC. In the test group, BOP decreased statistically significantly ($p < 0.05$) after 3 and 6 months, while in the control group the respective values decreased statistically significantly only at 3 months. Both treatments reduced statistically significantly the total bacteria counts (TBC) after 6 months ($p < 0.05$). At 6 months, the use of SRP and aPDT resulted in a statistically significant decrease in the number of all tested bacteria except *A. actinomycetemcomitans* while the use of SRP alone resulted only in a statistically significant decrease in the numbers of *P. gingivalis*, *T. denticola* and *T. forsythia.*
Conclusions: In periodontal patients enrolled in a maintenance program one single session of SRP followed by 3x application of aPDT, enhanced the clinical and microbiological outcomes compared to SRP alone.

1. Introduction

The main factor in the aetiology of periodontal diseases is the periodontal pathogenic bacterial biofilm [1]. The development of subgingival biofilm commences with planktonic cells adhering to the inert or living (abiotic or biotic) surface [2] and progresses to rapid colonization as a result of adhesion, growth and cell division. At present, *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* serotype b and c are considered as key stone periodontal pathogens involved in the pathogenesis of periodontitis [3–5].

Periodontal therapy aims at reducing the microbial load by mechanically disrupting the subgingival biofilm in orders to arrest or slow down the progression of periodontal disease [6,7]. In the majority of the clinical situations, mechanical debridement (scaling and root planing, e.g. SRP) alone by means of hand or/and ultrasonic instruments can predictably achieve this goal [8,9]. However, in more advanced cases, especially those associated with the presence of *A. actinomycetemcomitans* [10] and/or *P. gingivalis,* [11] SRP alone is often insufficient due to the ability of these periodontal pathogens to

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penetrate in the surrounding soft tissues. In such situations, in order to optimize the clinical outcomes, topical or systemic antibiotics are frequently used [12–16].

Despite the fact that antibiotics can substantially reduce the number of periodontal pathogens, their use may be is associated with a number of side effects including skin rash, itching, oral candidiasis or gastrointestinal problems such and nausea or vomiting [13]. These side effects may lead the patients to discontinue or deny antibiotic treatment [17]. Moreover, the frequent use of antibiotics may lead to an increase in developing bacterial resistance, an increasing public health issue with high economic and social costs [18,19]. Obviously, there is an increasing need for developing new treatment alternatives that can effectively reduce or eliminate periodontal pathogenic bacteria by causing fewer side effects [20].

One potential modality aiming to reduce the bacterial load, is antibacterial photodynamic therapy (aPDT) and consists of the use of red and infrared light of a particular wavelength varying between 650 and 900 nm, able to activate a photoactive substance, called photosensitizer [21]. Diode lasers of a wavelength between 635 nm and 670 nm are most frequently used, although in some studies also wavelengths of 808 nm [22] and 940 nm [23] have been evaluated. The transfer of energy leads to the release of free oxygen radicals, which subsequently destroy bacteria and their by-products [24–26]. The most commonly used photosensitizers in the treatment of periodontal, peri-implant, and endodontic infections are methylene blue (MB) and toluidine blue (TB) [27–32]. Toluidine blue and methylene blue display similar chemical and physicochemical characteristics and have been shown to be effective agents against both Gram-positive and Gram-negative bacteria [33,34]. Following their application in periodontal pockets and a persistence time of 1–5 min both, toluidine blue and methylene blue, have shown the best photodynamic properties for inactivating pathogenic bacteria after exposure to wavelengths between 630 nm and 660 nm, respectively [33,35]. On the other hand, green coloured photosensitizers such as indocyanine green have the highest absorption at a wavelength of 805 nm [36].

A number of studies have shown statistically significant reductions in the numbers of *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia* following the use of aPDT, thus pointing to the antibacterial effects of this approach [5,37–40]. The bactericidal effect of aPDT is explained in multiple ways, including breakdown of DNA structure, efflux of potassium ions, modification of cell membrane proteins, and disruptions in the cell-wall synthesis or bacterial cell destruction as a result of the reaction between photosensitizer and oxygen (photo-oxidative reaction) [41–43].

It needs however to be pointed out that the current literature provides conflicting results regarding the clinical and microbiological effects of aPDT: while some studies concluded that aPDT can be an useful adjunct to mechanical debridement [44–48], others have failed to show differences in the outcomes when the adjunctive use of aPDT to SRP was compared with the use of SRP alone [49]. Most studies [5,29,40,47,50–59] have also shown that the adjunctive use of aPDT to SRP markedly reduced the bacterial load. However, other studies have shown that the number of certain periodontal pathogens such as *T. denticola* [60], *P. gingivalis* and *T. forsythia* [39] increased statistically significantly following the application of aPDT compared with treatment with SRP alone. It has been also suggested that the strongest effects of aPDT were on the reduction of inflammation as evidenced by reduction of bleeding on probing [61–64]. A very recent systematic review including meta-analysis [65] has reported statistically significant effects of aPDT as an adjunct to SRP and concluded that aPDT as an adjunct to SRP is effective for probing depth (PD) reduction and clinical attachment level (CAL) gain. Moreover, aPDT was shown to be effective in preventing recolonization of subgingival area by periodontal pathogenic microorganisms [35].

Taken together, the major benefit of using aPDT as adjunct to mechanical debridement is related to the very limited to no side effects and the potential effects against bacteria, viruses, fungi, yeasts, and parasitic protozoa without increasing the risk for antibiotic resistance. Besides its bactericidal and detoxification activity, aPDT exhibits also positive effects on early wound healing which, in turn, may additionally improve the patient acceptance and clinical outcomes [66].

It needs, however, to be pointed out that in the great majority of studies, aPDT was either applied immediately after mechanical debridement or twice [5,22,45,50,63,67]. Thus, it cannot be excluded that the effects of aPDT may be diminished by bleeding and/or sulcular fluid at the inflamed sites. In order to overcome this problem, it has been suggested that the application of aPDT following subgingival mechanical debridement should be repeated at certain time intervals without any mechanical debridement. At present, very limited data are available on the clinical and microbiological outcomes obtained following repeated application of aPDT after one single mechanical debridement.

Therefore, the aim of this randomized, controlled, clinical study was to evaluate clinically and microbiologically the outcomes following one single session of subgingival mechanical debridement (scaling and root planing; e.g. SRP) and 1x immediate use of aPDT followed by 2 x additional application of aPDT without SRP.

2. Materials and methods

2.1. Ethical guidelines

The study was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) accompanied with the understanding and written consent of each experimental subject according to the above-mentioned principles. Regional Ethical Review Board approved the protocol (No74/KBL/OIL/2014).

2.2. Inclusion criteria

a) Age > 18 years
b) Patients diagnosed with chronic periodontitis having completed active periodontal treatment, receiving regularly supportive periodontal therapy (SPT) and exhibiting residual pockets of ≥5 mm at single rooted teeth.
c) Horizontal bone loss or the presence of an intrabony component of maximum 2 mm as measured on in x-rays
d) FMPS < 25 (Full-mouth plaque score)
e) FMBS < 25 (Full-mouth bleeding score)

2.3. Exclusion criteria

Subjects were excluded on the basis of the following criteria:

a) Presence of any relevant medical condition such as diabetes etc. that may influence periodontal status
b) Use of drugs such as immune-depressant, anti-epileptic, calcium antagonist
c) Smokers (5 cigarette or more per day)
d) Deep intrabony defects (e.g. intrabony component > 3 mm, as measured on the x-rays)
e) Use of systemic antibiotics in the last twelve months
f) Pregnancy

2.4. Study design

The study was designed and conducted as a randomized clinical trial of 6 months duration. A total of 40 subjects were recruited based on the following criteria: chronic periodontitis [68], probing pocket depth (PPD) of ≥5 mm at single rooted teeth and no signs of apical pathology. All subjects signed an informed consent. The subjects were assigned to the test or control group by a computer-generated randomization table.

Treatment was performed as follows by the same experienced periodontist (KGL):

1 20 patients receiving one session of scaling and root planing alone by means of ultrasonic and hand instruments (i.e. Gracey curettes) within one single session (control).
2 20 patients receiving one session of scaling and root planing followed by three sessions of aPDT application using a 635 nm diode laser (Smart M, Lasotronix, Poland) in a continuous wave mode and energy setting of 200 mW (test). The photosensitizer (Toluidine Blue 0.1%) was applied into the pocket using a disposable flexible tip with diameter 0.8 mm and left for 60 s. After 60 s, the photosensitizer was washed out through copious rinsing with sterile saline. Subsequently, the diode laser's tip with a diameter of 0.8 mm (PACT® Light Guides Universal, Cumdente, Tübingen, Germany) was inserted into the pocket for 30 s with a slow swiping movement to irradiate the photosensitizer. The delivered energy density was 117.64 J/mm^2.

Treatment with aPDT was performed in three sessions:

a) First session: scaling and root planing followed by immediate application of aPDT.
b) Second session after 7 days: application of aPDT alone.
c) Third session after 14 days: aPDT application of aPDT alone.

The post-treatment protocol consisted of supragingival maintenance every 4 weeks without any additional subgingival debridement or application of aPDT throughout the entire study period of 6 months. Follow up visits were scheduled at 3 and 6 months following the third session of aPDT.

2.5. Methods of assessment or measurement

The assessment was done at all-time points by the same calibrated examiner using an automated probe Pa-on® with constant probing force of 0.2 g of pressure (Orange Dental, Germany), which was calibrated before each measurement according to manufacturer's instructions. All treatments were performed by the same experienced clinician (KGL).

The following clinical parameters were recorded at baseline, at three and six months after treatment:

- Full-Mouth Plaque Scores (FMPS): the presence of plaque in percentage (%) at the cervical area of the tooth [70].
- Bleeding on Probing (BOP) recorded at the treated site, presence (+) or absence (−) of bleeding in percentage (%) 30 s after probe insertion in the periodontal pocket [69].
- Probing Pocket Depth (PPD): the distance from the free gingival margin to the bottom of the periodontal pocket.
- Clinical Attachment Level (CAL): the distance from the CEJ and the bottom of the sulcus or of the periodontal pocket includes both PPD and REC.
- Gingival Recession (REC): the distance from the cemento-enamel junction (CEJ) to the free gingival margin (respectively crown margin to the free gingival margin).

All measurements were made at six sites per included tooth (mesiobuccal/midbuccal/distobuccal/mesiopalatal/midpalatal/distopalatal). The measurements of PPD, REC, and CAL were performed by a fully automated periodontal probe pa-on (Pa-on, Orangedental, Germany), which was calibrated before each measurement according to manufacturer's instructions. According to the baseline PPD values, the periodontal pockets were classified as shallow (< 4 mm), medium (4–6 mm) or deep (> 6 mm).

2.6. Microbiological assessment

Microbiological samples were collected taken from the deepest periodontal pockets at baseline and six months after therapy. Sampling was performed by means of sterile paper points following removal of supra-gingival plaque at the sampling site. The paper points were inserted into the periodontal pockets and left inside for 30 s prior to careful removal in order to avoid contamination with blood or saliva.

Microbiological analysis consisted of a molecular test for the detection of nine periodontal pathogens including red and orange bacterial complexes. The test was performed with real-time PCR (polymerase chain reaction) method. Bacterial DNA was extracted using Pet Plus (MIP Pharma®, Germany) following the instructions of the manufacturer,

The following periodontal pathogenic species were analysed:

-
  - Total bacteria count (TBC)
  - *Fusobacterium nucleatum* (*F. nucleatum)*
  - *Prevotella intermedia* (*P. intermedia)*
  - *Porphyromonas gingivalis (P. gingivalis)*
  - *Tannerella forsythia (T. forsythia)*
  - *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans)*
  - *Eubacterium nodatum (E. nodatum)*
  - *Peptostreptococcus micros (P. micros)*
  - *Capnocytophaga gingivali (C. gingivalis)*

2.7. Statistical analysis

Clinical parameters before treatment and at 3 and 6 months after the treatment were compared between with baseline and between the groups. The primary outcome variable was BOP. In addition, the microbiological analysis of subgingival plaque samples before treatment and at 6 months were also analysed and compared to baseline and between the groups.

Friedman test as nonparametric analogue for repeated measures ANOVA and nonparametric Kruskal-Wallis test were used to compare values of FMPS, BOP, PPD, REC and CAL between two treatment groups at baseline, three and sixth months after treatment. Friedman test was also used to compare values of total bacteria count and a number of examined periodontal pathogenic bacteria between two treatment groups at baseline and six months after treatment. $\chi 2$ test was used to compare proportions of FMPS, BOP and proportions of shallow, medium and deep pockets in two treatment groups. The level of significance was set at $\alpha = 0.05$, and the statistical power was 0.80.

3. Results

3.1. General data

The distribution of both groups (SRP and aPDT groups) was homogenous regarding age and gender variance ($p > 0.05$). Out of 40 subjects 25 were female and 15 were male, aged between 32 and 79 years (Table 1).

According to the baseline PPD values the periodontal pockets were classified as shallow (< 4 mm), medium (4–6 mm) or deep (> 6 mm) periodontal pockets.

In total, there were 1052 periodontal pockets (426 periodontal pockets in SRP group (control), and 626 periodontal pockets in aPDT group (test). Samples collected in both groups included 176 from medium depth pockets (4–6 mm) and 45 from deep periodontal pockets (> 6 mm).

3.2. Clinical results

At 3 months, the following clinical parameters improved

Table 1
Demographic features of study population.

Variable	All $N = 40$		Group				**1** vs. **2** P-value
			1. Control $N = 20$		**2**. Test $N = 20$		
Age (years) $M \pm SD$:	50.3 ± 11.6		49.5 ± 13.2		51.1 ± 9.9		0.658[a]
Gender:	*n*	%	*n*	%	*n*	%	0.744[b]
Male	15	37.5%	7	35.0%	8	40.0%	
Female	25	62.5%	13	65.0%	12	60.0%	

M – mean, SD – standard deviation.
[a] Mann–Whitney test.
[b] χ^2 test.

statistically significantly ($p < 0.05$) in both groups: FMPS, PPD and CAL. However, RC did not show statistically significant changes compared to baseline in any of the two groups (n.s.). In the test group, BOP decreased statistically significantly ($p < 0.05$) at both 3 and 6 months compared to baseline with corresponding values of 15.7%–12.9% and 10.4%, respectively. In the control group, BOP decreased statistically non-significantly from 17.3% at baseline to 14.9% at 3 months and increased again to 16.3% at 6 months. Treatment with aPDT yielded statistically significantly lower BOP scores compared to the treatment with SRP alone (e.g. 10.4% vs.16.3%, $p = 0.007$).

There was no statistically significant difference between the test and control group regarding the distribution of shallow, medium and deep pockets at baseline ($p > 0.05$) (Table 2).

In both groups, the number of pockets < 4.0 mm increased statistically significantly ($p < 0.001$) after 3 months and remained stable after six months as compared to baseline ($p < 0.001$). There was no statistical significant difference between 3 and 6 months.

The number of pockets of 4–6 mm decreased statistically significantly after 3 months and remained stable after 6 months ($p < 0.001$). The difference between baseline and three months after the treatment as well as baseline and after six months was statistically not significant ($p > 0.05$) (Tables 3 and 4).

Table 2
Full Mouth Plaque Score, Bleeding on Probing Score and Probing Depth in SRP and aPDT groups of study population.

Clinical Parameters	All $N = 40$	Group		**1** vs. **2** Mann-Whitney Test P-value
		1. Control $N = 20$	**2**. Test $N = 20$	
FMPS ($M \pm SD$ *of %of sites*)				
base line	10.2 ± 6.2	9.6 ± 6.5	10.9 ± 5.9	0.363
after 3 months	7.2 ± 3.4	6.3 ± 3.1	8.1 ± 3.6	0.114
after 6 months	8.1 ± 4.2	8.0 ± 3.5	8.2 ± 4.8	0.775
Friedman Test P-value:	0.193	0.191	0.674	×
BOP ($M \pm SD$ *of %of sites*)				
base line	16.5 ± 6.8	17.3 ± 7.1	15.7 ± 6.5	0.504
after 3 months	13.9 ± 6.4	14.9 ± 6.2	12.9 ± 6.5	0.183
after 6 months	13.4 ± 7.3	16.3 ± 6.3	10.4 ± 7.3	0.007
Friedman Test P-value:	0.184	0.267	0.013	×
PD (mm, $M \pm SD$)				
baseline	2.33 ± 0.71	2.30 ± 0.74	2.37 ± 0.69	0.665
after 3 months	2.02 ± 0.64	2.01 ± 0.58	2.04 ± 0.70	0.818
after 6 months	2.03 ± 0.61	2.01 ± 0.52	2.05 ± 0.69	0.978
Friedman Test P-value:	0.002	0.074	0.015	×

M – mean, SD – standard deviation.

Table 3
Number of sites with probing depth (PD) < 4 mm or PD 4–6 mm or > 6 mm in SRP and aPDT groups.

PD (mm)	Baseline N = 426		After 3 months N = 426		After 6 months N = 426		
Control group	n	%	n	%	n	%	
< 4.0	264	62.0	314	73.7	330	77.5	< 0.001
4.0–6.0	113	26.5	92	21.6	75	17.6	
> 6.0	49	11.5	20	4.7	21	4.9	
PD (mm)	Baseline N = 606		After 3 months N = 606		After 6 months N = 606		Chi-square test
Test group	n	%	n	%	n	%	p-value
< 4.0	385	63.5	464	76.6	483	79.7	< 0.001
4.0–6.0	176	29.0	117	19.3	98	16.2	
> 6.0	45	7.4	25	4.1	25	4.1	

Table 4
Number and proportions of sites with probing depth (PD) < 4 mm or PD ≥ 4 mm in SRP and aPDT groups.

PD (mm)	Baseline N = 426		After 3 months N = 426		Chi-square test
Control group	n	%	n	%	p-value
< 4.0	264	62.0	314	73.7	0.0003
≥4.0	162	38.0	112	26.3	
PD (mm)	Baseline N = 606		After 3 months N = 606		Chi-square test
Test group	n	%	n	%	p-value
< 4.0	385	63.5	464	76.6	< 0.0001
≥4.0	221	36.5	142	23.4	

3.3. Microbiological results

Both treatments reduced statistically significantly the total bacteria counts (TBC) after 6 months ($p < 0.05$). At 6 months, the use of SRP and aPDT resulted in a statistically significant decrease in the number of all tested bacteria except *A. actinomycetemcomitans* while the use of SRP alone resulted only in a statistically significant decrease in the numbers of *P. gingivalis*, *T. denticola* and *T. forsythia.*

4. Discussion

The present study has compared clinically and microbiologically the outcomes following subgingival mechanical debridement (i.e. SRP) followed by 3 x application of aPDT with SRP alone in patients with chronic periodontitis receiving supporting periodontal therapy. The results have shown that repeated additional application of aPDT to mechanical debridement, yielded at both 3 and 6 months, statistically significantly higher reduction of inflammation as evidenced by the

decrease in BOP values, compared to one single session of SRP. The clinical outcomes are in line with the microbiological findings, thus indicating that despite the fact that both treatments reduced statistically significantly the TBC after 6 months, the additional use of aPDT has led to a statistically significant decrease in the number of all tested bacteria except *A. actinomycetemcomitans* while the use of SRP alone resulted only in a statistically significant decrease in the numbers of *P. gingivalis*, *T. denticola* and *T. forsythia.*

SRP is still considered as the gold standard in periodontal therapy [8,9]. However, SRP alone is not always sufficient to remove the subgingival biofilm, especially in deep pockets or at sites with difficult access such as intrabony defects or furcations. Increasing evidence from clinical studies suggests that the subsequent application of aPDT to SRP has been may be of benefit in such, difficult to reach, areas [57].

Another important aspect that needs to be discussed in conjunction with the use of aPDT and also other lasers, is the potential effect on the reduction of periodontal pathogenic bacteria and inflammatory mediators. Especially, the antibacterial effects are of increasing importance in the light of the dramatic increase in antibiotic resistance and the search for novel antimicrobial strategies [71].

The data on the outcomes following the application of aPDT as adjunct to SRP provide varying results depending on the used wavelength, number of applications and type of photosensitizer. The present study contributes to the body of research that uses a protocol that includes repeated application of aPDT (three times, i.e. immediately after SRP and at 7 and 14 days) with 635 nm wavelength and toluidine blue (0.1 mg/ml) as photosensitizer. Recent reviews report that the majority of studies evaluating the microbiological and clinical outcomes of adjunct laser treatment use wavelengths of 660–810 nm in single application and methylene blue or indocyanine green as a photosensitizer [6,15,65]. Birang et al. [50] reported 0.92 mm □CAL three months after SRP and double aPDT (indocyanine green) performed at baseline and after 2 weeks, while SRP alone yielded 0.83 mm. Monzavi et al. [72] used the same dye indocyanine green for aPDT as adjunct to treatment of chronic periodontitis. They applied aPDT four times, immediately after SRP, and after 7, 17 and 27 days and found after three months, 1.36 mm CAL gain in the aPDT group and 1.55 mm CAL gain in the SRP group after three months. However, four times aPDT reduced BOP 100% while one time SRP only 52%.

A number of previous clinical studies evaluating the use of aPDT in conjunction with non-surgical periodontal therapy in patients with chronic periodontitis included only single application of aPDT [56,58,60,73,74].

Twice application of aPDT was used as adjunct to ultrasonic debridement in residual periodontal pockets by Müller Campanile et al. [75] to evaluate the clinical, microbiological, and local biological effects of single or two times use of aPDT. After 6 months, all three treatments i.e. SRP, single aPDT and two times use of aPDT yielded favourable clinical and microbiological results, while the only statistically significant advantage was the lower value of C-reactive protein in group treated with two times application of aPDT ($p < 0.05$).

Bassir et al. [27] have used 3x application of 635 nm wavelength with toluidine blue (i.e. at baseline, after 7 and 14 days). They reported no statistically significant improvement in any clinical parameters in favour of aPDT as adjunct to SRP, whereas our study found statistically significant improvements favouring the test group for both BOP and PPD at six months.

The only study with a 12 months follow-up by Lulic et al. [76], used five times aPDT during maintenance phase (at baseline, 1,2,7 and 14 days), reported comparable results in terms of PDD reduction after 12 months (i.e. 0.27 mm) to those obtained in our study at 6 months (i.e. 0.32 mm). In the mentioned study, 5 x application of aPDT yielded BOP values of 20%, while in the present study, the BOP scores amounted to 5.3% after 3 x application of aPDT.

The detailed analysis of the PPD changes in our study revealed statistically significant differences in shallow and medium deep sites (e.g. up to 6 mm) thus suggesting that the main indication of using aPDT is for treating residual pockets in periodontal maintenance patients. This is in line with the results of a recent review on this topic which have indicated that in patients enrolled in periodontal maintenance (i.e. supportive periodontal therapy), the main effect of the additional use of PDT to SRP is on the reduction of inflammation as evidenced by statistically significantly higher reduction of BOP scores following SRP + aPDT when compared to SRP alone, and not necessarily on PPD reduction [77].

A number of *in vitro* and *in vivo* studies have demonstrated the effects of aPDT on the reduction of bacterial load. Chan and Lai [35], using a methylene blue 0.01% and light application of 665 nm at 100 mW, reported a statistically significant decrease of *P. gingivalis, F. nucleatum* and *A. actinomycetemcomitans.* Eick et al. [78] found that the combination of toluidine blue 0.1 mg/mL, wavelength 625–635 nm at 200 mW were effective in reducing the number of *A. actinomycetemcomitans*. These findings were corroborated by those of Novaes et al. [39] who reported a statistically significant decrease in the number of *A. actinomycetemcomitans* following treatment of patients with aggressive periodontitis with SRP and aPDT.

The above mentioned microbiological findings compare well to those obtained in the present study where both treatments reduced statistically significantly the total bacteria counts (TBC) after 6 months. However, at 6 months, the use of SRP and aPDT resulted in a statistically significant decrease in the number of all tested bacteria except *A. actinomycetemcomitans* while the use of SRP alone resulted only in a statistically significant decrease in the numbers of *P. gingivalis*, *T. denticola* and *T. forsythia.*

Furthermore, our results are also in agreement with those of Petelin et al. [5], who reported statistically significant clinical and microbiological improvements at 12 months following SRP and adjunctive use of aPDT.

Interestingly, other studies have failed to demonstrate statistically significant improvements in the clinical parameters following the use of 1x aPDT after SRP, but showed statistically significant reductions in periodontal pathogenic bacteria [40]. One potential explanation for the lack of differences in the clinical outcomes may be due to the single application of aPDT and the potential influence by the bleeding caused by mechanical trauma upon the light activation of the photosensitizer.

The potential beneficial effects of repeated aPDT application, have been also discussed in a review paper by Paula Eduardo et al. [79] suggesting that multiple laser applications may be more effective than single treatments.

In conclusion, the present results indicate that in periodontal patients receiving supportive periodontal therapy, one single session of SRP followed by 3x application of aPDT, may additionally enhance the clinical and microbiological outcomes compared to SRP alone.

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Conflict of interest

The authors declare to not have any conflict of interest regarding this study. All authors have read and approved the final manuscript.

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