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Transgingival photodynamic therapy (tg-aPDT) adjunctive to subgingival mechanical instrumentation in supportive periodontal therapy. A randomized controlled clinical study

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ABSTRACT

Background: Recent data from preclinical studies and case series suggest that transgingival irradiation with diode lasers may represent a novel modality for antimicrobial photodynamic therapy (aPDT). However, at present, there is lack of data from controlled clinical studies on the use of transgingival antimicrobial photodynamic therapy (tg-aPDT) in the treatment of periodontitis.

Objective: To evaluate the clinical effects of tg-aPDT used in conjunction with nonsurgical mechanical instrumentation during supportive periodontal therapy (SPT).

Materials and methods: Forty stage II and III periodontitis patients enrolled in SPT were randomly assigned to two groups of equal size. At baseline, study sites had to show sites with pocket probing depth (PPD) of $\geq$ 5 mm and Bleeding on Probing (BOP). Full mouth and site-specific Plaque-Index scores (PI), BOP, PPD, and Clinical Attachment Level (CAL) were recorded at baseline (BL), three months (3 M), and 6 months (6 M), respectively. The primary outcome variable was the change in the number of sites with BOP. Treatment was performed under local anaesthesia after random allocation to one of the following groups 1) Subgingival scaling and root planing (SRP) + tg-aPDT (test) or 2) SRP alone (control).

Results: Thirty-nine patients completed the study. Full mouth PI and BOP improved over six month, however without statistically significant difference between the groups. At 6 M, BOP-levels were statistically significantly lower in test sites (25.0 %) compared to the control sites (65.0 %), ($p < 0.025$). PPD improved in both groups with comparable mean values at 3 M (PPD test: 5.21 $\pm$ 0.92 mm; PPD control: 4.45 $\pm$ 1.36 mm) and 6 M (PPD test: 5.11 $\pm$ 1.10 mm; PPD control: 4.35 $\pm$ 1.14 mm). Additionally, CAL slightly improved in both groups with comparable mean values at 3 M (CAL test: 6.79 $\pm$ 1.72 mm; CAL control: 5.30 $\pm$ 2.43 mm) and 6 M (CAL test: 6.26 $\pm$ 1.70 mm; CAL control: 5.50 $\pm$ 2.33 mm).

Conclusions: Within its limits, the present results appear to indicate that the use of tg-aPDT adjunctive to SRP may represent a new modality for controlling inflammation and further bleeding in residual periodontal pockets.

1. Introduction

Periodontitis is an inflammatory multifactorial disease caused by "dysbiosis" in the polymicrobial subgingival biofilm characterized by progressive loss of the tooth`s supporting tissues, pocket formation and subsequent loss of clinical attachment [1–3]. Therefore, periodontal therapy aims at mechanically removing the supra- and subgingival microbial deposits from the root surfaces and from the periodontal pockets to arrest disease progression [4,5]. Nonsurgical periodontal therapy consisting of supra and subgingival scaling and root planing (SRP) by means of hand- and/or power-driven instruments is considered as the standard of care in the management of periodontitis. Ample evidence from the literature supports the effects of SRP on improving the clinical and microbiological outcomes thus yielding pocket depth reduction, gain of clinical attachment and a microbial shift compatible with periodontal health [4–6]. However, in specific clinical situations such as presence of deep periodontal pockets, intrabony defects or furcation involvements, the mechanical debridement alone may not be sufficient

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in completely removing or disrupting the microbial biofilm [7,8]. To overcome these limitations and improve the clinical outcomes, systemic and local antibiotics have been suggested as adjunctive agents to nonsurgical protocols, especially for the treatment of severe periodontitis [7,8].

However, in the last decades, there is a substantial increase in resistance against systemic antimicrobials, representing a serious global health problem, with more than 700′000 deaths annually being attributed to infections caused by antibiotic-resistant micro-organisms [9,10]. By 2050, infections caused by antibiotic-resistant micro-organisms are anticipated to cause 10 million deaths per year at a cumulative global cost of $100 trillion. Professional societies and international health agencies, including the United Nations, have declared escalating antimicrobial resistance as one of the most serious and urgent threats to global public health and issued calls for action [10]. Therefore, there is a stringent need for developing novel antimicrobial strategies which do not increase antibiotic resistance.

Antimicrobial photodynamic therapy (aPDT) has been introduced as a modality for destroying periodontopathogenic microorganisms by combining a low-level laser light and a photosensitizer [11–16]. The used low-level lasers for PDT are usually in the red/infrared light spectrum with a wavelength of 650−940 nm (i.e. Helium/Neon (He-Ne), Gallium-Aluminum-Arsenide (GaAlAs) laser) [11–16]. Photosensitizers are substances meant to absorb the laser light of a specific wavelength and transform it into useful energy. When photosensitizers are excited from a ground state to a triplet energy state they generate singlet oxygen molecules ($^1O_2$), which destroy the bacterial cell membranes [11–16]. These cytotoxic free oxygen radicals can maximally migrate to a distance of 0.02 μm not endangering molecules, cells or organs beyond this area [17].

The additional effects of aPDT when used in conjunction with mechanical debridement have been evaluated in numerous randomized controlled clinical trials (RCTs) and summarized in narrative and systematic reviews with meta-analyses [16,18–20]. Currently, the available evidence suggests that the adjunctive effects of aPDT to non-surgical mechanical instrumentation alone in untreated periodontitis patients is of rather limited benefit but may provide additional improvements in residual periodontal pockets in patients enrolled in maintenance therapy [16,18–24]. Recent evidence also indicates that one single session of SRP followed by repeated applications of aPDT may additionally enhance the clinical and microbiological outcomes compared to SRP alone, thus indicating a potential clinical benefit from a repeated use of aPDT for optimizing the outcomes [25–28].

However, from a practical point of view, the use of aPDT is often cumbersome, due to the need to repeatedly introduce the plastic tip of the laser into the pockets, in order to optimally irradiate the treated area and activate the photosensitizer.

A new approach for aPDT is the transgingival application of laser light, which allows an easier and faster treatment [29,30]. With this method, the laser light can be applied on the buccal and lingual side of the gingiva without the need of inserting the light into to periodontal pocket, thus facilitating the clinical application. Very recent findings from a preclinical study performed in pig lower jaws revealed that transgingival irradiation may be suitable for aPDT, since power transmission through the gingival tissue was observed in all specimens [30].

However, to the best of our knowledge, until now no data from controlled clinical studies are available evaluating the outcomes of aPDT when using transgingival irradiation (tg-aPDT).

Hence, the aim of this randomized, controlled, clinical study was to evaluate the clinical outcomes following SRP and additional use of tg-aPDT in periodontitis patients enrolled in supportive periodontal therapy (SPT).

2. Material and methods

2.1. Subject sample

Forty stage II and III periodontitis patients [31] enrolled in SPT at the Department of Periodontology, School of Dental Medicine, University of Bern, Switzerland were randomly assigned to two groups of equal size (test [n20] and control [n = 20]) for the duration of six months. The study protocol was submitted to and approved by the Ethical Committee of the Canton of Bern, Switzerland (number KEK-BE: 050/11).

2.2. Screening

Subjects were asked to read and sign the informed consent. Demographic information and entrance criteria were assessed and personal medical history information was recorded (Fig. 1). During screening, an oral examination including the measurements of Pocket Probing Depths (PPD) and Clinical Attachment Level (CAL) were performed using a manual periodontal probe with a diameter of 0.5 mm (UNC 15, Hu-Friedy, Chicago IL, USA). Additionally, the presence of supragingival plaque as well as bleeding on probing was recorded.

SPT patients were enrolled when presenting with BOP-positive sites and PPD of ≥ 5 mm at single rooted teeth or multi-rooted teeth without furcation involvement. Included patients additionally agreed to return for the clinical visits 3 and 6 months following their baseline. Following screening, patients were excluded in case they presented with ≥25 % PI and ≥25 % BOP, respectively, or in case they were smoking more than 10 cigarettes per day.

A power analysis was performed based on the results reported by Chondros et al. (2009) [20]. Using changes in bleeding on probing scores reported by Lang et al. (1990) [32] as the primary endpoint and calculating the allocation ratio of 1 and the means of 0.48 ± 36 in their group 1 and 0.19 ± 0.22 in their group 2, respectively, a total of 36 subjects was needed with $\alpha = 0.05$ and a power of 0.8 (1-β). Thus, a total of 20 subjects were to be recruited for each group.

2.3. Randomization method (balance and assignment system)

A computer randomization of the treatment modalities for each patient was performed.

2.4. Supportive periodontal therapy (SPT)

Subgingival re-instrumentation of sites with residual PPD of ≥ 5 mm and BOP was performed under local anaesthesia using a combination of Gracey curettes (Deppeler SA, Rolle, Switzerland) and piezoelectric ultrasonic instruments (EMS - Electro Medical Systems S.A., Nyon, Switzerland) during SPT by four well trained dental hygienists after randomized allocation to one of the two groups with 1) SRP + tg-aPDT (Orcos Medical, Küsnacht, Switzerland) (test) or 2) SRP alone (control).

2.5. Clinical measurements

At baseline, and at 3 and 6 months after treatment, the following clinical measurements were performed using a manual periodontal probe with a diameter of 0.5 mm (UNC 15, Hu-Friedy, Chicago IL, USA) at six sites (mesiobuccal, midbuccal, distobuccal, mesiopalatal, midpalatal, distopalatal) per tooth: PPD, CAL, Presence or absence of supragingival plaque (PI), and BOP. Clinical measurements were made by two blinded (i.e. the examiners were not aware of the performed treatment) and calibrated examiners (CAR and GES).

Examiner calibration was performed as follows: five patients, not enrolled in the study, and showing at least 4 teeth with probing depths ≥ 6 mm on at least one aspect of each tooth, were evaluated by the examiner on 2 separate occasions, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were similar to the millimeter at

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram

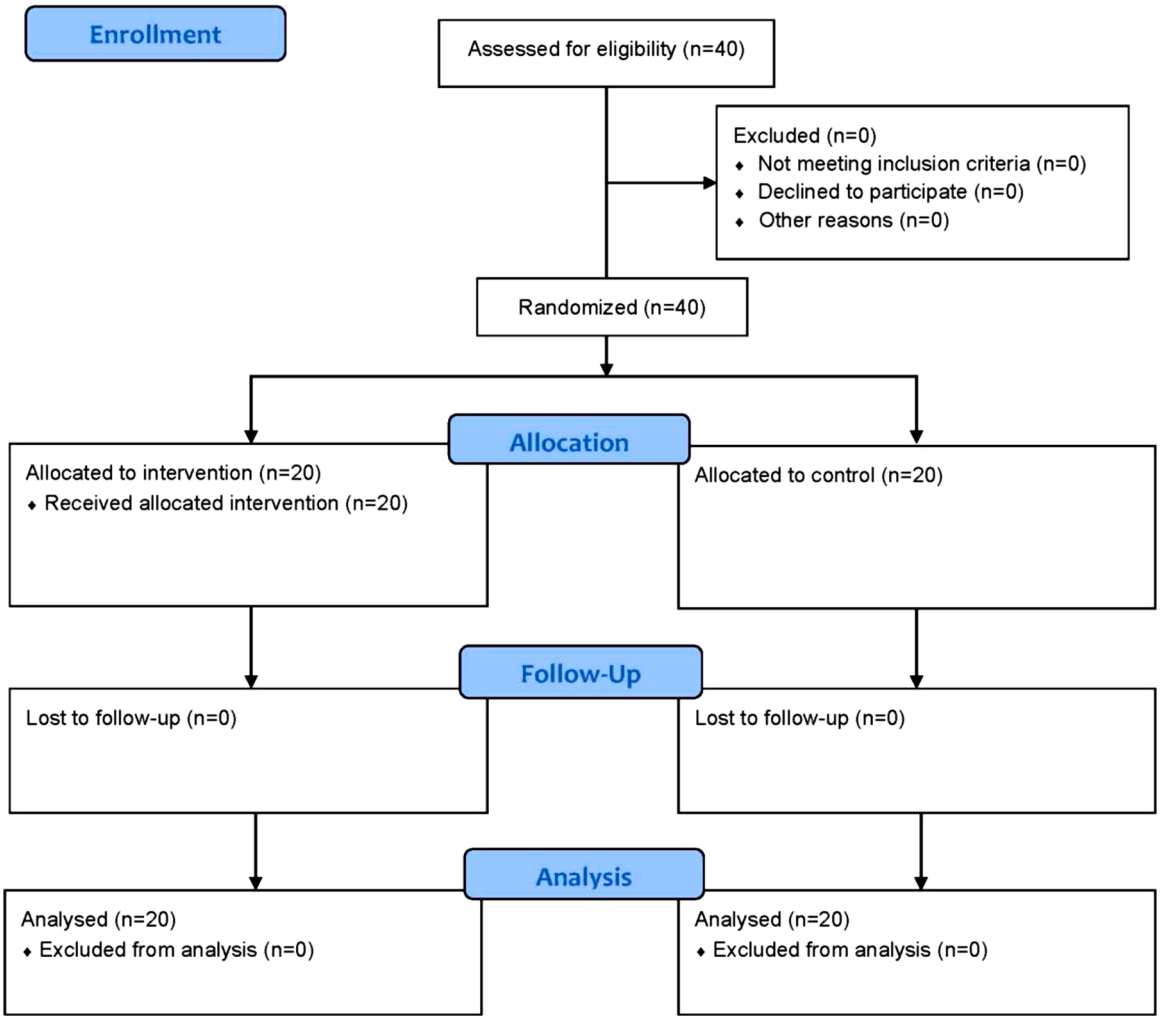

Fig. 1. Consort flow diagram reporting the enrolled, treated and evaluated patients.

≥ 90 %.

2.6. Tg-aPDT

Following SRP under local anaesthesia, the pockets in the test group were treated with tg-aPDT as follows:

As photosensitizer, a 1.0 % solution of phenothiazine chloride (Orcos Medical, Küsnacht, Switzerland) was used. The photosensitizer was applied from the bottom of the pocket in coronal direction with 1 mL syringe and a plastic needle. After 2 min, the pockets were irrigated with distilled water in order to wash out the free photosensitizer.

The laser included a hand-held diode laser (MED 701, Orcos Medical, Küsnacht, Switzerland) with a wavelength of 670 nm and a power of 330 mW. The laser tip diameter measured 8 mm with a laser tip surface of 0.5cm^2 that was brought in contact with the gingival tissues. The duration of the laser application was 60 s at single rooted teeth and of 120 s in molars. Thus, we used the following applications on both vestibular and oral surfaces: a) at single rooted teeth: 330 mW x60 sec = 19.8 J/ 0.5 cm^2 with 39 J/ cm^2, b) at molars: 330 mW X120 sec with 58 J/ cm^2. Therefore, the applied laser power of 330 mW on the soft tissue surfaces was about 4 times higher compared to that obtained with conventional laser tips (70–90 mW) used subgingivally in other studys.

2.7. Statistical analysis

All analyses were conducted with IBM SPSS (Version 18.0.0, Polar Engineering and Consulting, Armonk, NY, USA). Summary statistics (e. g. means, standard deviations, frequencies, etc.) of the demographic

characteristics and each measurement were calculated for both groups and visits. The primary outcome variable was the difference in BOP between the two groups and time points. Secondary outcome variables were the differences in PPD and CAL. Further analysis was performed using the Students-T-Test or ANOVA, respectively. The significance level was set to $p = 0.05$.

3. Results

3.1. Demographic characteristics of the subject sample

Out of the 40 enrolled and treated patients, 39 completed the study, while one patient was lost due to follow-up.

Patient`s demographic characteristics are depicted in Table 1.

3.2. Site-based bleeding on probing (BOP)

The baseline frequency of site-based bleeding on probing in both groups was 100 %. BOP improved in both groups over six months. As revealed by the Fisher's exact test, site-based BOP-levels were statistically significantly lower in test sites (25.0 %) compared with control sites (65.0 %) at the six-month follow-up ($p < 0.05$) (Fig. 2A).

3.3. Site-based presence of plaque

The baseline presence of plaque frequency was 40.0 % in the test groups and 55.5 % in the control group, respectively. During follow-up at 3 and 6 months, presence of plaque improved slightly, however, as revealed by the Fisher's exact test, without reaching statistical significant difference between the groups (Fig. 2B).

3.4. Site-based probing pocket depth (PPD)

PPD improved with no between-groups statistically significant difference at the 3-month follow-up (PPD test: 5.21 ± 0.92 mm; PPD control: 4.45 ± 0.61 mm) and at 6 months (PPD test: 5.11 ± 1.10 mm; PPD control: 4.35 ± 1.14 mm). In both groups, PPD reduction reached statistical significance between baseline and 3 months or baseline and six months, respectively (Fig. 3A).

3.5. Site-based clinical attachment level (CAL)

CAL improved with no statistically significant between-group difference at the 3-month follow-up (CAL test: 6.79 ± 1.72 mm; CAL control: 5.30 ± 2.43 mm) and at 6 months (CAL test: 6.26 ± 1.70 mm; CAL control: 5.50 ± 2.33 mm). In the control group, the improvement of CAL reached statistical significance between baseline and 3 months or baseline and six months, respectively (Fig. 3B).

Table 1
Baseline Demographics.

	All patients		Test (tPDT)		Control	
	n = 40		n = 20		n = 20	
	mean (±SD)	*min. - max.*	mean (±SD)	*min. - max.*	mean (±SD)	*min. - max.*
Age	59.0 (±10.5)	*26 - 81*	60.8 (±10.7)	*34 - 81*	57.3 (±10.3)	*26–72*
Gender (female)	17 (42.5 %)		9 (45.0 %)		8 (20.0 %)	
Smokers	8 (20.0 %)		5 (12.5 %)		3 (15.0 %)	

SD: standard deviation.

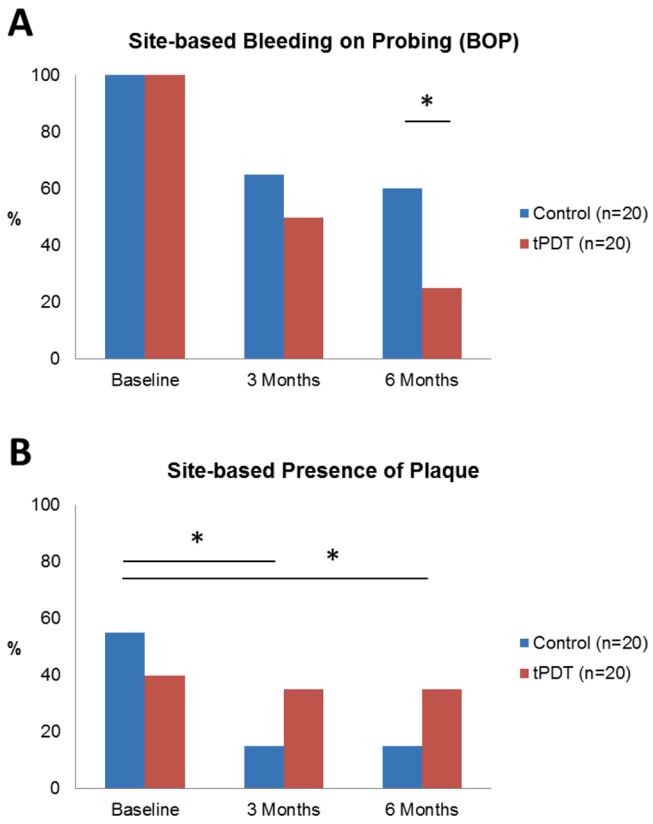

Fig. 2. Frequency (%) of site-based bleeding on probing (BOP) (A) and site-based presence of plaque (B) in n = 20 per group (test and control). *: Statistically significant difference (Fisher's exact Test, $p < 0.05$).

4. Discussion

The present randomized, controlled, clinical study has evaluated the potential clinical effects of using tg-aPDT additionally to subgingival mechanical instrumentation in periodontitis patients enrolled in SPT. Compared to baseline, both treatments led at 3 and 6 months to statistically significant improvements in the evaluated clinical parameters. The additional use of tg-aPDT to SRP resulted at 6 months in statistically significantly higher BOP reductions compared to that obtained with SRP alone (i.e. (25.0 % vs. 65 %). However, in terms of PPD and CAL changes, despite the fact that there was higher tendency for improvement in the group treated with tg-aPDT, the difference between the 2 groups did not reach statistical significance. These results are in agreement with those of 2 previous studies by our group, suggesting that the use of aPDT additionally to SRP may rather lead to a reduction of inflammation than to major changes in terms of PPD and CAL [19,20]. Using a similar design to that of the present study, Christodoulides et al. (2008) [19] and Chondros et al. (2009) [20] evaluated the use of aPDT in conjunction with SRP in untreated periodontitis patients and in patients enrolled in SPT, respectively. In both studies, the results have shown a statistically significantly higher reduction in bleeding on probing (BOP) following one single application of PDT following SRP, compared with SRP alone, but have failed to reveal statistically significant differences in any of the other evaluated parameters (i.e. PPD and CAL changes) [19,20].

These results are also in agreement with the conclusions of previous controlled clinical studies indicating that the adjunctive effects of aPDT to non-surgical subgingival mechanical instrumentation alone in untreated periodontitis patients is of rather limited benefit, but may provide additional improvements in residual periodontal pockets in patients enrolled in SPT [16,18–24]. Generally, most of the available data suggest that the main effect of PDT is rather related to the reduction

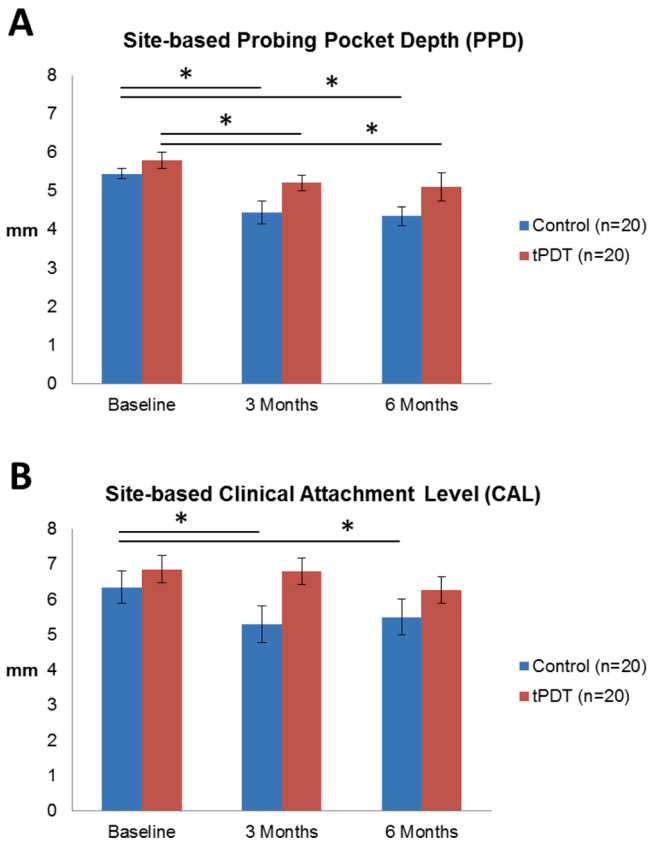

Fig. 3. Mean site-based probing pocket depth (PPD) (A) and mean site-based clinical attachment level (CAL) (B) in n = 20 per group (test and control) at baseline.
*: statistically significant difference ($p < 0.05$).

of inflammatory response evidenced by reduction of BOP than on other parameters such as changes in PPD and CAL. Since in periodontal patients enrolled in SPT, the main goal is to ensure periodontal stability by reducing periodontal inflammation, it appears that the main indication for using aPDT in conjunction with mechanical subgingival instrumentation is the treatment of residual periodontal pockets that persist after initial nonsurgical therapy [16,18–24].

However, when interpreting the clinical relevance of the statistically significantly higher improvements in terms of BOP levels in favor of the test group, one cannot be rule out the possibility that these results might have been also influenced by the slightly higher numbers of smokers included in the test group (i.e. there were 5 smokers in the test group versus 3 patients in the control group, respectively). Substantial evidence is available demonstrating that in patients enrolled in SPT, smokers show lower mean BOP concomitantly with an increased prevalence of residual PPDs [33]. Therefore, it may be speculated that in non-smokers enrolled in SPT, mechanical debridement and tg-aPDT might have resulted in even higher improvements in BOP values compared to those obtained in the present study.

The rationale to develop tg-aPDT is related to the difficulties in placing the laser tip directly into the subgingival area, an often cumbersome and time-consuming procedure. By using the same type of photosensitizer in the subgingival environment (i.e. phenothiazine chloride), the transgingival application of laser light enables an easier and faster treatment [29]. The feasibility of transgingival laser irradiation during aPDT and the possibility to activate the photosensitizer was recently demonstrated in a preclinical study in pig lower jaws [30]. Four diode laser settings were assessed for transgingival irradiation: 120 mW, 80 mW, 60 mW, and 40 mW, respectively. Transgingival laser irradiation (average soft-tissue thickness: 0.84 ± 0.06 mm) resulted in power transmission through the gingival tissue in all specimens, thus indicating that transgingival irradiation may be suitable for aPDT [30]. The authors reported a spot size at the target surface of 0.25 cm^2 and an application time of 30 s with an effective power transmission of 40 mW (energy density 4.87 J/ cm^2; power density 0.16 W/ cm^2), as defined by the company that developed the technique. It was therefore hypothesized that an effective power of at least 40 mW also needs to be achieved transgingivally in order to activate the photosensitizer dye.

The laser power used in our study was 330 mW at the surface, with a duration of 60 s at single rooted teeth and of 120 s in molars, while the laser tip surface was 0.5 cm^2. Thus, we used the following applications on both vestibular and oral surfaces: a) at single rooted teeth: 330 mW x 60 s = 19.8 J/ 0.5 cm2 with 39 J/ cm^2, b) at molars: 330 mW X120 sec with 58 J/ cm^2. In this respect, Wenzler et al. 2019 [30] reported that a minimum of 40 mW was needed to activate the dye in the pocket. With 120 mW laser plus photosensitizer they only achieved 23 mW while with our laser power of 330 mW we applied about 3 times more energy (i.e. we used 3 × 23 mW = 69 mW), which obviously fullfills the requirements.

Additionally, in order to further control the loss of energy from the pockets, irrigation of the pockets was performed for 1 min with destilled water to wash out the free photosensitizer. For the same reason we kept the outer surface of the tissues free of the photosensitizer in order to avoid any filter for the laser light before penetrating the tissue. Furthermore, there were no sites with pigmented mucosa.

Another relevant aspect that needs to be addressed is the possibility of a large amount of energy that might have been absorbed in the soft tissues before penetrating the periodontal pockets and could activate the dye. Subsequently, the absorbed laser energy in the tissues might act as low-level laser photochemically induced biostimulation or biomodulation. However, the effects of biomodulation in the tissue are always taking place when a 670 nm laser light is used, independently whether a photosensitizer is applied or not. Therefore, it should be kept in mind that the use of a-PDT is associated with the following effects: 1. Decontamination and killing of the stained bacteria, and 2. Photobiomodulation in the peripheral tissues [29]. On the other hand, in order to definitively elucidate this important issue, further clinical studies, including a group treated solely by means of low level laser irradiation in addition to mechanical debridement, that might serve as second control group, should be performed.

The potential clinical effects of tg-aPDT as adjunctive to mechanical subgingival instrumentation was first evaluated in patients with untreated periodontitis by Mettraux and Husler (2011) [29]. In 19 patients with untreated periodontitis, one test and one control pocket were treated with scaling and root planing. The test sites received additional tg-aPDT at baseline, after 2 and 6 months, while the control sites were rinsed with “Ringer`s” solution. At 6 months, the results revealed in both groups statistically significant improvements in terms of PPD reduction and CAL gain compared to baseline and higher, but statistically not significant, improvements in the group treated with tg-aPDT [29]. However, when interpreting the data, it has to be kept in mind that in the aforementioned study, treatments were performed in patients with untreated periodontitis while the additional use of a-PDT appears to provide limited additional benefit compared to subgingival mechanical instrumentation alone [22].

Another important aspect to be discussed is the possibility of the remaining bacteria to develop resistance mechanisms against oxidative stress by synthesizing different dismutase enzymes. However, according to the best of our knowledge, until now, no development of resistance to aPDT has been demonstrated. On the other hand, since the targets of aPDT are cell wall components (e.g. lipopolysaccharide, cytoplasmic membrane) and DNA, it cannot be excluded that bacteria may develop strategies to modify these targets and subsequently, to become less sensitive to the action of oxygen species.

The fact that in the present study, no adverse effects were reported in any of the cases treated with tg-aPDT, coupled with the comparable

clinical outcomes obtained with the conventional use of aPDT (i.e. subgingival laser irradiation), appear to suggest that tg-aPDT may represent a realistic alternative to conventional aPDT [16,18–24].

Another aspect that needs to be considered when interpreting the present results is the potential decrease in laser power caused by the bleeding that occurs following subgingival instrumentation. Therefore, in order to overcome this potential limitation, it has been suggested to repeat the use of aPDT after the first session of subgingival mechanical instrumentation. The potential beneficial effects of repeated PDT applications were evaluated in a number of clinical studies which appear to suggest that one single session of SRP followed by repeated applications of aPDT may additionally enhance the clinical outcomes compared either with SRP alone or followed by one single use of aPDT [25–28]. Obviously, since at present it is unknown to what extent the repeated use of t-aPDT may additionally influence the outcomes, further studies are needed to shed light on this important clinical aspect related to treatment with t-aPDT.

In conclusion, within their limits the present results indicate that the use of tg-aPDT adjunctive to SRP may represent a novel modality for controlling inflammation and further bleeding in residual periodontal pockets.

Statement of sources of funding for the study

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Declaration of Competing Interest

The authors report no declarations of interest.

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