Effect of repeated adjunctive antimicrobial photodynamic therapy on subgingival periodontal pathogens in the treatment of chronic periodontitis

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Abstract  The aim of this study was to compare the effect of subgingival ultrasonic scaling followed by repeated (three times) antimicrobial photodynamic therapy (PDT), ultrasonic scaling alone (US), and scaling and root planing with hand instruments (SRP) for initial periodontal treatment. Twenty-seven non-smoking systemically healthy chronic periodontitis patients were included. Residual pockets ≥4 mm deep and bleeding on probing were debrided either with SRP, US alone, or US followed by a single episode of PDT during supportive periodontal treatment. Probing pocket depth (PPD), bleeding on probing (BOP), and clinical attachment level (CAL) were monitored over 12 months. The presence of five periodontal pathogens in the pockets was determined by a commercially available micro-IDent test. Intergroup and intragroup statistical analysis was performed. All three treatments resulted in a significant clinical improvement. Additional application of PDT to US failed to result in further improvement in terms of PPD reduction and CAL gain. However, it resulted in a higher reduction of BOP at 3 and 12 months comparing to US alone or SRP (PDT from 25 to 13 and to 9 %, US from 23 to 16 and to 12 %, and SRP from 17 to 10 and to 9 %, respectively). PDT reduced the proportion of positive sites after 6 months for Treponema denticola (TD) significantly more effectively than US or SRP (p<0.0001). Additionally, PDT resulted in a greater reduction of Aggregatibacter actinomycetemcomitans (AA), Tannerella forsythia (TF), and TD in medium pockets (4–6 mm) (p<0.02) and of TD in deep pockets (>6 mm) compared to mechanical debridement alone (p<0.05).

Keywords  Chronic periodontitis · Photodynamic therapy · Non-surgical periodontal treatment · Periodontal pathogens · Supportive periodontal therapy

Introduction

Periodontal diseases result from inflammation of the supporting structures of the teeth in response to chronic infections caused by various periodontal pathogenic bacteria. Periodontal pockets, a unique environment for colonizing microorganisms, contain as many as 400 species of bacteria, which are organized in biofilms [1]. The subgingival biofilm differs markedly in periodontal health and in periodontitis [2]. The predominant early colonizers of the subgingival plaque biofilms are the Actinomyces sp. and streptococci [3] which develop a complex microbial community within only a few days [4]. A number of secondary colonizers, in particular Fusobacterium nucleatum and Porphyromonas gingivalis, can bind both to early colonizers and to other, later colonizers [5]. The main goal of periodontal therapy is to eliminate bacterial deposits by removing the supra- and subgingival biofilms [6]. Generally, this objective is achieved by mechanical debridement, which consists of hand- or ultrasonic instrumentation of the affected sites. Studies have shown comparable clinical outcomes for the use of hand instruments or ultrasonic scalers employed in mechanical debridement [7–9]. Despite the improvement in terms of clinical parameters, neither of these techniques removes bacterial plaque...
biofilm entirely because of the bacterial invasion into the adjacent soft tissue, root cement, and dentinal tubules and also because of the possible recolonization of periodontal pockets from other affected areas [10–14]. Therefore, a variety of new methods have been introduced in the last decade, antimicrobial photodynamic therapy (PDT) with diode laser as an adjunctive to mechanical debridement demonstrated additional clinical benefits. Recent studies have shown improvement in all clinical periodontal parameters, especially reduction of bleeding on probing [15–20] and a significant reduction in the percentage of sites positive for periodontal pathogenic bacteria [21]. On the other hand, some studies failed to show an additional benefit of PDT on clinical periodontal parameters and subgingival microbial flora [22, 23]. Similarly, the results of a recent meta-analysis suggest that PDT as an adjunct to scaling and root planing in patients with chronic periodontitis provides only a modest clinical improvement, and no significant microbiological changes were shown [24].

In view of the conflicting results in the literature, the purpose of this study was to evaluate the antimicrobial effects of repeated PDT (three times), performed after ultrasonic debridement on periodontal clinical parameters as well as microbiological composition of subgingival microbial flora in patients with chronic periodontitis. In addition, added benefits of PDT as an adjunct to the ultrasonic scaling of residual pockets in supportive periodontal treatment were evaluated.

Materials and methods

Study population

Patients were recruited from the pool of the Department for Oral Medicine and Periodontology, University of Ljubljana, Faculty of Medicine, between October 2010 and June 2012. The inclusion criteria were plaque index (PI) less than 20 % and at least four teeth with increased probing pocket depth (PPD) (≥4 mm) in each quadrant. The following conditions led to exclusion from the study: smoking, antibiotic use in the previous 6 months, pregnancy or breast-feeding, and presence of systemic disease.

Study design

This study was a single-center, randomized clinical trial of 12 months duration. National Medical Ethics Committee of the Republic of Slovenia approved the protocol (No: 144/02/11). Supra-gingival deposits were removed in all teeth with an ultrasonic device 14 days before treatment. All patients were instructed on oral hygiene practices. At the baseline, the following periodontal parameters were evaluated: periodontal probing depth (PPD), bleeding on probing (BOP), and clinical attachment level (CAL) all at six sites on each tooth. All measurements were carried out by one experienced person (KP), intra-examiner calibration score was 0.80.

A total of 27 patients signed informed consent. Initially, subgingival debridement was performed with an ultrasonic device (NSK Varios 970, Japan). Afterwards, patients were randomly divided into three groups of nine subjects (Table 1). In the first group, subgingival scaling and root planning using Gracey curettes was performed (SRP), in the second group, subgingival deposits were removed using only an ultrasonic scaler (US), and the third group of patients received combined treatment with an ultrasonic scaler, followed by three episodes of antimicrobial photodynamic therapy (first, third, and seventh day after the ultrasonic debridement) (PDT). The protocol with three applications of PDT was selected based on the results of a preliminary study (results are not shown). The photosensitizer phenothiazine chloride (HELBO-Blue Photosensitizer) was applied from the bottom of the periodontal pocket towards the crown on all affected teeth. After 3 min of action, the photosensitizer was rinsed with sterile distilled water. The pocket was then exposed to the laser light (Helbo TeraLite, 660 nm, 60 mW/cm2) using a fiber optic application for 1 min (HELBO-3D Pocket Probe). Control measurements were performed at 3, 6, 9, and 12 months after the initial treatment. Supportive periodontal therapy (SPT) for sites with remaining PPD (≥4 mm) and positive BOP continued every 3 months. In the first group (SRP), subgingival scaling and root planing with curettes was done; in the second group (US), subgingival plaque biofilm was removed by an ultrasonic scaler only; and the third group (PDT) received combined treatment with an ultrasonic scaler followed by a single episode of PDT.

Microbiological assessment

In each of the four quadrants, subgingival plaque samples were collected from one periodontal pocket at baseline, 1 week, 3, and 6 months after the treatment. Two samples from each patient were collected from medium pockets (4–6 mm) and two samples from deep pockets (≥6 mm). Plaque samples were collected with sterile paper points after the removal of supra-gingival plaque at the sampling site. In total, 432 (144 samples in each group: 72 from medium and 72 from deep periodontal pockets) samples were obtained. The presence of five periodontal pathogens—Aggregatibacter actinomycetemcomitans (AA), P. gingivalis (PG), Prevotella intermedia (PI), Tannerella forsythia (TF), and Treponema denticola (TD)—was qualitatively determined in each sample by multiplex polymerase chain reaction (PCR), followed by hybridization against species-specific DNA probes using a
commercially available micro-IDent test (Hain Lifescience, Nehren, Germany) according to the manufacturer’s instructions, as described earlier [25, 26]. PCR amplification was carried out in a reaction volume of 25 μL consisting of 2.5 μL of template DNA and 22.5 μL of reaction mixture containing 17.5 μL of primer–nucleotide mix from the micro-IDent kit, 2.5 μL of 10× PCR buffer, 2.5 μL of 25 mmol/L MgCl₂, and 1 U FastStart Taq polymerase (Roche Diagnostics, Mannheim, Germany). PCR cycling was carried out in the Mastercycler Personal (Eppendorf, Hamburg, Germany). The cycling conditions comprised of an initial denaturation step at 95 °C for 5 min; 20 cycles at 95 °C for 30 s and at 58 °C for 2 min; 20 cycles at 95 °C for 25 s, at 53 °C for 40 s, and at 70 °C for 40 s; and a final extension step at 70 °C for 8 min. According to the manufacturer’s instructions, for the subsequent reverse hybridization, the biotinylated amplicons were denatured and incubated at 45 °C with hybridization buffer and strips coated with two control lines and five species-specific probes. After PCR products had bound to their respective complementary probe, a highly specific washing step removed any non-specifically bound DNA. Streptavidin-conjugated alkaline phosphatase was added, the samples were washed, and hybridization products were visualized by adding a substrate for alkaline phosphatase. According to the manufacturer, the cut-off of the test is set to 103–104 genome equivalents [25].

Statistical analysis

Statistical analysis of the data was performed using ANOVA and χ² test. Clinical periodontal parameters before treatment and 3, 6, 9, and 12 months after the treatment were compared within the groups and between the groups. In addition, the microbiological analysis of subgingival plaque samples before treatment, 1 week, 3, and 6 months after the treatment, were compared within the groups and between the groups. Differences were considered statistically significant when \( p<0.05 \).

Table 1 Demographic characteristics of the patient population at baseline

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SRP</th>
<th>US</th>
<th>PDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>6/3</td>
<td>4/5</td>
<td>5/4</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>43 (37–51)</td>
<td>51 (42–64)</td>
<td>47 (36–59)</td>
</tr>
<tr>
<td>Mean number of teeth with PPD ≥4 mm</td>
<td>16</td>
<td>16</td>
<td>18</td>
</tr>
</tbody>
</table>

m = male; f = female; SRP = scaling and root planning; US = ultrasonic scaling; PDT = ultrasonic scaling followed by photodynamic therapy

Results

Clinical outcomes

All 27 patients were followed up to 12 months. No differences in PPD and CAL were observed at baseline between the groups, however, statistically significant lower baseline percentage of BOP in SRP group compared to US and PDT group was found (\( p<0.05 \)). Table 2 shows clinical status in the study sites after the initial treatment (at 3 months) and during SPT (at 6, 9, and 12 months). All three treatment modalities resulted in a statistically significant clinical improvement from baseline to 3 months (\( p<0.001 \)) and to 12 months (\( p<0.00001 \)) bleeding on probing reduction was statistically significant from baseline to 3 months in the PDT group (\( p<0.01 \)) and from baseline to 12 months for all treatment modalities (\( p<0.05 \)). However, there were no statistically significant differences between the groups, besides BOP reduction at 12 months being greater in PDT group than in SRP group (\( p<0.05 \)). The proportion of the shallow (<4 mm), moderate (4–6 mm), and deep pockets (>6 mm) at baseline to 3, 6, 9, and 12 months after the treatment was evaluated (Fig. 1a). The proportion of shallow pockets increased in all three groups, the proportion of moderate pockets in SRP and PDT groups decreased, but increased in US group, and the proportion of deep pockets decreased in all three groups. The effect of different treatment modalities on different initial

Table 2 Clinical findings at baseline, and at 3, 6, 9 and 12 months after scaling and root planning (SRP), ultrasonic scaling (US), or ultrasonic scaling followed by photodynamic therapy (PDT), \( n=27 \)

<table>
<thead>
<tr>
<th>Therapy mode</th>
<th>Time (month)</th>
<th>PPD</th>
<th>CAL</th>
<th>BOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRP</td>
<td>0</td>
<td>3.8±0.2</td>
<td>4.7±0.3</td>
<td>17.0±2.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.5±0.2</td>
<td>4.4±0.3</td>
<td>10.5±2.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.4±0.2</td>
<td>4.2±0.2</td>
<td>10.6±2.3</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>3.3±0.2</td>
<td>4.0±0.3</td>
<td>7.8±1.5</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.3±0.2</td>
<td>4.0±0.2</td>
<td>9.0±1.4*</td>
</tr>
<tr>
<td>US</td>
<td>0</td>
<td>3.6±0.2</td>
<td>4.3±0.3</td>
<td>23.0±2.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.1±0.2</td>
<td>3.7±0.3</td>
<td>16.5±2.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.1±0.2</td>
<td>3.7±0.2</td>
<td>17.8±2.3</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>3.1±0.2</td>
<td>3.8±0.3</td>
<td>13.5±1.5</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.0±0.2</td>
<td>3.7±0.2</td>
<td>12.2±1.4*</td>
</tr>
<tr>
<td>PDT</td>
<td>0</td>
<td>3.4±0.2</td>
<td>4.2±0.3</td>
<td>24.9±2.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.0±0.2</td>
<td>3.7±0.3</td>
<td>13.1±2.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.0±0.2</td>
<td>3.6±0.2</td>
<td>11.2±2.3</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>3.0±0.2</td>
<td>3.7±0.3</td>
<td>9.7±1.5</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.9±0.2</td>
<td>3.7±0.2</td>
<td>9.4±1.4*</td>
</tr>
</tbody>
</table>

PPD probing pocket depth; CAL clinical attachment level; BOP bleeding on probing. Mean±standard deviation, * Different from baseline, \( p<0.001 \), a Different from baseline, \( p<0.00001 \), b Different from baseline, \( p<0.01 \), c Different from baseline, \( p<0.05 \), * Difference between groups, \( p<0.05 \)
pocket depths is shown in Fig. 1b. Deeper pockets showed the greatest decrease in PPD after 12 months. The decrease was significantly greater in PDT group compared to US or SRP groups \( (p<0.05 \text{ and } p<0.001, \text{ respectively}) \). Intermediate sites showed moderate improvement in PPD (PDT vs. US; \( p<0.01 \)) while shallow pockets exhibited the least change (PDT vs. SRP; \( p<0.001 \)).

Microbiological analysis

Figure 2 shows microbiological analysis of subgingival plaque samples for five periodontal pathogens (AA, PG, PI, TF, and TD). Proportion of positive sites decreased for all bacteria 1 week after the initial treatment, regardless of the treatment modality (Fig. 2a, b, c, d, e). SRP reduced proportion of positive sites for all five periodontal pathogens 3 months after the initial treatment, but sites positive for AA increased above baseline level at 6 months (Fig. 2a). US reduced proportion of positive sites for AA and PI 3 months after the initial treatment and for AA, PG, and PI at 6 months, but proportion of sites positive for TF and TD increased above baseline level (Fig. 2d, e). PDT reduced proportion of positive sites for PI, TF, and TD 3 months after the initial treatment and for all five periodontal pathogens at 6 months. In addition, proportion of sites positive for TD was statistically significantly reduced compared to US alone or SRP \( (p<0.0001) \) (Fig. 3e).

Figure 3 shows the proportion of moderate (4–6 mm) and deep pockets (>6 mm) at baseline, at 1 week, 3 months, and 6 months positive for five periodontal pathogens. In the SRP group, 3 and 6 months after the treatment, the proportion of sites positive for PI and TF in moderate pockets decreased, but increased for AA. However, there was no effect on reduction of sites positive for PG and TD (Fig. 3a). At 3 and 6 months, the proportion of all periodontal pathogens in deep pockets decreased (Fig. 3b). In the US group, 3 months after the initial treatment, there was a reduction in the proportion of sites positive for PI and TF in moderate pockets decreased, but increased for AA. However, there was no effect on reduction of sites positive for PG and TD (Fig. 3a). In deep pockets, at 3 and 6 months, a reduction in the proportion of sites positive for TF and TD was found, and there was no effect on the sites positive for PG, TF, and TD (Fig. 3b). In PDT group, 3 months after the treatment, there was a reduction in the proportion of sites positive for TF and TD and an elevation in the proportion of sites positive for AA and PI in moderate pockets. Six months after the treatment numbers of all five periodontal pathogenic bacteria were reduced (Fig. 3a). In deep pockets, 3 months after the treatment, there was a reduction in the proportion of sites positive for PI, TF, and TD but no effect was observed regarding sites positive for AA and PG. After 6 months, the numbers of all periodontal pathogenic bacteria were reduced (Fig. 3b). In moderate pockets, 6 months after the treatment, PDT statistically significantly reduced the proportion of sites positive for AA, TF, and TD \( (p<0.02) \) (Fig. 3a), and in deep pockets sites positive for TD


Discussion

Scaling and root planning using hand instruments is one of the most common procedures in the treatment of periodontal diseases and has been used as the ‘gold standard’ against which other therapies have been compared. The study of Kaldahl et al. [27] reported this treatment to improve both clinical and microbial parameters. In the last years, the ultrasonic debridement emerged as a new non-surgical treatment based on the knowledge that removing the tooth structure is not a prerequisite for periodontal healing, because bacterial lipopolysaccharide is easily removed from the root surface [28, 29]. Ioannou et al. [9] reported comparable clinical and microbial outcomes using hand instruments or ultrasonic scalers for mechanical debridement. However, mechanical instrumentation cannot remove subgingival biofilm entirely. Photodynamic therapy results in microbial reduction which may improve periodontal treatment results in areas of difficult access, such as deep pockets and furcations.

The aim of the present study was to investigate the clinical and microbiological effects of repeated PDT as compared to mechanical debridement in initial periodontal treatment of chronic periodontitis and benefit of a single application of PDT in maintenance care. Photodynamic therapy as an adjunct treatment to US failed to result in further improvement in terms of PPD reduction and CAL gain; however, it resulted in a higher reduction of BOP at 12 months compared to SRP ($p<0.05$).

The patients in this study were randomly divided into three groups, however, statistically significant lower baseline percentage of BOP in SRP group compared to US and PDT group was found. In addition, microbiological analysis showed lower baseline proportion of TF and TD in SRP group. It seems that TF and TD have some influence on BOP. It has been shown that TF was detected more frequently and in higher numbers in active periodontal lesions compared to inactive lesions [30] and association between decreased BOP and the decreased proportion of TF was found after SRP [31]. $T. denticola$ was found to be more common in periodontally affected compared to healthy sites [32]. Patients in the present clinical trial were high motivated for oral home care, with plaque index below 20% over the entire study. The effect of any treatment was assessed by comparing the differences between the baseline values and the values at
different time intervals. However, it is difficult to compare the differences between baseline value of the BOP and the value after 12 months in the SRP group with the differences of other treatment modalities as BOP was significantly lower already at the baseline. If only the average values of the BOP in the SPT phase, i.e. after 6, 9, and 12 months in SRP group (10.6, 7.8, and 9.5 %, respectively) are taken into consideration, they are comparable to those in the same time intervals in PDT group (11.2, 9.7, and 9.4 %, respectively). The average values of the BOP in US remained slightly higher during the SPT phase (BOP value at 6 months was 17.8 %, at 9 months was 13.5 % and at 12 months 12.2 %). This is in accordance with the proportions of positive sites for TF and TD

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Fig. 2 Microbiological analysis of subgingival plaque samples for five periodontal pathogens. a Proportion of sites positive for A. actinomycetemcomitans (AA), b for P. gingivalis (PG), c for P. intermedia (PI), d for T. forsythia (TF), and e for T. denticola (TD) at baseline, and at 1 week, 3 months, and 6 months after the treatment. SRP reduced proportion of positive sites for all five periodontal pathogens 3 months after the initial treatment, but sites positive for AA increased above the baseline level 6 months post supportive treatment. US reduced proportion of positive sites for AA and PI 3 months after the initial treatment and for AA, PG, and PI 6 months post supportive treatment, but the proportion of sites positive for TF and TD increased above the baseline level. PDT reduced proportion of positive sites for PI, TF, and TD 3 months after the initial treatment and for all five periodontal pathogens 6 months post supportive treatment. n=432. SRP scaling and root planing; US ultrasonic scaling; PDT ultrasonic scaling followed by photodynamic therapy.
at 3 and 6 months in US group, which were higher than the baseline levels.

The results of the present study indicate that most clinical changes occurred during the first 3 months post any treatment and continued to show improvement during the maintenance period. In a study of Balata et al. [23], where single application of PDT in initial periodontal therapy was tested against US, no additional clinical benefits were found. Andersen et al. [15] and Braun et al. [16] reported that single application of PDT associated with SRP promotes a statistically significant improvement of CAL and BOP 3 months after the treatment. In contrast, Berakdar et al. [20] also achieved the greater reduction of PPD by a combination of SRP and PDT 6 months after the treatment.

Fig. 3 Proportion of moderate (4–6 mm) (a) and deep pockets (>6 mm) (b) at baseline, at 1 week, 3, and 6 months after the initial and supportive treatment positive for A. actinomycetemcomitans (AA), P. gingivalis (PG), P. intermedia (PI), T. forsythia (TF), and T. denticola (TD). In the SRP group, the proportion of sites positive for AA increased, but there was no effect on reduction of sites positive for PG and TD in moderate pockets. The proportion of all periodontal pathogens in deep pockets decreased, the effect on TD was minimal. In the US group, there was an increase in proportion of sites positive for TF and TD in moderate pockets. In deep pockets, there was no effect on the sites positive for PG, TF, and TD. In PDT group, numbers of all five periodontal pathogenic bacteria were reduced in moderate and deep pockets. In deep pockets, the effect on AA and PG was minimal. $n=216$ moderate pockets, $n=216$ deep pockets. SRP scaling and root planing; US ultrasonic scaling; PDT ultrasonic scaling followed by photodynamic therapy.
Photodynamic therapy uses a laser light of appropriate wavelength, in the presence of oxygen, to activate the photosensitizer. Activated photosensitizer bound on bacterial cells reacts with molecular oxygen, resulting in the formation of reactive oxygen species (ROS), which damage bacterial cell components and cause cell lysis [33]. Reactive oxygen species half-life is very short, a few nanoseconds to a few microseconds, which is too short to significantly interact with the surrounding (host) molecules [34]. Therefore PDT has no side effects on the local surrounding tissues. Due to simple molecular form of ROS, the possibility of bacterial resistance development is very low [35]. Photosensitizer itself has been reported to have no bactericidal effects on periodontal pathogens [36]. In addition to bactericidal effects of PDT, the diode laser application added benefits to faster periodontal healing because of its biostimulative effects [37].

In most previous studies, initial treatment with mechanical debridement has been followed by a single application of PDT, with an exception being the study of Lulic et al. [38] where PDT was applied five times in 2 weeks but in periodontal maintenance phase. In the present study, US have been followed by three episodes of PDT, namely first, third, and seventh day after mechanical debridement. This protocol has been selected basing on our preliminary study, which showed a significant reduction of BOP (p<0.05) and greater reduction of subgingival microbial flora at 3 months in the group with three repeated PDT episodes, compared to the group that received two repeated PDT episodes (first and seventh day). With selected time window, the aim was to prevent bacterial recolonization at three critical stages. After removal of the supra-and subgingival plaque, gram-positive coccus *Streptococcus oralis* reached the baseline level in one single day. *S. oralis* is a numerically important member of the commensal oral microbiota, isolated from all intra-oral surfaces and a pioneer organism involved in the colonization of the primary dentition [39]. Surface receptors on the deposited gram-positive cocci and rods allow subsequent adherence of gram-negative organisms, which otherwise express only poor ability to attach directly to the pellicle. Therefore, *F. nucleatum* and *P. intermedia* also reach the baseline values third to fourth day after removal of plaque biofilm. On the seventh day, the baseline values of bacteria are exceeded [40].

A significant effect on subgingival microbial composition was expected after repeated adjunctive PDT in initial therapy; therefore, additional subgingival samples were obtained after 1 week. All three treatment modalities (SRP, US, and PDT) reduced the proportion of pathogens, except for SRP, which had no effect on AA, and PDT having no effect on PI after 1 week. However, none of these species were completely eliminated from subgingival area by any of the treatment modalities. The proportions of different bacteria were increased after 3 months and decreased again after 6 months. The biofilm formation is very complex and community development is controlled by programmed patterns of gene expression and multilevel regulation of protein expression and activity [41]. The subgingival area is colonized by biofilm communities shortly after any treatment. The initial clinical improvements and long-term stability were associated with limited changes in the subgingival microbiota. The data suggest that the maintenance phase of therapy may be essential in consolidating microbiological improvements.

The clinical studies have shown that the impact of PDT on the periodontal pathogens is contradictory. Some studies failed to show an impact of PDT on the microbial biofilm [22, 42]. In contrast, a positive effect of a single application of PDT has been confirmed by Theodoro et al. [21]. Six months after the treatment, they found a significant reduction of five periodontal pathogens (AA, PG, PI, TF, and FN). The efficiency of photodynamic maintenance treatment on periodontal pathogens has also been proven [43, 44].

In the present study, initial periodontal treatment with repeated PDT decreased proportion of positive sites for PI, TF, and TD at 3 months, and a reduction in numbers of all five pathogens (AA, PG, PI, TF, and TD) was found 6 months after maintenance care. Compared to mechanical debridement alone, statistically significantly greater reduction in TD was observed at 6 months (p<0.0001). Additionally, PDT was effective in both medium (4–6 mm) and deep pockets (>6 mm) and resulted in a statistically significantly greater reduction of AA, TF, and TD in medium (p<0.02) and TD in deep pockets (p<0.05) comparing to US alone or SRP. It seems that US alone or SRP have a little or no effect on reduction of some periodontal pathogens in medium and deep pockets. The data presented here suggest that PDT as an adjunct to US results in statistically significant reductions in some of the key periodontal pathogens, but does not improve the results of mechanical debridement alone in terms of PPD and CAL. However, it reduces BOP after initial treatment and during SPT. Photodynamic therapy proved to have additional benefits and may therefore be recommended for the maintenance treatment of residual pockets.

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**Conflict of interest** The authors declare no conflicts of interest in this study.

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