

# Full-Mouth Antimicrobial Photodynamic Therapy in *Fusobacterium nucleatum*-Infected Periodontitis Patients

Bernd W. Sigusch,\* Marcus Engelbrecht,\* Andrea Völpel,\* Anne Holletschke,\* Wolfgang Pfister,† and Juliane Schütze‡

**Background:** The purpose of this study is to clinically and microbiologically evaluate the effect of photodynamic therapy (PDT) as a full-mouth procedure in *Fusobacterium nucleatum*-infected patients with periodontitis.

**Methods:** In the present study, PDT is administered adjvantly after scaling and root planing (SRP) to patients with localized chronic periodontitis (LCP). Twenty-four patients, in whom only *F. nucleatum* was detected by baseline polymerase chain reaction (PCR) after SRP, were randomly assigned to PDT and control groups. PDT was carried out once as a full-mouth disinfection in the test group. The control group was treated with the photosensitizer solution, but without laser irradiation. In all subjects, the clinical parameters plaque index, reddening, bleeding on probing (BOP), probing depth (PD), gingival recession, and clinical attachment level (CAL) were determined at the baseline examination and at 1, 4, and 12 weeks after PDT. Quantitative analysis of the *F. nucleatum* DNA concentration was performed by competitive PCR. All clinical indices were calculated for each test and control subject as were the median and interquartile range of each group.

**Results:** In patients with LCP who received PDT treatment, significant reductions in reddening, BOP, and mean PD and CAL were observed during the observation period and with respect to controls. Four and 12 weeks after PDT, the mean PD and CAL showed significant differences from baseline values and from those of the control group. In the PDT group, 12 weeks after treatment, the *F. nucleatum* DNA concentration was found to be significantly reduced compared to the baseline level.

**Conclusion:** The results of this study show that the adjuvant application of the described PDT method is appropriate to reduce periodontal inflammatory symptoms and to successfully treat infection with *F. nucleatum*. *J Periodontol* 2010;81:975-981.

## KEY WORDS

Biofilm; *Fusobacterium nucleatum*; inflammation; photodynamic therapy.

The presence of a bacterial biofilm of periodontopathogenic species on the tooth or root surface is the major cause of gingivitis and periodontitis.<sup>1-3</sup> It is well accepted that mechanical removal of dental biofilms is the basis of any adjunctive periodontal therapy. Currently, researchers search increasingly for efficient adjuvant anti-infection treatment modalities with the least possible side effects. Local and systemic administrations of antibiotics may lead to resistance and gastrointestinal and other disorders, and patient compliance is often an added problem.<sup>4-6</sup>

A new, alternative method of adjunctive antimicrobial treatment is provided by photodynamic therapy (PDT), which involves the use of a photosensitizer (PS) that is activated by exposure to light of a specific wavelength in the presence of oxygen. The exposure of the PS to light results in the formation of oxygen species such as singlet oxygen and free radicals, the antimicrobial effects of which are known.<sup>7</sup> There are also several in vitro studies<sup>8-11</sup> on the effectiveness of PDT against periodontopathogenic bacteria. However, only a few studies<sup>12,13</sup> investigated the efficacy of PDT in animal models or clinical trials. Kömerik et al.<sup>12</sup> proved the successful use of PDT in the rat model, in which toluidine blue-mediated PDT sufficiently suppressed *Porphyromonas gingivalis*. Sigusch et al.<sup>14</sup> established a clinical infection model using beagle dogs. The animals were infected with

\* Department of Conservative Dentistry, University Hospital Jena, Jena, Germany.

† Institute for Medical Microbiology, University Hospital Jena.

‡ Department of Fundamental Science, University of Applied Sciences Jena, Jena, Germany.

*Fusobacterium nucleatum* and *P. gingivalis* in all periodontal sites. PDT was performed with two chlorine-based sensitizers and BLC1010, followed by illumination with a diode laser (wavelength: 662 nm). The treatment resulted in a significant reduction of inflammation signs like redness and bleeding on probing (BOP). It was also proven that BLC1010 photosensitization suppressed *P. gingivalis* more efficiently than it suppressed *F. nucleatum*. In a recent study by de Oliveira et al.,<sup>15</sup> antimicrobial PDT, together with scaling and root planing (SRP), was applied in a split-mouth design in patients with aggressive periodontitis. The experiments used a laser source with a wavelength of 660 nm and a phenothiazine-based PS. Phenothiazine dyes include, among others, methylene blue and toluidine blue. In the study by de Oliveira et al.,<sup>15</sup> PDT (without SRP) and SRP showed similar clinical results in the non-surgical treatment of patients with aggressive periodontitis over an observation period of 3 months. However, the authors<sup>15</sup> did not report any results of microbiologic monitoring of the periodontopathogenic species. Azarpazhooh et al.<sup>16</sup> also held that there were not enough data to show that phenothiazine-based PDT is efficacious.

For these reasons, the aim of the present study is to investigate the clinical and microbiologic efficacy of the phenothiazine-based PDT method as a full-mouth procedure in *F. nucleatum*-infected patients with localized chronic periodontitis (LCP).

## MATERIALS AND METHODS

### Patient Population

The experimental protocol was reviewed and approved by the Ethics Committee of the University of Jena (B1671-12/05).

Examinations in the present study were carried out from April 2006 through February 2008 on patients with LCP from the patient pool of the Department of Conservative Dentistry, University of Jena. Patients with LCP were characterized by <30% of sites with probing depths (PDs) >3.5 mm.<sup>17</sup> After meticulous supra- and subgingival SRP and microbiologic monitoring,<sup>18</sup> 24 patients (age range: 32 to 58 years) with LCP were included in the study (Table 1). All subjects had complete dentitions. We selected only those patients in whom *F. nucleatum* was detected 3 weeks after SRP. After randomization by drawing lots, 12 persons each were assigned to the test and control groups. Patients with systemic diseases, smokers, and patients who had received periodontal treatment with antibiotics in the previous 6 months were excluded from the study. All participants signed informed consent forms.

### Clinical Measurements

Clinical findings were taken on six sites of each tooth at the baseline visit (3 weeks after SRP) and after 1, 4,

**Table 1.**

### Basic Demographic Data in PDT and Control Groups

Demographic	PDT Group			Control Group		
	Female	Male	Total	Female	Male	Total
N	8	4	12	9	3	12
Mean age (years)	39.75	45.00	41.50	44.22	42.67	43.83
SD	10.33	12.06	10.69	8.21	5.03	7.36
Minimum	32	32	32	34	38	34
Maximum	55	58	58	58	48	58

and 12 weeks by an experienced masked periodontist (BWS) who was not involved in the treatment phase. The clinical findings recorded were the plaque index (PI),<sup>14</sup> presence and/or absence of BOP and redness, PD, gingival recession, and clinical attachment level (CAL) (measured using a 1-mm-graduated probe<sup>§</sup>).<sup>14,18-20</sup>

### Microbiologic Monitoring

At the baseline visit and the other clinical examination times, subgingival plaque was taken with endodontic paper tips (International Standard Organization [ISO] 30) from the deepest site per quadrant and subsequently pooled separately for the upper and lower jaw. At baseline, qualitative analysis with regard to the periodontopathogenic species *F. nucleatum*, *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*), *P. gingivalis*, *Tannerella forsythia* (previously *T. forsythensis*), and *Treponema denticola* was made by means of polymerase chain reaction (PCR).<sup>14</sup> The study exclusively included subjects in which only *F. nucleatum* was detected. To obtain a quantitative measure of the bacterial DNA concentration of *F. nucleatum* before and after PDT, we used the method of competitive PCR. A known concentration of a specific competitor DNA and a template of the DNA to be analyzed were simultaneously amplified in a PCR batch. Quantification was possible by means of step-by-step decadic dilution of the initial competitor DNA concentrations and the subsequent comparison of the bands in the agarose gel of the competitor and template amplificates.<sup>21,22</sup>

### PDT Procedure

The antimicrobial effect of the PDT method is based on the combination of a blue PS with laser light with

§ Hu-Friedy, Chicago, IL.

a 660-nm wavelength. We used a soft diode laser<sup>||</sup> and a phenothiazine chloride PS solution.<sup>¶</sup> A fiber-optic applicator<sup>#</sup> with a 0.6-mm diameter was used as a laser applicator to direct the laser light into the gingival crevice or the periodontal pocket. The power density measured at the surface of that laser applicator was ~60 mW/cm<sup>2</sup>.

In the course of the study, the PDT method was applied in one session as a one-stage full-mouth treatment on all periodontal sites of the test subjects with LCP (Table 2). The PS solution was applied by placing the cannula tip of the PS applicator to the bottom of the periodontal pocket and delivered continuously during the removal of the tip toward the coronal side, and the upper surface of the tongue was wetted with PS solution. After an action time of 1 minute, the excess was removed by careful rinsing of all sites with physiologic saline solution. Immediately thereafter, six sites of each tooth were irradiated. Each site was exposed to the laser light using the fiber-optic applicator<sup>\*\*</sup> for 10 seconds for a total of 1 minute per tooth. This was carried out as a full-mouth treatment that covered all teeth and the tongue. The tongue was irradiated in six segments, each for 10 seconds. Control subjects were also treated with the PS solution but without laser irradiation (Table 2).

### Statistical Analyses

The clinical indices of plaque, redness, BOP, PD, gingival recession, CAL, and DNA amount of *F. nucleatum* (maxillary and mandible) were calculated for each test and control subject, as were the median and interquartile range of each group. Global time effects were studied using the Friedman non-parametric analysis of variance. Paired comparisons of time effects were analyzed by the Wilcoxon signed-rank test with Bonferroni adjustment. The significance of the difference between the test and control groups was evaluated on the basis of the Mann-Whitney U test. Results were considered significant at  $P < 0.05$ . A software package was used for statistical calculations.<sup>††</sup>

**Table 2.**

### Number of Treated Sites in PDT and Control Groups

Statistical Parameters	Number of Sites 3.5 to 5.5 (mm)		Number of Sites >5.5 (mm)	
	PDT	Controls	PDT	Controls
Median	41.50	43.50	5.00	5.50
Interquartile range	6.50	9.50	5.75	7.75
Minimum	29	35	0	0
Maximum	48	50	9	11

### RESULTS

In patients with LCP who were infected with the periodontopathogenic species *F. nucleatum*, the PDT method led to a manifest reduction in the periodontal inflammation symptoms within the 3-month observation period.

The PI, which did not differ between the test and control groups at baseline, was found to be significantly reduced in both groups at all times after treatment (PDT procedure or sole application of the PS solution for the controls). Twelve weeks after the treatment, a significant difference between groups was detected (Fig. 1).

In the PDT group, a significant reduction of the redness-positive sites from 45% (baseline) to 3% (post-treatment) was observed after 12 weeks (Fig. 2). This post-therapy difference was also significant compared to the control group ( $P < 0.001$ ).

A similar good result with a significant difference from the baseline value and the control group was also observed for the BOP parameter. After 12 weeks, the test group showed a significant reduction of the mean bleeding score from 66% to 18%, whereas it increased from 68% to 72% in the control group (Fig. 3).

Regarding the mean PD and CAL, a significant reduction was ascertained in the test group during the observation period and with respect to controls (Fig. 4; Table 3). Twelve weeks after PDT, the mean PD of the deepest site per quadrant showed significant differences from the baseline value and from the control group (Fig. 5). As for gingival recession and CAL, no significant changes were detected 12 weeks after the treatment (Table 3).

Microbiologic monitoring revealed that PDT had a clearly detectable effect on the periodontopathogenic species *F. nucleatum*. As shown in Figure 6, the comparison of the *F. nucleatum* DNA quantities of the maxillary and mandible with the baseline value still revealed a significant reduction 12 weeks after PDT. The difference was also significant compared to the control group (Fig. 6).

### DISCUSSION

In this study, we proved that application of the PDT method resulted in a significant reduction of the clinical inflammatory signs in patients with LCP and *F. nucleatum* infection. Moreover, to our knowledge, this was the first clinical study that proved that PDT can reduce PD and significantly suppress *F. nucleatum* in cases of LCP.

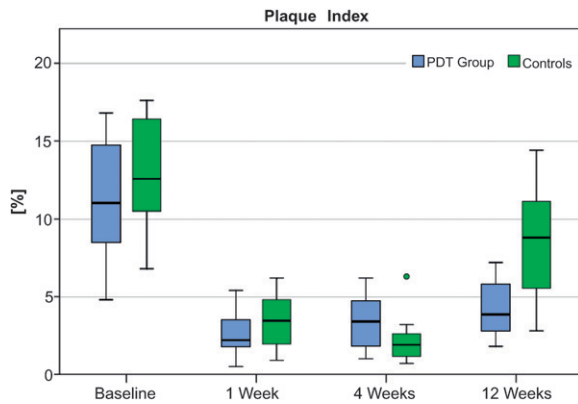
<sup>||</sup> Helbo-TheraLite Laser, Helbo Photodynamic Systems, Grieskirchen, Austria.

<sup>¶</sup> Helbo-Blue Photosensitizer, Helbo Photodynamic Systems.

<sup>#</sup> Helbo-3D Pocket Probe, Helbo Photodynamic Systems.

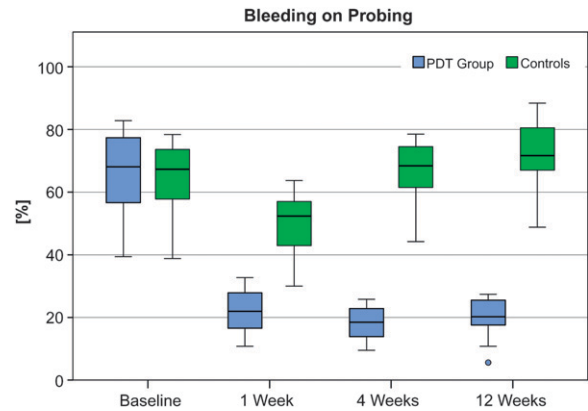
<sup>\*\*</sup> Helbo-3D Pocket Probe, Helbo Photodynamic Systems.

<sup>††</sup> SPSS 17.0 for Windows, SPSS, Chicago, IL.



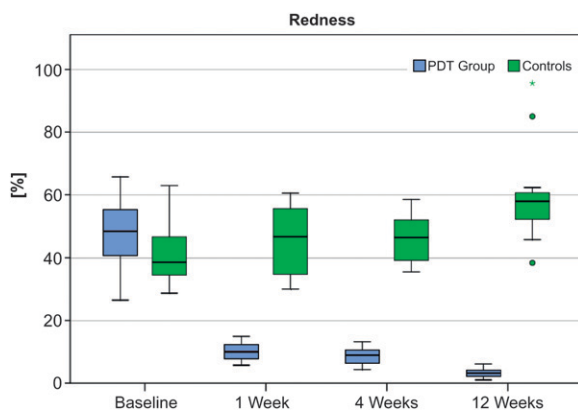
**Figure 1.**

PI in PDT and control groups from baseline to 12 weeks after treatment. There was a significant reduction in both groups at all times. Bonferroni-adjusted P values for the PDT versus control groups (Wilcoxon signed-rank test): 1 week:  $P = 0.001$  versus  $P = 0.001$ ; 4 weeks:  $P = 0.001$  versus  $P = 0.001$ ; and 12 weeks:  $P = 0.001$  versus  $P = 0.02$ . There was a significant difference between the PDT and control groups after 12 weeks ( $P = 0.003$ ; U test). In Figure 1, each box indicates the lower and the upper quartiles; the central line is the median; whiskers indicate 10% and 90% quartiles; and circles represent outliers.



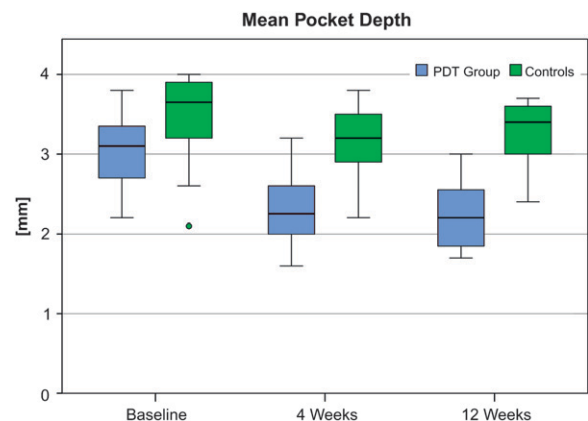
**Figure 3.**

BOP in the PDT and control groups from baseline to 12 weeks after treatment. There was a significant reduction in the PDT group at all times and in the control group at week 1 only. Bonferroni-adjusted P value for the PDT group at 1 week:  $P = 0.001$ , at 4 weeks:  $P = 0.001$ , and at 12 weeks:  $P = 0.001$ . Bonferroni-adjusted P value for the control group at 1 week:  $P = 0.001$ . There was a significant difference between PDT and control groups after 12 weeks:  $P < 0.001$ . In Figure 3, each box indicates the lower and the upper quartiles; the central line is the median; whiskers indicate 10% and 90% quartiles; and circles represent outliers.



**Figure 2.**

Redness in PDT and control groups from baseline to 12 weeks after treatment. There was a significant reduction in the PDT group after 12 weeks and a significant increase in the control group after 12 weeks. Bonferroni-adjusted P value for the PDT and control groups at 12 weeks:  $P = 0.001$ . There was a significant difference between PDT and control groups at all times:  $P < 0.001$ . In Figure 2, each box indicates the lower and the upper quartiles; the central line is the median; asterisk and whiskers indicate 10% and 90% quartiles; and circles represent outliers.



**Figure 4.**

Mean PD in the PDT and control groups from baseline to 12 weeks after treatment. There was a significant reduction in the PDT group at all times after PDT and after 4 weeks in the control group. Bonferroni-adjusted P value for the PDT group at 4 weeks:  $P = 0.002$  and at 12 weeks:  $P = 0.003$ . Bonferroni-adjusted P value for the control group at 4 weeks:  $P = 0.013$ . There was a significant difference between the PDT and control groups 12 weeks after baseline:  $P < 0.002$ . In Figure 4, each box indicates the lower and the upper quartiles; the central line is the median; whiskers indicate 10% and 90% quartiles; and circles represent outliers.

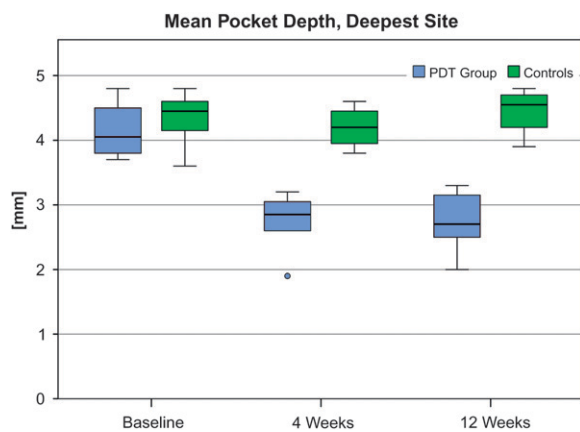
The microbiologic and clinical efficacy of PDT against periodontopathogenic species and periodontal inflammation *in vitro*<sup>11</sup> and in animal experiments<sup>14</sup> and in patients<sup>22</sup> was already observed in previous studies; de Oliveira et al.<sup>15</sup> and Braun et al.<sup>23</sup> also reported on the efficacy of PDT against the clinical inflammatory signs compared to scaling and root planing. To our knowledge, only one clinical study<sup>24</sup> of periodontitis patients included microbio-

logic monitoring, although different attempts were made in recent years to introduce antimicrobial PDT as an additional methodology for the efficient control of periodontal disease. Christodoulides et al.<sup>24</sup> reported that, in patients with chronic periodontitis (mean PD >5 mm), a single episode of PDT, based on the same procedure used in the present study, led to a significantly higher reduction in bleeding

**Table 3.****CAL and Gingival Recession in PDT and Control Groups Over the 12-Week Observation Time**

Visits	CAL (mm)				Gingival Recession (mm)			
	PDT Group		Control Group		PDT Group		Control Group	
	Median	Interquartile Range	Median	Interquartile Range	Median	Interquartile Range	Median	Interquartile Range
Baseline	3.40	0.58	3.75	0.52	0.20	0.00	0.20	0.10
4 weeks	2.55	0.52	3.40	0.67	0.15	0.18	0.25	0.18
12 weeks	2.45	0.68	3.55	0.55	0.20	0.18	0.20	0.10

There was a significant change in CAL in the PDT group after PDT versus the control group at 4 weeks ( $P < 0.002$ ; Mann-Whitney U test) and 12 weeks ( $P < 0.001$ ; Mann-Whitney U test).

**Figure 5.**

Mean PD of the deepest site per quadrant in the PDT and control groups from baseline to 12 weeks after treatment. There was a significant reduction in the PDT group 12 weeks after treatment. Bonferroni-adjusted P value at 12 weeks:  $P = 0.001$ . There was a significant difference between the PDT and control groups 12 weeks after baseline:  $P = < 0.001$ . In Figure 5, each box indicates the lower and the upper quartiles; the central line is the median; whiskers indicate 10% and 90% quartiles; and circles represent outliers.

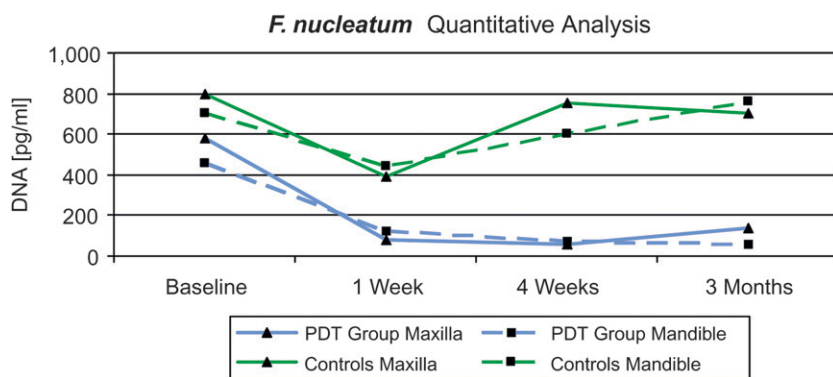
scores compared to SRP alone, although semiquantitative PCR did not reveal any statistically significant changes of the periodontopathogenic species.

In various studies<sup>11,14,25</sup> on PDT, PDT was capable of suppressing the periodontopathogenic species in vitro and in vivo. However, whether PDT as an adjuvant anti-infection method will be successful in the long run will have to be ascertained in controlled clinical studies. To our knowledge, the present study is one of the first to prove PDT efficacy in periodontitis patients. In addition to the clinically evident reduction of the redness and BOP parameters caused by phenothiazine-based photosensitization, the present study proved that PDT successfully suppressed the periodontopathogenic species *F. nucleatum* in patients with LCP.

The pathogenic potential of *F. nucleatum* and its significance in the development of periodontal diseases have gained great interest in recent years.<sup>18</sup> Shenker<sup>26</sup> proposed a model of immunologic dysfunction in the early stages of periodontitis. In this immunologic dysfunction and the subsequent persistence of the inflammation (a poor response to SRP), *F. nucleatum* may possibly play an important role. This positive antimicrobial effect was accompanied with a significant reduction in the mean PD and mean CAL throughout the 12-week observation period.

However, the initial results of a hitherto unpublished clinical and microbiologic study of generalized chronic periodontitis patients conducted by our team showed that the repeated application of phenothiazine-based PDT seemed to be necessary in more severe cases to ensure a clinical and microbiologic success. This might be the reason why a recently published review<sup>16</sup> of the effect of PDT in periodontitis cases found that PDT as an adjunct to SRP using phenothiazine-based PSs was not superior to SRP treatment alone. Apart from that, in the studies cited in the review,<sup>16</sup> appropriate microbiologic monitoring and exact patient classification were missing.

In the investigated LCP cases of the present study, we observed a significant reduction in the mean PD, mean CAL, and mean PD of the deepest site per quadrant compared to controls, even after a one-stage application of PDT. After 12 weeks, the mean PD of the deepest site per quadrant of the PDT group was  $2.7 \pm 0.5$  mm, whereas the mean PD of the deepest site per quadrant of the control group was  $4.3 \pm 0.7$  mm. The results of the PDT group were also statistically significant compared to baseline values. de Oliveira et al.<sup>15</sup> stated that the reduction of gingival recession in a PDT group may have been attributed to the atraumatic use of optic fibers. In the present study, no statistically significant influence of PDT on the gingival recession parameter is detected.



**Figure 6.**

*F. nucleatum* DNA concentration in the PDT group ( $n = 8$ ) and controls ( $n = 8$ ) over the 12-week observation time. There was a significant reduction in the DNA quantity of *F. nucleatum* in the PDT group 12 weeks after PDT compared to baseline levels:  $P < 0.001$ . There was a significant difference in the DNA quantity of *F. nucleatum* between the PDT and control groups 12 weeks after treatment:  $P = < 0.014$ .

Periodontitis is an infectious disease, and a current concept for treating periodontitis is primarily focused on eliminating the infection.<sup>2</sup> The management of periodontal disease includes many treatment modalities such as conventional therapies by surgery and/or non-surgical methods. Although several studies<sup>17,27-30</sup> considered SRP as the basic prerequisite for a long-term treatment success, the relevant literature lacks any definition of a sufficiently planed and bacterially reduced root surface from a clinical point of view. In particular, the complete removal of the bacterial biofilm by exclusively mechanical methods seems too insufficient. Therefore, it is interesting that PDT successfully suppressed periodontopathogenic species in animal experiments.<sup>10,12</sup> Given that periodontopathogenic bacteria can also invade the periodontal tissue and often (as with the subjects in the present study) are still evident after careful SRP, with clinically and still relatively high levels of redness and BOP, special treatment concepts combining mechanical and alternative anti-infection modalities are required. It is known, for example, that PSs are capable of penetrating through the epithelium and connective tissue.<sup>15</sup> Several authors recommend<sup>19,20,27,30</sup> the adjuvant application of antibiotics in addition to mechanical therapy in cases of severe periodontitis; adjuvant PDT could become clinically relevant at least in less severe cases of periodontitis.<sup>19,31</sup> It can be assumed that the reduction in the DNA quantity of *F. nucleatum* after PDT in the present study was closely associated with the distinctly reduced inflammatory signs and the decrease in mean PD and mean CAL because *F. nucleatum* is known to be one of those periodontopathogenic species associated with periodontitis, and especially with BOP.<sup>18</sup>

Previously, Sigusch et al.<sup>14</sup> reported the significant suppression of *F. nucleatum* and *P. gingivalis* by PDT in an animal experiment. After the infection of periodontally healthy animals and the establishment of a microbiologic steady state, PDT was carried out with various PSs. A significant reduction of *F. nucleatum* and *P. gingivalis* was made evident by culture methods and quantitative PCR.

On the basis of a case report of a patient with generalized chronic periodontitis, we demonstrated a distinct suppression of the bacterial DNA of *F. nucleatum*, *P. gingivalis*, *T. forsythia*, *T. denticola*, and *A. actinomycetemcomitans*, in addition to the reduction of clinical inflammatory signs and PDs after application of the PDT method on

three successive days.<sup>22</sup> In 2001, Dörtbudak et al.<sup>13</sup> reported the antimicrobial efficacy of PDT in peri-implantitis patients. The species *A. actinomycetemcomitans*, *P. gingivalis*, and *Prevotella intermedia* were distinctly suppressed on the implant surfaces.

Because the bacterial flora of periodontitis differs only slightly from that of peri-implantitis, there is a noticeable parallel in the therapeutic approach, i.e., antimicrobial PDT is employed as an adjuvant after mechanical therapy. This method is suitable for use in both diseases.<sup>27,32</sup> In the present study, we recruited patients with LCP in which SRP alone was not sufficient to reduce the clinical inflammatory symptoms and suppress *F. nucleatum*. But the adjuvant application of phenothiazine-based PDT as a full-mouth procedure led to a significant improvement of the clinical and microbiologic parameters after 12 weeks.

## CONCLUSION

The results of this study show that the adjuvant application of the PDT method described was appropriate to reduce periodontal inflammatory symptoms and to successfully treat infection with *F. nucleatum*.

## ACKNOWLEDGMENTS

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- Correspondence: Dr. Bernd W. Sigusch, Friedrich Schiller University of Jena, Department of Conservative Dentistry, University Hospital Jena, An der Alten Post 4 D-07740, Jena, Germany. Fax: 49-3641-934582; e-mail: bernd.w.sigusch@med.uni-jena.de.
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