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Indocyanine green-based adjunctive antimicrobial photodynamic therapy for treating chronic periodontitis: A randomized clinical trial

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ABSTRACT

Background: The objective was to evaluate the efficiency of indocyanine green (ICG)-based adjunctive antimicrobial photodynamic therapy (aPDT) in a prospective clinical study regarding non-surgical treatment of chronic periodontitis.

Methods: Affected teeth of twenty patients were treated with scaling and root planing (control group). Using a split-mouth design, two quadrants received additional ICG-based (perio green®, 0.1 mg/ml) aPDT (test group) with a diode laser at 808 nm (100 mW at 2 kHz). Clinical assessment of bleeding on probing (BOP), sulcus fluid flow rate (SFFR) and microbiological analysis were performed at baseline, two weeks, three and six months after treatment. Relative attachment level (RAL), probing depths (PD) and gingival recession (GR), were also analyzed.

Results: At baseline, none of the assessed parameters showed significant differences between the test and control groups. Median values for BOP, RAL, PD, decreased significantly in both groups after three months of treatment ($p \leq 0.05$) without significant difference between the groups. Two weeks after treatment, the SFFR showed significantly lower mean values in the test group (aPDT).

Conclusion: Within the study limits, the only significant difference between the control group and the aPDT group was a transient smaller amount of SFFR in the latter during the first follow-up. With the applied parameters, this study does not conclusively support ICG-based aPDT, though it is promising because no adverse effects occurred. The precise modes of action of ICG must be elucidated, and further clinical trials are needed.

1. Introduction

Periodontal diseases are caused by a microbial biofilm-induced inflammation of the connective tissue and alveolar-bone [1]. The main objective of periodontal treatment, apart from improving oral hygiene, is to remove the microbial biofilm, thereby eliminating periodontopathogenic bacteria from the periodontal pocket [2]. Even so, conventional methods such as sonic, ultrasonic, scaling and root planing (SRP) do not completely remove calculus and the bacterial biofilm from periodontal pockets [3]. Consequently, occasional non-surgical periodontal therapy does not relevantly improve clinical parameters such as bleeding on probing (BOP), sulcus fluid flow rate (SFFR), relative attachment level (RAL) and probing depths (PD). Moreover, risk factors such as diabetes, smoking or furcation involvement may cause adverse treatment outcomes [3,4]. To improve clinical results of periodontal therapy, systemic administration of antibiotics is a common method for periodontal indications [5,6]. However, stemming from demographic shifts, today dentistry is faced with an increasing number of geriatric patients suffering from systemic adverse effects by antibiotics and drug interactions through multimedication [7].

As a result of the overprescription and incorrect use of antibiotics in general medicine and dentistry, an increasing and sustained resistance of pathogenic microorganisms to antibiotics has emerged over the last years [8]. Promoting new and effective therapeutic treatment strategies is crucial to directly address this alarming problem of antibiotic resistance [9]. Laser-based antimicrobial photodynamic therapy (aPDT) could serve as an alternative to conventional antimicrobial agents in general medicine and dentistry and could help preserve the efficacy of antibiotics to combat life-threatening diseases [10].

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The antimicrobial effect of aPDT is based on particular interaction of a specific dye which attaches to the surface of microorganisms and the radiation emitted by a laser source. Due to the high absorption of radiation energy by the dye, this process ultimately leads to a functional disintegration of microorganisms [11]. Several dyes, also referred to as "photosensitizers" are firmly established in dentistry. The effects of aPDT are based, among others, on the transfer of energy by the photosensitizer to oxygen in the surrounding tissue, thereby generating singlet oxygen ($1O_2$) [11,12]. In addition to these effects, the absorption of laser light by the photosensitizer results in thermal and photobiostimulative reaction [13,14] - collectively referred to as photodynamic effects [15], which ultimately causes irreversible protein modification, cell membrane and mitochondria damage.

Several studies showed successful periodontal treatment without any side effects or systemic effects [16,17]. Irrespective of the photosensitizer applied, aPDT reveals temporary and locally limited effects. However, aPDT with phenothiazine-based dyes (such as methylene blue) exhibit, even without application of laser light, a slightly antimicrobial activity [18]. Recently, the photosensitizer indocyanine green (ICG) has been additionally used to treat periodontitis besides scaling and root planing [19]. Basic research indicated the antimicrobial effects of ICG-based aPDT on periodontopathogenic organisms [20].

Different antimicrobial effects on gram-positive and -negative microorganisms appear to be linked with the electric charge of the photosensitizer used [21]. Due to the cationic charge of ICG, the effect on gram-negative bacteria and the precise molecular mode of action are still controversial [22]. However, both kinds of photosensitizers - phenothiazine-based dyes in comparison with ICG – could reduce the viability of fungal cells [23].

Until now, few systemic clinical trials on ICG-based adjunctive periodontitis treatment have been performed [24,25]. Therefore, this current study aims to evaluate the ICG-based adjunctive aPDT compared to conventional non-surgical therapy to treat chronic periodontitis in a randomized clinical study.

2. Materials and methods

To ensure international ethical and scientific quality standards, the clinical trial was conducted in adherence with the guidelines of *the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and the Good Clinical Practice* (ICH-GCP), the Declaration of Helsinki and in line with regulations of the *Medicinal Products Act* (MPG) and of the *Federal Data Protection Act*. The study was approved by the *Ethics Committee of Bonn University* (reference number: 324/12).

2.1. Patients

Inclusion criteria were stipulated according to the *Ethics Committee of Bonn University*. The consulting biometrician supported a cohort number of at least 20 patients according to the results of a previous aPDT study with comparable clinical parameters [16]. Based on this study [16], we expected a distribution of the differences in the change of BOP (bleeding on probing) from baseline to 3 months between the differentially treated regions with a standard deviation of 10%. By assuming the data to be close enough to a normal distribution to apply a *t*-test (two-sided at 5%), this would result in a power of 80% to detect a difference of 6.6% in the reduction of BOP between the treatments. Taking into account the reduced efficiency of the intended nonparametric test, we expected to detect differences of at least 7.5% with a probability of 80%. Therefore, 20 medically healthy, non-smoking patients with chronic periodontal disease (PSI ≥ 3 in all quadrants) were recruited by the *Department of Periodontology, Operative and Preventive Dentistry at the Dental Faculty of Bonn University*. The evaluation of PSI (Periodontal Screening Index in Germany, corresponding to PSR®-Periodontal Screening & Recording) permitted an overview of the periodontal situation of the respective patient and the need for periodontal treatment [26,27].

The prerequisite for participation was the presence of at least one single and one multi-rooted tooth with at least 4 mm probing depth in each quadrant. Only well-compliant probands with good domestic oral hygiene were included (documented with a full mouth plaque index of ≤ 30% before starting the clinical trial). Exclusion criteria were probands with diseases or on medication with an inhibitory or promoting effect on periodontal healing including anticoagulants, anti-inflammatories and antibiotics within the last six months. Moreover, pregnant or nursing women and patients receiving periodontal therapy within the last six months or having existing allergies to the test product were excluded.

The given cohorts (17 female and 3 male test persons) were randomized by considering the inclusion and exclusion criteria. The mean age of the probands was 61.1 years. Hereby the weighting of the gender distribution in the study population was waived, because up to now, there were no indications of various gender-specific efficacies of the test product [28,29]. Furthermore, during the follow-up, no test person dropped out of the study.

2.2. Clinical parameters

The clinical condition of the test persons was quantitatively evaluated using parameters that reflect the whole periodontal state: bleeding on probing (BOP – primary outcome variable of the study) [30], sulcus fluid flow rate (SFFR) [31], periodontal-status with probing depths (PD), gingival recession (GR) and relative attachment level (RAL) [32,33]. Furthermore, quantitative molecular biological determination by real-time chain reaction (RTD-PCR) of following periodontal pathogenic microorganisms was conducted: *Aggregatibacter actinomycetemcomitans* (A.a.), *Porphyromonas gingivalis* (P.g.), *Prevotella intermedia* (P.i.), *Tannerella forsythia* (T.f.) *and Treponema denticola* (T.d.).

After relative drying, the sampling with sterile paper tips (ISO 40) was conducted for a period of respectively 30 s avoiding provoking bleeding. The sampled paper tips were placed quadrant-wise as pooled samples into microreaction tubes. A blinded investigator performed the quantification in a certified laboratory (*Dr. Bauermeister & Co., Moers, Germany*).

The parameters bleeding on probing (BOP), sulcus fluid flow rate (SFFR) and microbial analysis were clinically assessed at baseline (BL), two weeks (U_1), three months (U_2) and six months (U_3) after treatment. The SFFR and microbiological tests were examined on single and multi-rooted teeth with the highest PD in each quadrant.

The relative attachment level (RAL) with probing depths (PD) and gingival recession (GR) were measured at baseline, and three months and six months after treatment. Intraoral photo documentation as well as control examinations were made at baseline, and after three and six months of follow-up therapy, respectively.

With a pressure-calibrated probe (*Click-probe®, Kerr GmbH, Bioggio, Switzerland*) periodontal status was evaluated as well as bleeding on six sites per tooth, 30 s following probing [34]. The SFFR and microbiological pooled sampling were performed in periodontal pockets with maximum initial probing depths. After relative isolation of teeth, the sulcus fluid was collected for 30 s by using PerioPaper Strips® (*Oraflow Inc., New York, USA*). The SFFR was assessed with Periotron 8000® (*Oraflow Inc., New York, USA*) [35].

2.3. Treatment procedures

The study was based on a split-mouth design. Each participant received standard therapy and adjunctive aPDT. The patients received pre-treatment with professional tooth cleaning and precise instructions to ensure domestic oral hygiene. Plaque-revelators were used for

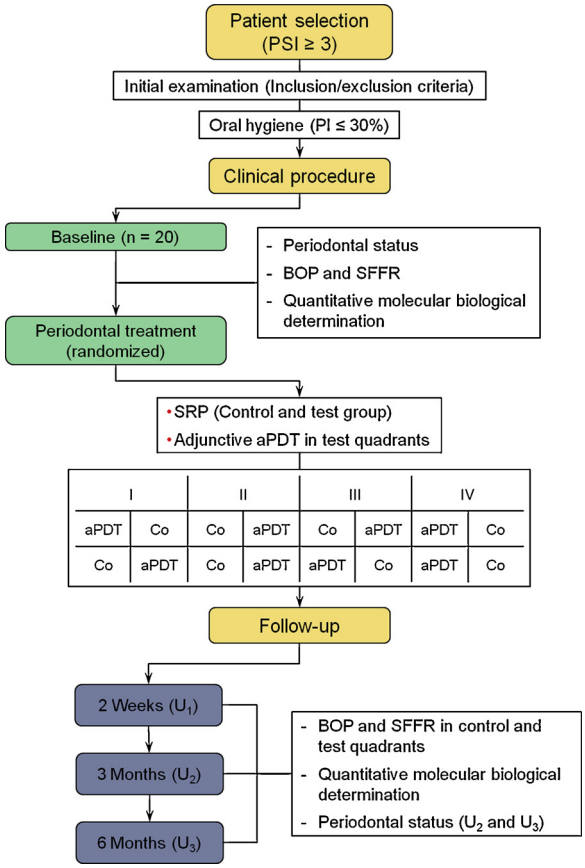

Fig. 1. Study design in accordance to the split-mouth model with random allocation to groups I–IV (control group with standard therapy (Co) and test group with adjunctive aPDT) for the observation period of 6 months following therapy. Examinations were carried out at baseline, after 2 weeks (U_1), 3 months (U_2) and 6 months (U_3). Investigated variables were bleeding on probing (BOP), sulcus fluid flow rate (SFFR), probing depth, gingival recession, clinical attachment level and quantitative molecular biological determination.

verification.

Affected teeth were manually treated with conventional scaling and root planing (SRP) (*Gracey-curettes, Hu-Friedy Mfg. B.V., Rotterdam, Netherlands*) and additionally with a piezoelectric ultrasonic system (*Sirosonic L, Sirona, Bensheim, Germany*).

After this non-surgical periodontal treatment, patients were randomly allocated into four different groups according to split-mouth design. Treatment procedures differed per quadrant in the respective groups (Fig. 1). Two quadrants received an additional ICG-based aPDT (test group), further quadrants were treated with SRP alone as control group (Co). After conventional periodontal treatment, this random allocation to different groups was carried out by a computer-generated block randomization using a randomization box. The real aPDT procedure was completely performed by a single operator in the selected quadrants.

The photosensitizer system perio green® (*elexxion AG, Radolfzell, Germany*) in combination with a diode laser (*elexxion claros pico®, elexxion AG, Radolfzell, Germany*) at 808 nm was used to perform aPDT. As advised by the manufacturer, one pill of perio green® (containing of 0.2 mg ICG) with sterile water was dissolved by carefully tilting. Ready-to-use solution had a concentration of 0.1 mg/ml ICG (Fig. 2b). The total administered dose depended on the periodontal pockets to be treated.

After the ICG-suspension was created, a syringe (Fig. 2c) was used to drip the liquid into the periodontal pockets of the test-quadrants. Excess photosensitizer was rinsed out with water after an exposure time of 60 s. Irradiation with diode laser (Fig. 2d) was executed utilizing an intrasulcular fiber (∅ = 300 µm) for 20 s with an average output power of 100 mW and a pulse repetition rate of 2 kHz. Thus, the applied irradiation dose was 2829 J/cm^2 per periodontal pocket.

Referring to the approval of the *Ethics Committee* to minimize the risk of adverse effects, only a single application was performed in this study.

2.4. Statistical analysis and data verification

Statistical analysis was performed in collaboration with the *Institute for Medical Biometry, Informatics and Epidemiology at Bonn University*. The analysis was conducted with IBM SPSS Statistics® (*version 22, IBM, New York, USA*).

The statistical unit of the study was based on the site-specific data of the investigated parameters. Since normal distribution was not assumed, a non-parametric test for paired samples was used. The data were evaluated using the Wilcoxon-Test. Distinction was drawn between test and control group. Furthermore, differences within the treatment modalities were obtained for control examinations and, additionally, for the course of therapy. Differences were considered as statistically significant at a value of $p \leq 0.05$.

For quality assurance and GCP-compliant trial documentation, data collection was confirmed by a monitoring committee. Collected data were reviewed with visual record verification and 30% double data entry [36].

3. Results

At baseline (BL), no significant differences in any parameter values were observed between treatment modalities of the control and test group ($p > 0.05$). Furthermore, in neither case were there particular indications for side effects upon using indocyanine green in this clinical study.

3.1. Bleeding on probing (BOP)

Two weeks (U_1) after treatment, values for BOP decreased significantly in the test and control groups (Table 1). At U_1, a slightly favorable tendency towards the test group (aPDT) could be observed ($p = 0.136$). This short-term effect in favor of the test group equalized at the evaluation after three months. Corresponding p-values are given in Table 1 and shown in Fig. 3. Three months (U_2) and six months (U_3) after treatment, a significant decrease in BOP ($p \leq 0.05$) relative to the baseline values was detected with no differences between treatment modalities U_1/U_3 and U_2/U_3 ($p > 0.05$ in Table 1). In general, a correlation between BOP and PD at baseline could be observed in both the test group and control group (Table 2).

3.2. Sulcus fluid flow rate (SFFR)

Values for SFFR decreased significantly two weeks (U_1), three months (U_2) and six months (U_3) after treatment relative to baseline (BL) ($p \leq 0.05$, Table 1). Thereby a significant difference between treatment modalities at U_1 with lower values in the test group (aPDT) was revealed ($p \leq 0.05$, Fig. 4). Upon taking the whole period into consideration, the difference between treatment groups was a short-term effect. Regarding the values of U_2 and U_3, the differences between treatment modalities did not remain. However, the significant decrease in SFFR persisted in both groups at all follow-up-examinations (Fig. 4).

3.3. Probing depths (PD), gingival recession (GR) and relative attachment level (RAL)

Three months (U_2) and six (U_3) months after therapy, both treatment modalities resulted in a significant reduction in probing depths

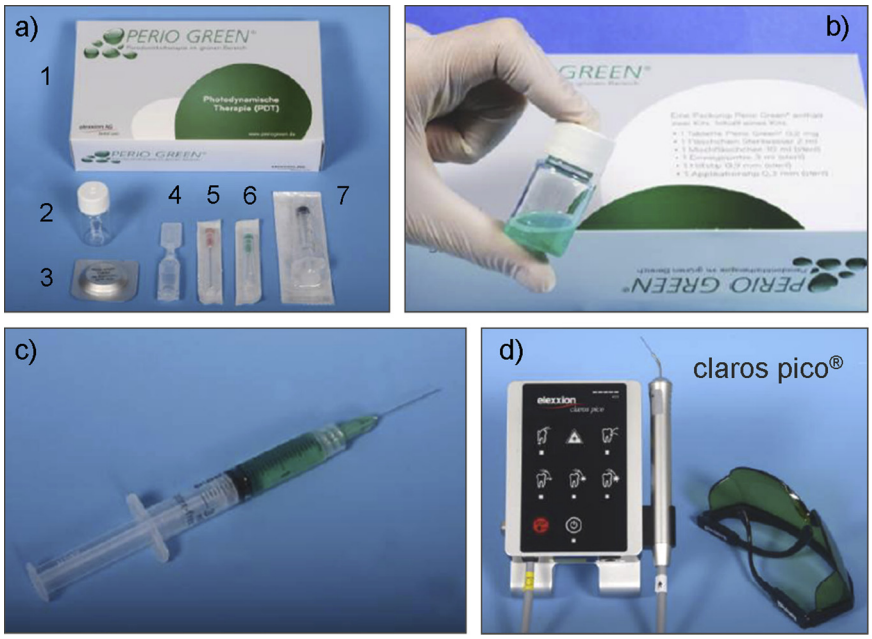

Fig. 2. Treatment equipment for aPDT. a) shows the used product *perio green*®: 1) Product packaging, 2) Mixing bottle, 3) ICG tablet 0.2 mg (blistered), 4) 2 ml water (sterile), 5) Auxiliary tip 0.9 mm (sterile) 6) Application tip 0.3 mm (sterile), 7) 3 ml syringe (sterile); b) shows the ready-to use solution (photosensitizer) in mixing bottle; c) Photosensitizer ready-made for the application inside the syringe; d) diode laser used for aPDT treatment.

relative to baseline values ($p \leq 0.05$, Fig. 5).

The gingival recession values increased after periodontal treatment in both groups significantly ($p \leq 0.05$). In terms of successful therapy, loss of clinical attachment decreased significantly regarding baseline values compared to values of U_2 and U_3 ($p \leq 0.05$).

Neither for probing depths nor gingival recession nor clinical attachment levels were significant differences between treatment modalities observed ($p > 0.05$). A more detailed analysis of the relative attachment level (RAL) depending on the probing depth (PD) at baseline showed an increased gain of attachment in deeper pockets (PD > 6 mm) after treatment in both groups. In the test group this effect was more pronounced than in the control group (Table 3).

3.4. Quantitative analysis of periodontal pathogenic microorganisms

Baseline (BL) content of A.a. was below the levels detectable by methods of analysis. During the observation period, no differences between control (Co) and test group (aPDT) could be found ($p > 0.05$). After a period of six months (U_3), significant effects for P.g. in both treatment modalities ($p \leq 0.05$) were detected in relation to baseline. Following aPDT, there was a significant reduction in P.i. and T.d. compared to control group. Detailed information are given in the Supplementary Data.

4. Discussion

Several studies revealed that especially the combination of SRP and systemically administered antibiotics result in a positive clinical outcome of periodontal treatment. The induced disintegration of microbial

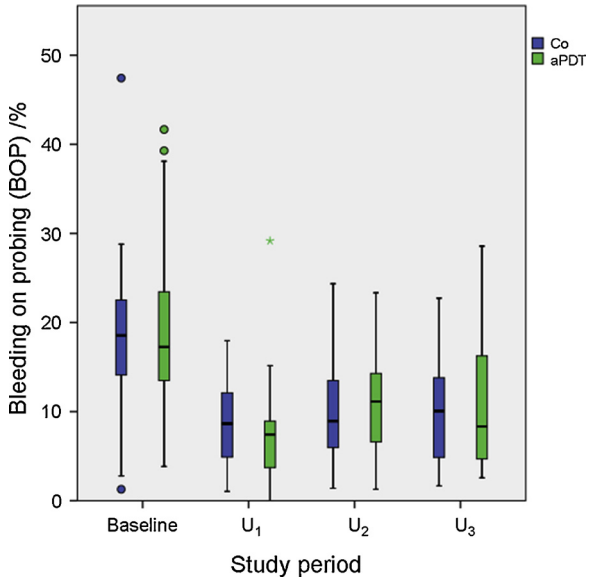

Fig. 3. Temporal development of bleeding on probing (BOP) in control (Co) and test group (aPDT) at baseline, two weeks (U_1), three (U_2) and six months (U_3) following therapy. At baseline no significant differences have been observed between the treatment arms of control and test group ($p > 0.05$). Three (U_2) and six (U_3) months after therapy both treatment modalities resulted in a significant reduction of BOP ($p \leq 0.05$), given in Table 1.

Table 1
Results of the statistical evaluation (Wilcoxon-Test) for bleeding on probing (BOP) and for sulcus fluid flow rate (SFFR). Regarding to the baseline (BL) a statistical significant reduction of the BOP value could be observed. Between 2 weeks (U_1) and 3 months (U_2) as well as 6 months (U_3) slightly favourable tendencies for the control and aPDT are noticeable. For SFFR in almost all time periods a significant reduction was given. Only between 2 weeks (U_1) and 3 months (U_2) compared to aPDT a strong tendency for the control was observed.

		BL/U_1	BL/U_2	BL/U_3	U_1/U_2	U_1/U_3	U_2/U_3
BOP	Control	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p = 0.153$	$p = 0.145$	$p = 0.970$
	aPDT	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p = 0.076$	$p = 0.904$
SFFR	Control	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p = 0.055$	$p \leq 0.05$	$p \leq 0.05$
	aPDT	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p = 0.796$	$p \leq 0.05$	$p \leq 0.05$

Table 2
Bleeding on probing BOP before and after treatment in correlation to the probing depth (PD) at baseline (BL) of control and test group. A decrease of BOP after treatment was observed in the control and test group within the observation period. The values depend on the extend of the probing depth at baseline.

	Probing depth (PD)	Number of sites	BOB (BL)	BOP (U_1)	BOP (U_2)	BOP (U_3)
Control group	PD (1-3 mm)	1172	13.0%	5.3%	8.6%	7.9%
	PD (4-6 mm)	379	35.0%	16.3%	14.5%	17.0%
	PD (> 6 mm)	21	47.6%	42.9%	33.3%	28.6%
Test group	PD (1-3 mm)	1160	12.2%	4.1%	6.4%	6.9%
	PD (4-6 mm)	385	39.0%	14.1%	19.6%	19.4%
	PD (> 6 mm)	33	73.5%	50.0%	57.1%	66.7%

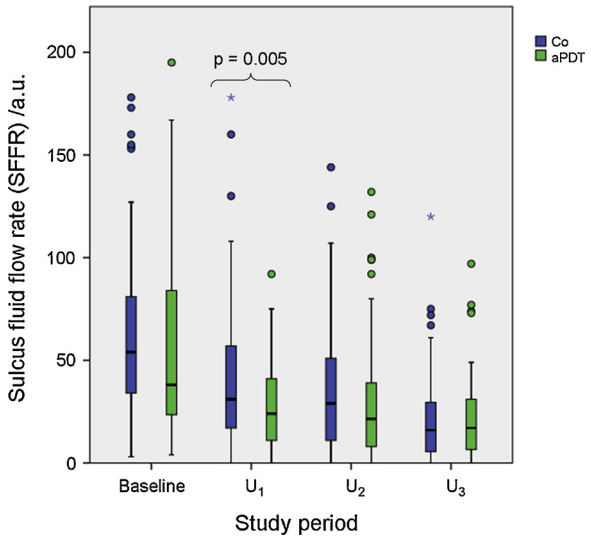

Fig. 4. Development of sulcus fluid flow rate (SFFR) in control (Co) and test group (aPDT) at baseline (BL), two weeks (U_1), three (U_2) and six months (U_3) following therapy. At baseline, no significant differences have been observed between the treatment arms of control and test group ($p > 0.05$). Values for SFFR decreased significantly two weeks after treatment (BL/U_1, $p \leq 0.05$). Thereby, significant differences between treatment modalities with lower value in test group were revealed at U_1 ($p \leq 0.05$). Taking the whole period into consideration, values decreased significantly irrespective of treatment modality ($p \leq 0.05$, Table 1).
a.u. scores: Volumes of the sulcus fluid are determined by the measurement of the electrical capacitance of wet PerioPaper Strips® placed between the jaws of the used Periotron 8000®. Acquisition time for sulcus fluid was 30 s. Scores obtained from the instrument are without a physical unit [35].

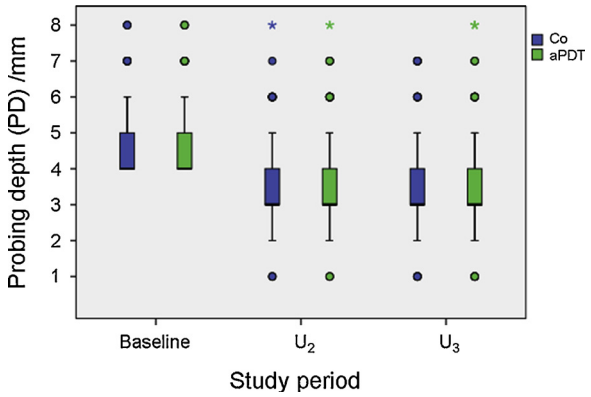

Fig. 5. Temporal development of probing depths (PD ≥ 4 mm) in control (Co) and test group (aPDT) at baseline, three (U_2) and six months (U_3) following therapy. At baseline no significant differences have been observed between treatment arms of control and test group ($p > 0.05$). Three (U_2) and six (U_3) months after therapy both treatment modalities resulted in significantly reduction of baseline probing depths ($p \leq 0.05$). No significant differences between treatment modalities occurred ($p > 0.05$).

biofilm thereby leads to an improvement in effectivity [37]. Since antimicrobial resistance has reached alarming levels, alternative and effective therapeutic treatment strategies are urgently required [9,38] for preserving efficacy of antibiotics in life-threatening diseases [10]. Reviewing the relevant literature regarding aPDT, it was shown that even if at its current stage, aPDT cannot replace antibiotics yet, even though it may be an effective adjunctive antimicrobial treatment option [10,17].

Indocyanine green which has already been proven and tested for years in clinical diagnostic and therapy in human medicine [39,40], has now been developed as a photosensitizer in dentistry. Due to rare systemic adverse effects and negligible toxicity, ICG is considered to be safe, and it does not bear the disadvantages of antibiotics [10,41]. Owing to less coloration of oral structures compared to phenothiazine dyes (such as methylene blue), ICG-based photosensitizers inherently help improve a patient's comfort [20].

Periodontal pockets with increasing probing depths enable the transition to a more anaerobic environment. Analysis of elemental composition determined a significant increase in calcium content under anaerobic conditions [42]. The presence of bivalent cations results in a reduced intracellular uptake of the photosensitizer methylene blue by bacterial cells. In contrast, bivalent cations promote an increase in absorption of ICG for both gram-positive and gram-negative bacteria [42]. Hence, because of the high mineral content in deep periodontal pockets, the applicability of methylene blue-based aPDT could be limited; by contrast, applying an anionic photosensitizer such as indocyanine green could be indicated instead.

Nevertheless, the effects of ICG on gram-negative bacteria, which are associated with deep periodontal pockets, are still a point of contention and underscore the fact that the research of precise modes of action of ICG is indispensable [20,38]. Many studies have investigated the antimicrobial effects and several modes of action by ICG-based aPDT. Critical points concerning the direct comparison between basic *in vitro* research, general medicine and dental application are the use of planktonic bacteria as well as delivery methods, laser parameters and concentrations of the photosensitizer [43,44]. Apparently, there is a shift from exclusively photothermal and photo-oxidative effects (generation of singlet oxygen) to those progressing simultaneously [45]. Photo-induced reactions and bactericidal effects seem to be a function of dye concentration and physical parameters such as applied light dose [46,47].

The present prospective clinical trial was one of the few clinical studies of ICG-based aPDT in non-surgical periodontal treatment. Latest clinical and *in vitro* studies [48,49] support the positive effect of adjunctive ICG-based aPDT. However, different ICG concentrations and laser parameters were applied in these studies, whereby higher ICG concentrations and laser powers of more than 100 mW, as we applied in the present study, could be advantageous [20]. Furthermore, certain additives like vitamins could enhance the bactericidal effect on periodontopathogenic microorganisms [49].

Although studies indicate effects of aPDT on bacteria organized in biofilms [50], they exhibited lower susceptibility in contrast to planktonic bacteria. Against this background and supplemented by several clinical trials, in the current study aPDT was applied using a split-mouth

Table 3
Relative attachment level (RAL) before and after treatment in correlation to the probing depth (PD) at baseline (BL) of control and test group (mean values and standard deviations (SD) are given in mm). A decrease of RAL and PD after treatment was observed in the control and test group within the observation period (GR: Gingival recession).

	Probing depth (PD)	Number of sites	PD (BL)	GR (BL)	RAL (BL)	PD (U_2)	GR (U_2)	RAL (U_2)	PD (U_3)	GR (U_3)	RAL (U_3)
Control group	PD (1-3 mm) SD	1172	2.15 0.74	1.45 1.07	3.61 1.28	2.08 0.79	1.71 1.10	3.78 1.31	2.14 0.80	1.62 1.12	3.76 1.33
	PD (4-6 mm) SD	379	4.34 0.64	1.38 1.18	5.72 1.36	3.32 1.06	1.72 1.19	5.03 1.61	3.18 1.04	1.59 1.14	4.77 1.53
	PD (> 6 mm) SD	21	8.05 1.69	1.90 1.30	9.95 2.20	6.71 2.10	2.33 1.02	9.05 2.52	6.67 1.80	1.95 1.32	8.62 2.65
Test group	PD (1-3 mm) SD	1160	2.17 0.72	1.59 1.19	3.76 1.32	2.04 0.75	1.75 1.13	3.79 1.33	2.10 0.73	1.71 1.19	3.81 1.35
	PD (4-6 mm) SD	385	4.38 0.63	1.49 1.22	5.86 1.42	3.32 1.09	1.83 1.18	5.14 1.72	3.22 1.09	1.71 1.20	4.93 1.74
	PD (> 6 mm) SD	33	8.68 1.57	2.53 2.09	11.21 2.73	6.95 2.18	2.71 1.82	9.67 2.97	6.48 1.99	2.43 1.72	8.90 2.76

design adjunctive to non-surgical periodontal treatment [16,51].

In terms of a successful non-surgical therapy, a regression in periodontal inflammation, reduction in probing depths and gain in clinical attachment level was documented in all groups. This was shown in a positive development for investigated parameters SFFR, BOP, PD, GR and RAL for either standard therapy as well as adjunctive aPDT. The results of the present trial concur with corresponding literature values [52].

The clinical parameters BOP and SFFR represent signs of inflammation and markers for the quantity of microbial biofilm [53,54]. With respect to the examined development of SFFR, there is a statistically significant difference between adjunctive aPDT (test group) and standard therapy (control) two weeks after the single application of ICG-based aPDT.

As a result, the reduction in periodontal pathogenic microorganisms only showed minor changes and revealed only slight differences between the treatment modalities. These results suggest that the applied power and concentration as well as irradiation time and repetitions of treatment applications did not suffice to efficiently reduce microbial counts [43]. In general, the evaluated parameters show high standard deviations. This could imply that by raising the sample size, tendencies could be statistically determined.

Corresponding to related studies, the present clinical trial reveals a temporary improvement in inflammation indicated only by SFFR [16,55]. Previous studies using phenothiazine-based dyes have emphasized advantages of repeated applications [56], whereas single treatments with ICG-based aPDT do not result in a persistent improvement [57]. Especially treatments of moderate to severe inflammatory forms of acne vulgaris lead to an antiphlogistic impact [29].

The clinical studies of ICG-based aPDT in periodontitis support these observations. Monzavi et al. reported significant improvements in bleeding on probing (BOP) as well as in probing depth (PD), by repeating the procedure thrice. Moreover, they used a higher power setting of 200 mW in CW mode and a concentration of 1 mg/ml ICG, promoting the thermal effects [24,46]. Just like in the current study, no additional advantages in the clinical attachment gain could be observed [24]. Most likely, repetitive application would not be harmful, because no adverse effects could be observed in all available studies including the current trial. Concerning clinical parameters, the results of Shingnapurkar et al. surpasses the results of the current study in reducing probing depth (PD) more effectively and showing a significantly better outcome with regard to the relative attachment level (RAL) [25]. These improved clinical outcomes can be related to the particularly high concentration of ICG (5 mg/ml). The comparison of the studies highlights the fact that the choice of parameters (laser output power, photosensitizer concentration, repetitive applications) can relevantly affect the results of clinical studies. It becomes clear here that an evidence-based clinical protocol cannot be derived from the currently available results. Consequently, further research to identify the precise modes of action of ICG and further clinical prospective trials are indispensable.

Conflict of interest

All authors declare to have no conflict of interest.

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Ethical approval

To ensure international ethical and scientific quality standards, the clinical trial was conducted in adherence with the guidelines of *the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and the Good Clinical Practice* (ICH-GCP), the Declaration of Helsinki and in line with German regulations of the *Medicinal Products Act* (MPG) and of the *Federal Data Protection Act*. The study was approved by the *Ethics Committee of Bonn University* (reference number: 324/12).

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pdpdt.2019.02.019.

References

[1] A. Cekici, A. Kantarci, H. Hasturk, T.E. Van Dyke, Inflammatory and immune pathways in the pathogenesis of periodontal disease, Periodontol. 2000 64 (1) (2014) 57–80, https://doi.org/10.1111/prd.12002.
[2] P.D. Marsh, D.J. Bradshaw, Dental plaque as a biofilm, J. Ind. Microbiol. 15 (3) (1995) 169–175.
[3] P.A. Adriaens, L.M. Adriaens, Effects of nonsurgical periodontal therapy on hard and soft tissues, Periodontol. 2000 36 (1) (2004) 121–145, https://doi.org/10.1111/j.1600-0757.2004.03676.x.
[4] S.G. Grossi, F.B. Skrepcinski, T. DeCaro, J.J. Zambon, D. Cummins, R.J. Genco, Response to periodontal therapy in diabetics and smokers, J. Periodontol. 67 (Suppl. 10) (1996) 1094–1102, https://doi.org/10.1902/jop.1996.67.10s.1094.
[5] American Academy of Periodontology, Parameter on aggressive periodontitis, J. Periodontol. 71 (5 Suppl) (2000) 867–869, https://doi.org/10.1902/jop.2000.71.5-S.867.

[6] American Academy of Periodontology, Treatment of plaque-induced gingivitis, chronic periodontitis, and other clinical conditions, J. Periodontol. 72 (12) (2001) 1790–1800, https://doi.org/10.1902/jop.2001.72.12.1790.

[7] K. Johnell, J. Fastbom, Multi-dose drug dispensing and inappropriate drug use: a nationwide register-based study of over 700,000 elderly, Scand. J. Prim. Health Care 26 (2) (2008) 86–91, https://doi.org/10.1080/02813430802022196.

[8] A.J. van Winkelhoff, E.G. Winkel, Microbiological diagnostics in periodontics: biological significance and clinical validity, Periodontol. 2000 39 (1) (2005) 40–52, https://doi.org/10.1111/j.1600-0757.2005.00116.x.

[9] G.M. Rossolini, E. Mantengoli, Antimicrobial resistance in Europe and its potential impact on empirical therapy, Clin. Microbiol. Infect. 14 (Suppl. 6) (2008) 2–8, https://doi.org/10.1111/j.1469-0691.2008.02126.x.

[10] T. Maisch, S. Hackbarth, J. Regensburger, A. Felgenträger, W. Bäumler, M. Landthaler, B. Röder, Photodynamic inactivation of multi-resistant bacteria (PIB) – a new approach to treat superficial infections in the 21st century, J. Dermatol. Ges. 9 (5) (2011) 360–366, https://doi.org/10.1111/j.1610-0387.2010.07577.x.

[11] C. Schweitzer, R. Schmidt, Physical mechanisms of generation and deactivation of singlet oxygen, Chem. Rev. 103 (5) (2003) 1685–1757, https://doi.org/10.1021/cr010371d.

[12] F. Cieplik, L. Tabenski, W. Buchalla, T. Maisch, Antimicrobial photodynamic therapy for inactivation of biofilms formed by oral key pathogens, Front. Microbiol. 12 (5) (2014) 405, https://doi.org/10.3389/fmicb.2014.00405.

[13] A. Klein, W. Baumler, M. Koller, G. Shafirstein, E.A. Kohl, M. Landthaler, P. Babilas, Indocyanine green-augmented diode laser therapy of telangiectatic leg veins: a randomized controlled proof-of-concept trial, Lasers Surg. Med. 44 (5) (2012) 369–376, https://doi.org/10.1002/lsm.22022.

[14] T. Qadri, L. Miranda, J. Tuner, A. Gustafsson, The short-term effects of low-level lasers as adjunct therapy in the treatment of periodontal inflammation, J. Clin. Periodontol. 32 (7) (2005) 714–719, https://doi.org/10.1111/j.1600-051X.2005.00749.x.

[15] J. Meister, G. Hill, C. Dehn, M. Frentzen, M. Hopp, Die Photodynamik in der dentalen Anwendung - Definition und Wegweiser, Laser Journal 17 (3) (2014) 14–16 [German].

[16] A. Braun, C. Dehn, F. Krause, S. Jepsen, Short-term clinical effects of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial, J. Clin. Periodontol. 35 (10) (2008) 877–884, https://doi.org/10.1111/j.1600-051X.2008.01303.x.

[17] H. Gursoy, C. Ozcakir-Tomruk, J. Tanalp, S. Yilmaz, Photodynamic therapy in dentistry: a literature review, Clin. Oral Investig. 17 (4) (2013) 1113–1125, https://doi.org/10.1007/s00784-012-0845-7.

[18] M. Wilson, T. Burns, J. Pratten, G.J. Pearson, Bacteria in supragingival plaque samples can be killed by low-power laser light in the presence of a photosensitizer, J. Appl. Microbiol. 78 (5) (1995) 569–574, https://doi.org/10.1111/j.1365-2672.1995.tb03101.x.

[19] S. Parker, The use of diffuse laser photonic energy and indocyanine green photosensitiser as an adjunct to periodontal therapy, Br. Dent. J. 215 (4) (2013) 167–171, https://doi.org/10.1038/sj.bdj.2013.790.

[20] T.K. Boehm, S.G. Ciancio, Diode laser activated indocyanine green selectively kills bacteria, J. Int. Acad. Periodontol. 13 (2) (2011) 58–63.

[21] K. Konopka, T. Goslinski, Photodynamic therapy in dentistry, J. Dent. Res. 86 (8) (2007) 694–707, https://doi.org/10.1177/154405910708600803.

[22] S. George, M.R. Hamblin, A. Kishen, Uptake pathways of anionic and cationic photosensitizers into bacteria, Photochem. Photobiol. Sci. 8 (6) (2009) 788–795, https://doi.org/10.1039/b809624d.

[23] V.G. Barghi, R. Fekrazad, A.P.B. Mir, M.S. Ghahfarokhi, In vitro photodynamic inactivation of Candida albicans by phenothiazine dye (New methylene blue) and Indocyanine green (EmunDo®), Photodiagnosis Photodyn. Ther. 12 (1) (2015) 52–57, https://doi.org/10.1016/j.pdpdt.2014.12.006.

[24] A. Monzavi, Z. Chinipardaz, M. Mousavi, R. Fekrazad, N. Moslemi, A. Azaripour, O. Bagherpasand, N. Chiniforush, Antimicrobial photodynamic therapy using diode laser activated indocyanine green as an adjunct in the treatment of chronic periodontitis: a randomized clinical trial, Photodiagnosis Photodyn. Ther. 14 (2016) 93–97, https://doi.org/10.1016/j.pdpdt.2016.02.007.

[25] S.H. Shingnapurkar, D.K. Mitra, M.S. Kadav, R.A. Shah, S.V. Rodrigues, S.S. Prithyani, The effect of indocyanine green-mediated photodynamic therapy as an adjunct to scaling and root planing in the treatment of chronic periodontitis: a comparative split-mouth randomized clinical trial, Indian J. Dent. Res. 27 (6) (2016) 609–617, https://doi.org/10.4103/0970-9290.199598.

[26] D. Ziebolz, A. Priegnitz, G. Hasenfuss, H.J. Helms, E. Hornecker, R.F. Mausberg, Oral health status of patients with acute coronary syndrome–a case control study, BMC Oral Health 12 (17) (2012), https://doi.org/10.1186/1472-6831-12-17.

[27] R.G. Landry, M. Jean, Periodontal Screening and Recording (PSR) Index: precursors, utility and limitations in a clinical setting, Int. Dent. J. 52 (1) (2002) 35–40.

[28] M.S. Fineman, J.I. Maguire, S.W. Fineman, W.E. Benson, Safety of indocyanine green angiography during pregnancy: a survey of the retina, macula, and vitreous societies, Arch. Ophthalmol. 119 (3) (2001) 353–355, https://doi.org/10.1001/archopht.119.3.353.

[29] V.V. Tuchin, E.A. Genina, A.N. Bashkatov, G.V. Simonenko, O.D. Odoevskaya, G.B. Altshuler, A pilot study of ICG laser therapy of acne vulgaris: photodynamic and photothermolysis treatment, Lasers Surg. Med. 33 (5) (2003) 296–310, https://doi.org/10.1002/lsm.10211.

[30] N.P. Lang, M.A. Schätzle, H. Löe, Gingivitis as a risk factor in periodontal disease, J. Clin. Periodontol. 36 (Suppl 10) (2009) 3–8, https://doi.org/10.1111/j.1600-051X.2009.01415.x.

[31] J.M. Goodson, Gingival crevice fluid flow, Periodontol. 2000 31 (1) (2003) 43–54, https://doi.org/10.1034/j.1600-0757.2003.03104.x.

[32] A. Haffajee, S. Socransky, J. Lindhe, R. Kent, H. Okamoto, T. Yoneyama, Clinical risk indicators for periodontal attachment loss, J. Clin. Periodontol. 18 (2) (1991) 117–125, https://doi.org/10.1111/j.1600-051X.1991.tb01700.x.

[33] M. Listgarten, Periodontal probing: what does it mean? J. Clin. Periodontol. 7 (3) (1980) 165–176, https://doi.org/10.1111/j.1600-051X.1980.tb01960.x.

[34] F. Hunter, Periodontal probes and probing, Int. Dent. J. 44 (5 Suppl 1) (1994) 577–583.

[35] M. Ciantar, D.J. Caruana, Periotron 8000: calibration characteristics and reliability, J. Periodontal Res. 33 (Suppl. 5) (1998) 259–264, https://doi.org/10.1111/j.1600-0765.1998.tb02198.x.

[36] D.W. King, R. Lashley, A quantifiable alternative to double data entry, Control. Clin. Trials 21 (2) (2000) 94–102, https://doi.org/10.1016/S0197-2456(00)00042-8.

[37] T. Berglundh, L. Krok, B. Liljenberg, E. Westfelt, G. Serino, J. Lindhe, The use of metronidazole and amoxicillin in the treatment of advanced periodontal disease. A prospective, controlled clinical trial, J. Clin. Periodontol. 25 (5) (1998) 354–362, https://doi.org/10.1111/j.1600-051X.1998.tb02455.x.

[38] A. Nagahara, A. Mitani, M. Fukuda, H. Yamamoto, K. Tahara, I. Morita, C.C. Ting, T. Watanabe, T. Fujimura, K. Osawa, S. Sato, S. Takahashi, Y. Iwamura, T. Kuroyanagi, Y. Kawashima, T. Noguchi, Antimicrobial photodynamic therapy using a diode laser with a potential new photosensitizer, indocyanine green-loaded nanospheres, may be effective for the clearance of Porphyromonas gingivalis, J. Periodontal Res. Suppl. 48 (5) (2013) 591–599, https://doi.org/10.1111/jre.12042.

[39] V. Melikian, J.D. Eddy, A. Paton, The stimulant effect of drugs on indocyanine green clearance by the liver, Gut 13 (10) (1972) 755–758.

[40] G.R. Cherrick, S.W. Stein, C.M. Leevy, C.S. Davidson, Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction, J. Clin. Invest. 39 (4) (1960) 592–600, https://doi.org/10.1172/JCI104072.

[41] R. Benya, J. Quintana, B. Brundage, Adverse reactions to indocyanine green: a case report and a review of the literature, Cathet. Cardiovasc. Diagn. 17 (4) (1989) 231–233, https://doi.org/10.1002/ccd.1810170410.

[42] S. George, A. Kishen, K.P. Song, The role of environmental changes on monospecies biofilm formation on root canal wall by Enterococcus faecalis, J Endod 31 (12) (2005) 867–872, https://doi.org/10.1097/01.don.0000164855.98346.fc.

[43] G.S. Omar, M. Wilson, S.P. Nair, Lethal photosensitization of wound-associated microbes using indocyanine green and near-infrared light, BMC Microbiol. 8 (2008) 111, https://doi.org/10.1186/1471-2180-8-111.

[44] N. Topaloglu, M. Gulsoy, S. Yuksel, Antimicrobial photodynamic therapy of resistant bacterial strains by indocyanine green and 809-nm diode laser, Photomed. Laser Surg. 31 (4) (2013) 155–162, https://doi.org/10.1089/pho.2012.3430.

[45] E. Engel, R. Schraml, T. Maisch, K. Kobuch, B. König, R.M. Szeimies, J. Hillenkamp, W. Bäumler, R. Vasold, Light-induced decomposition of indocyanine green, Invest. Ophthalmol. Vis. Sci. 49 (5) (2008) 1777–1783, https://doi.org/10.1167/iovs.07-0911.

[46] J. Meister, M. Hopp, J. Schäfers, J. Verbeek, D. Kraus, M. Frentzen, Indocyanine green (ICG) as a new adjuvant for the antimicrobial photo-dynamic therapy (aPDT) in dentistry, Lasers in Dentistry XX, Proc SPIE 8929:89290T-1–89290T-10 (2014), https://doi.org/10.1117/12.2057433.

[47] G. Shafirstein, M. Moreno, A. Klein, E. Siegel, P. Babilas, M. Landthaler, W. Baeumler, Treatment of leg veins with indocyanine green and lasers investigated with mathematical modelling, Int. J. Hyperthermia 27 (8) (2011) 771–781, https://doi.org/10.3109/02656736.2011.602037.

[48] K. Srikanth, R.V. Chandra, A.A. Reddy, B.H. Reddy, C. Reddy, A. Naveen, Effect of a single session of antimicrobial photodynamic therapy using indocyanine green in the treatment of chronic periodontitis: a randomized controlled pilot trial, Quintessence Int. 46 (5) (2015) 391–400.

[49] S. Kranz, M. Huebsch, A. Guellmar, A. Voelpel, S. Tonndorf-Martini, B.W. Sigusch, Antibacterial photodynamic treatment of periodontopathogenic bacteria with indocyanine green and near-infrared laser light enhanced by Trolox(TM), Lasers Surg. Med. 47 (4) (2015) 350–360, https://doi.org/10.1002/lsm.22336.

[50] M. Schneider, G. Kirfel, M. Berthold, M. Frentzen, F. Krause, A. Braun, The impact of antimicrobial photodynamic therapy in an artificial biofilm model, Lasers Med. Sci. 27 (3) (2012) 615–620, https://doi.org/10.1007/s10103-011-0998-7.

[51] R. Andersen, N. Loebel, D. Hammond, M. Wilson, Treatment of periodontal disease by photodisinfection compared to scaling and root planing, J. Clin. Dent. 18 (2) (2007) 34–38.

[52] C.M. Cobb, Non-surgical pocket therapy: mechanical, Ann. Periodontol. 1 (1) (1996) 443–490, https://doi.org/10.1902/annals.1996.1.1.443.

[53] C.M. Champagne, W. Buchanan, M.S. Reddy, J.S. Preisser, J.D. Beck, S. Offenbacher, Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases, Periodontol. 2000 31 (1) (2003) 167–180, https://doi.org/10.1034/j.1600-0757.2003.03110.x.

[54] R. Vanooteghem, L.H. Hutchens, S. Garrett, R. Kiger, J. Egelberg, Bleeding on probing and probing depth as indicators of the response to plaque control and root debridement, J. Clin. Periodontol. 14 (4) (1987) 226–230, https://doi.org/10.1111/j.1600-051X.1987.tb00971.x.

[55] P. Chondros, D. Nikolidakis, N. Christodoulides, R. Rossler, N. Gutknecht, A. Sculean, Photodynamic therapy as adjunct to non-surgical periodontal treatment in patients on periodontal maintenance: a randomized controlled clinical trial, Lasers Med. Sci. 24 (5) (2009) 681–688, https://doi.org/10.1007/s10103-008-0565-z.

[56] M. Lulic, I. Leiggener Görög, G.E. Salvi, C.A. Ramseier, N. Mattheos, N.P. Lang, One-year outcomes of repeated adjunctive photodynamic therapy during periodontal maintenance: a proof-of-principle randomized-controlled clinical trial, J. Clin. Periodontol. 36 (8) (2009) 661–666, https://doi.org/10.1111/j.1600-051X.2009.01432.x.

[57] E.A. Genina, A.N. Bashkatov, G.V. Simonenko, O.D. Odoevskaya, V.V. Tuchin, G.B. Altshuler, Low-intensity indocyanine-green laser phototherapy of acne vulgaris: pilot study, J. Biomed. Opt. 9 (4) (2004) 828–834, https://doi.org/10.1117/1.1756596.