Photodiagnosis and Photodynamic Therapy 27 (2019) 433–439

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Comparison of different laser-based photochemical systems for periodontal treatment

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ARTICLE INFO

Keywords:
Laser irradiation
Antimicrobial photodynamic therapy (aPDT)
Antimicrobial photothermal therapy (aPTT)
Methylene blue
Indocyanine green (ICG)
Curcumin

ABSTRACT

Purpose: The main aim in periodontitis treatment is to remove supragingival and subgingival biofilm. Mechanical treatment to eliminate pathogenic bacteria is limited by morphological conditions on the root surface. This study assessed the antibacterial effectiveness of different laser-based photochemical systems, particularly a novel curcumin-based option.

Methods: Ninety-one titanium bars were inoculated with an artificial biofilm of common pathogenic periodontal bacteria and inserted into an artificial periodontal pocket model. The following groups (n = 13) were tested: 1, curcumin solution plus SLB laser irradiation (C + L; 445 nm, 0.6 W, 25% duty cycle, 100 Hz, 10 s); 2, curcumin solution (Cur); 3, dimethyl sulfoxide solution (DMSO); 4, SiroLaser Blue (SLB) — laser irradiation (445 nm, 0.6 W, 25% duty cycle, 100 Hz, 10 s); 5, antimicrobial photodynamic therapy (aPDT); 6, antimicrobial photothermal therapy (aPTT); 7, control. The samples were stored in Eppendorf tubes and analyzed microbiologically using quantitative real-time polymerase chain reaction (PCR). The main parameter for analyzing group differences was the total bacterial load. Statistical analysis was performed with nonparametric methods.

Results: Statistically significant reductions in bacterial count were observed in all experimental groups ($p < 0.05$). The mean percentage reductions were as follows: SLB, 95.03%; aPDT, 83.91%; DMSO, 95.69%; C + L, 97.15%. No statistically significant differences in bacteria reduction were observed for laser alone (SLB), DMSO, or curcumin with or without additional laser irradiation.

Conclusions: The greatest antibacterial efficacy was observed in samples treated with aPTT. Using curcumin as a photosensitizing agent for 445 nm laser irradiation did not result in improved antibacterial effectiveness in comparison with laser alone.

1. Introduction

Periodontitis is an inflammatory disease of the tissue surrounding the teeth, caused by bacteria in the plaque biofilm. It causes pocket formation in the gum tissue, loss of attachment, bone destruction, and ultimately tooth loss — affecting both the soft tissue initially and also the hard tissue of the periodontium at the end of the infection process. Up to 30–50% of adults in industrialized nations are affected by periodontitis [1]. Due to the high prevalence of the condition, it represents a serious public health concern that is increasing with demographic changes and rising numbers of remaining teeth in geriatric patients. Periodontal disease is multifactorial and is affected by numerous risk factors, such as oral hygiene, everyday habits such as nutrition and smoking, and also genetic predisposition. The main goal in periodontitis therapy is to reduce or eliminate infectionand prevent disease progression [2].

In conventional nonsurgical periodontal treatment, an attempt is made to achieve infection-free conditions by removing bacterial deposits in the supragingival and subgingival biofilm through mechanical treatments such as scaling and root planing (SRP). Although effective, this treatment approach is limited by morphological conditions on the subgingival tooth surface such as difficult access to furcations, concavities, grooves, and the distal location of the molars [3,4]. The approach is not able to completely eliminate periodontal pathogens [4], which can persist in the root cement and dentin tubules and can serve as a reservoir for recolonization of root surfaces that have been treated [5]. Due to these limitations of conventional mechanical treatment, it has been proposed that adjuvant therapies such as systemic or local

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https://doi.org/10.1016/j.pdpdt.2019.06.009
Received 25 March 2019; Received in revised form 8 June 2019; Accepted 14 June 2019
Available online 15 July 2019
1572-1000/

antibiotic therapy may provide additional benefit [3–6].

Due to the side effects of antibiotics and increasing bacterial resistance when they are frequently used [7], a new adjuvant treatment strategy is needed in order to supplement mechanical therapy and allow effective removal of residual pathogens. The use of laser-based photochemical systems has therefore been suggested.

Laser therapy was first introduced into dentistry in 1960 [8] and it is now well established not only in periodontal treatment, but in all fields of dentistry. Various types of laser can be used, both for hard-tissue applications such as cavity preparation and bleaching, or for diagnostic purposes, and also for soft-tissue applications. The latter include surgery, photodynamic and photothermal therapy, as well as low-level laser therapy [9].

Photodynamic therapy is based on the principle that light has chemical effects [8]. A photosensitizer or photoactivatable agent such as methylene blue is applied to infected tissue. Exposing the tissue to light at the appropriate wavelength in the presence of molecular oxygen then generates the formation of reactive oxygen species (ROS) that cause nonthermal cytotoxic effects by damaging the proteins, cell membrane, and organelles of microorganisms [1,10]. In addition to having antibacterial effects, photosensitizers are also effective in inactivating fungi and are highly photovirucidal [11], providing a strong potential for disinfection. Several in vitro [12–14] studies, as well as randomized clinical trials (RCTs) [15,16] have investigated the effectiveness of aPDT.

As aPDT is an oxygen-dependent process, its use in deep pocket sides with a limited oxygen supply may be limited. Antimicrobial photothermal therapy (aPTT) — e.g., with indocyanine green activated by a laser source that emits green light — might be more effective in eliminating microorganisms through a local hyperthermal mechanism [17]. Few data on the efficacy of aPTT are as yet available [18].

The 445 nm laser has been the subject of substantial research interest for general applications. Previous investigations have demonstrated the phototoxic effect of blue light for effectively reducing periodontal pathogens [19] with no need for a photoactivatable agent, and further potential applications in periodontal treatment may therefore also be possible.

The modern revival of interest in natural medicines has drawn attention to curcumin, as an ancient remedy, in Western scientific and medical circles [6]. Curcumin, a component of the turmeric rhizome (*Curcuma longa*) that is primarily known as a spice, has various beneficial properties, including anti-inflammatory, antioxidant, chemopreventive, and chemotherapeutic activities; its most favorable characteristics may be its ability to suppress acute and chronic inflammation [20]. Used in traditional medicine, as well as in cooking and in fabric dyeing for more than 2000 years, it is extremely promising as a therapeutic agent for a variety of conditions, such as cancer, psoriasis, Alzheimer disease [21], and also for dental problems [22]. In dentistry, curcumin gel applied as an adjunct to SRP in the treatment of periodontal disease has been shown to be more effective than chlorhexidine gel, which is still regarded as the standard treatment [23].

aPDT performed using curcumin as the photoactivatable agent with an LED light in the blue region has shown promising results for inactivating multispecies biofilms, as reported by Quishida et al. in 2016 [24].

Few data are as yet available reporting comparisons of the various systems in clinical use [25,26]. The aims of the present study were therefore to evaluate the effect of curcumin as a photosensitizer for the 445 nm laser and its effectiveness against periodontal pathogenic microorganisms in multispecies biofilms; and also to collate data on the antimicrobial efficacy in vitro of various established photochemical treatment systems. The null hypothesis was that curcumin used as a photosensitizer for aPDT does have an antimicrobial effect.

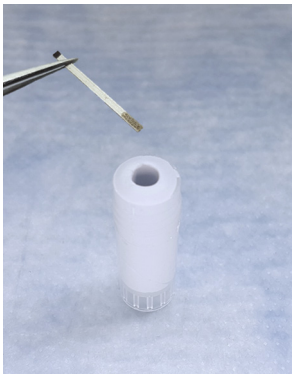

Fig. 1. Periodontal pocket model with one inoculated titanium bar being inserted.

2. Materials and methods

2.1. Periodontal pocket model

Seventy-eight periodontal pocket samples of identical size (10 × 3 mm in diameter) were prepared from commonly used silicone. Therefor standard Eppendorf tubes were filled with uncured silicone and the recesses for the artificial pockets were created by inserting plastic cylinders of standardized dimensions into the middle of each tube. After curing the cylinders were removed and the finished samples were divided into six groups, with each sample only being used once in order to prevent contamination between the different groups (Fig. 1).

2.2. Simulated biofilm

The lower 5 mm of 91 titanium bars, 1.5 mm wide and 2.5 mm long (Dentaurum GmbH & Co. KG, Ispringen, Germany), were sterilized and then inoculated with an artificial biofilm consisting of common pathogenic periodontal bacteria such as *Aggregatibacter actinomycetemcomitans (A.a.)*, *Campylobacter rectus* (*C.r.*), *Eikenella corrodens (E.c.)*, *Fusobacterium nucleatum (F.n.), Porphyromonas gingivalis (P.g.), Prevotella intermedia (P.i.), Parvimonas micra (P.m.), Treponema denticola (T.d.),* and *Tannerella forsythis (T.f.)*. The bacteria used for the artificial biofilm were extracted from patient samples which were collected from the gingival sulcus using paper points and analyzed by an external microbiology laboratory (Oro-Dental Microbiology ODM, Kiel, Germany). The prepared bacterial suspensions were mixed with human sterile-filtered saliva to generate an appropriate matrix which was sufficiently stable for inoculation of the used titanium bars.

The bars were separately stored in clean Eppendorf tubes labeled with consecutive numbering, and were divided into seven groups consisting of 13 trials each. One group of inoculated but untreated titanium bars was retained as a negative control group. In the study, the bars were inserted into the prepared periodontal pocket model and treated according to their group affiliation. They were subsequently re-stored in Eppendorf tubes and microbiologically analyzed.

2.3. Photosensitizer

For the present investigation, a curcumin solution at 100 $\frac{mg}{L}$ (Merck KGaA, Darmstadt, Germany) was prepared freshly on the day of the trial and stored in a dark glass bottle with light excluded, in order to prevent light effects. In view of its insolubility in water, 0.5 mL dimethylsulfoxide (DMSO; Sigma Aldrich, St. Louis, Missouri, USA), an organic solvent, was used as an emulsifier, as a 0.5% DMSO solution.

Light in the blue region of the spectrum (445 nm) was used for activation, as curcumin has an absorption peak in the range of 300–500 nm (Fig. 2) [27].

In the aPDT group, the photosensitizer HELBO Blue® (Helbo

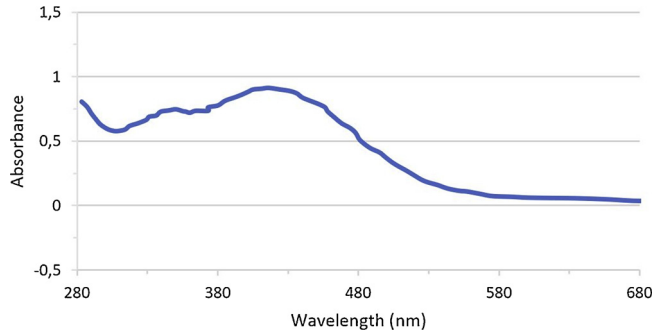

Fig. 2. Absorption spectrum of curcumin dye (modified according to Paschoal et al., 2013 [27]).

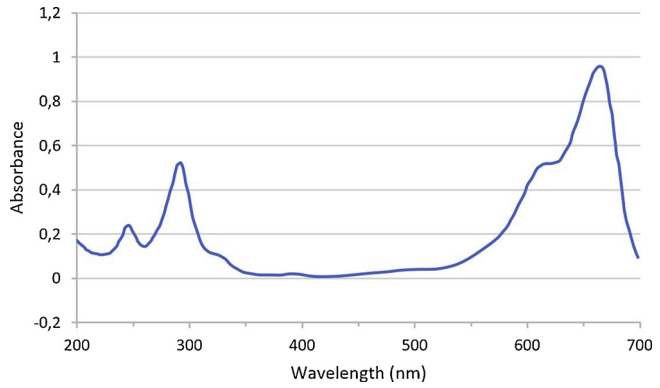

Fig. 3. Absorption spectrum of methylene blue (modified according to Melgoza et al. 2009 [55]).

Bredent, Walldorf, Germany), was used. It contains phenothiazine chloride [phenothiazine-5-ium,3,7-bis(dimethylamino)-chloride], a derivative of methylene blue, as its active constituent. The absorption spectrum of methylene blue can be seen in Fig. 3.

In the aPTT group, the photoactivatable agent EmunDo® (A.R.C. Laser, Nuremberg, Germany) was used. It contains indocyanine green (ICG) [1,7-bis(1,1-dimethyl-3-[4-sulfobutyl]-1*H*-benz[e]indol-2-yl) heptamethinium-betaine-Na]; its absorption spectrum is shown in Fig. 4. The agent was activated using a green light–emitting laser source (FOX Q810plus, A.R.C. Laser, Nuremberg, Germany).

2.4. Light-emitting source

Light was applied using a light-emitting diode (LED) laser (SiroLaser BLUE®, Sirona Dental Systems GmbH, Bensheim, Germany) at a wavelength of 445 nm (± 5 nm). Calibrated at 100 Hz, 600 mW and with a 25% duty ratio, an active output of 100 mW was generated and checked in advance using a digital power meter (PM100D model S314C, Thorlabs GmbH, Dachau/Munich, Germany).

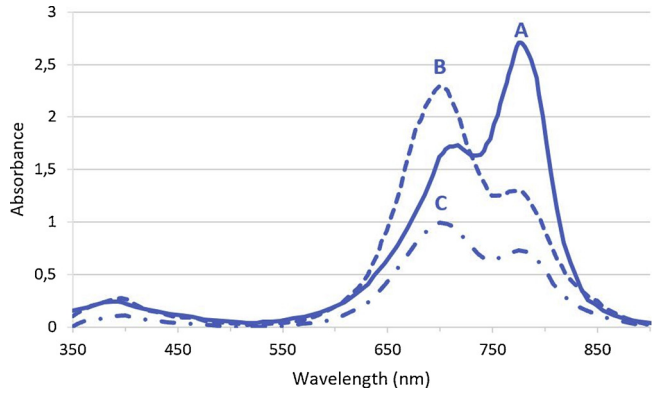

Fig. 4. Absorption spectrum of indocyanine green depending on the concentration (A > C > B) (modified according to Urbanska et al. 2002 [56]).

A fiber optic with a tip diameter of 320 µm (EasyTip 320 µm, Sirona Dental Systems GmbH, Bensheim, Germany) was linked to the device. For application, the tips were inserted as far as the bottom of the pocket and the laser was initiated, moved upwards along the titanium bar, and set to stop automatically after 10 s.

In the aPDT group, the relevant laser system (HELBO TheraLite® Laser, Helbo Bredent, Walldorf, Germany) with a wavelength of 665 nm and an appropriate laser tip for three-dimensional illumination were used (HELBO® 3D Pocket Probe, Helbo Bredent, Walldorf, Germany). The same application procedure was used.

In the aPTT group, the relevant laser system (FOX Q810plus®, A.R.C. Laser, Nuremberg, Germany) with wavelength of 810 nm and an appropriate laser fiber were applied. The same application procedure was used.

2.5. Experimental groups

The following seven groups were analyzed (Fig. 5):

Group I (C + L): A 0.5 mL prepared curcumin solution was applied to the periodontal pocket samples. After an application time of 3 min, it was also rinsed out with 5 mL 0.9% NaCl solution. This was followed by laser irradiation using the SiroLaser BLUE (445 nm, 0.6 W, 25% duty cycle, 100 Hz, 10 s).

Group II (Cur): A 0.5 mL prepared curcumin solution was applied to the pocket samples. After an application time of 3 min, the pockets were rinsed out with 5 mL 0.9% NaCl solution. No laser light was applied.

Group III (DMSO): 0.5 mL of a second prepared 0.5% dimethylsulfoxide solution without any curcumin was inserted into the pocket model using an Eppendorf pipette. After an application time of 3 min, the pockets were rinsed out with 5 mL 0.9% NaCl solution. No laser light was applied.

Group IV (SLB). The prepared pockets were also rinsed with 5 mL 0.9% NaCl solution (Fresenius SE & Co. KG, Bad Homburg, Germany) in order to wet the material before laser irradiation was applied with the SiroLaser BLUE® (Sirona Dentsply, Bensheim, Germany) (445 nm, 0.6 W, 25% duty cycle, 100 Hz, 10 s). A drug solution was not applied.

Group V (aPDT) was treated with a conventional photodynamic therapy system (Helbo, Bredent, Walldorf, Germany). The Helbo BLUE® photosensitizer was therefore applied to the periodontal pocket samples as described, with an application time of 3 min measured using a stopwatch before rinsing with a 5 mL 0.9% NaCl solution. Laser irradiation was carried out using a HELBO TheraLite® laser (665 nm, 10 s).

Group VI (aPTT) was treated with a conventional photothermal therapy system (EmunDo®, A.R.C. Laser, Nuremberg, Germany). The photoactivatable agent EmunDo® was applied to the periodontal pocket samples. No exposure time or rinsing is prescribed as necessary for this system. Irradiation was carried out using a FOX Q810plus® laser (810 nm, 10 s).

Group VII (control). This was the untreated control group.

In all of the groups, the bars were inserted into the prepared pocket models and treated as described above before being re-stored in their Eppendorf tubes and sent for microbiological analysis. Contamination between different groups was prevented by exchanging the tips and carrying out disinfection between different pocket samples.

2.6. Microbiological analysis

The samples were analyzed microbiologically in an external microbiology laboratory (Oro-Dental Microbiology ODM, Kiel, Germany) using a real-time quantitative polymerase chain reaction. The main parameter for analyzing the groups was the total bacterial load (TBL).

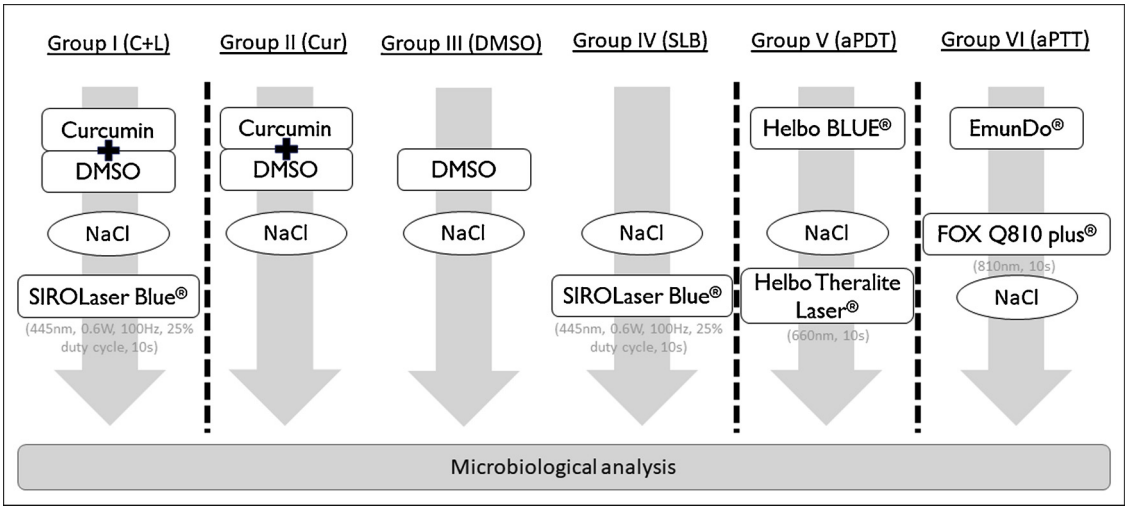

Fig. 5. Flow-chart showing the treatment procedure of the experimental groups.

2.7. Statistical analysis

A power analysis was performed prior to the study. Therefore, the effect size was set to 0.5 according to Cohen [28]. For an alpha-error of 0.05 and a power of 0.8 a sample size of at least 11 specimens in each group was calculated. Normal distribution of the values was assessed with the Shapiro-Wilk test. Since not all data were normally distributed, values were analyzed with non-parametric pairwise comparisons (Wilcoxon, Mann-Whitney. Differences were considered as statistically significant at $p < 0.05$.

3. Results

Statistically significant reductions of the bacterial counts were observed in all experimental groups ($p < 0.05$). Highest reduction occured in group VI (aPTT) with a median of 1.78E5 CFU and a reduction of 99.63% (minimum 97.09%, maximum 99.74%), which was statistically significant higher than the antibacterial efficacy of antibacterial photodynamic therapy (aPDT), DMSO solution (DMSO) and curcumin solution (Cur) without additional laser irradiation ($p < 0.05$). Second highest reduction of the bacterial count was observed in group II (Cur) with a reduction of 97.35% (minimum 96.54%, maximum 96.85%), with a statistically significant higher reduction than for antimicrobial photodynamic therapy (aPDT) ($p < 0.05$). Lower reductions in the bacterial count were observed in group I (C + L) with 97.15% (minimum 96.29%, maximum 99.48%), group III (DMSO) 95.69% (minimum 96.39%, maximum 98.12%) and group IV (SLB) with a reduction of 95.03% (minimum 94.29%, maximum 99.51%). Antibacterial efficacy of laser alone (SLB) showed no statistically significant difference to all other groups ($p > 0.05$) except the untreated control group ($p < 0.05$). No statistically significant differences in bacterial reductions were observed for laser alone (SLB), DMSO solution (DMSO), or curcumin solution with (C + L) or without (Cur) additional laser irradiation ($p > 0.05$). Lowest antibacterial efficacy occurred for samples treated with antibacterial photodynamic therapy (aPDT) with a reduction of 83.91% (minimum 44.46%, maximum 99.79%). Antibacterial efficacy was statistically significantly lower than in all other groups ($p < 0.05$) (Fig. 6, Table 1).

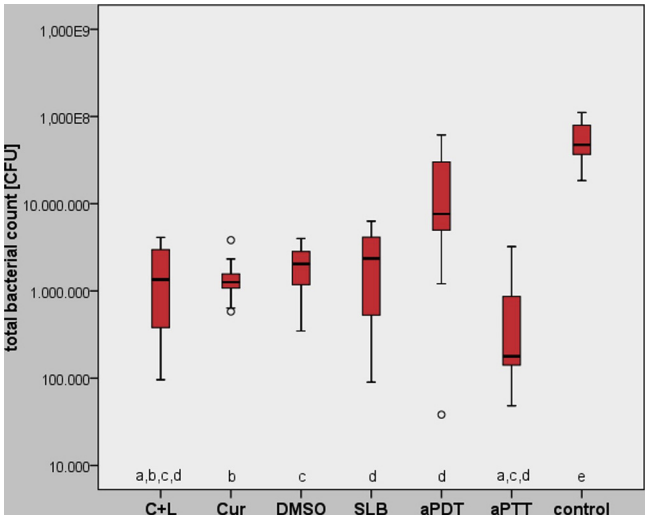

Fig. 6. Reduction in total bacterial load (TBL) in all of the study groups (same lowercase letters: $p > 0.05$, Mann-Whitney U-test).

4. Discussion

Several studies have investigated the use of lasers as a treatment strategy for antimicrobial purposes [25,26,29,30]. It is extremely difficult to compare the findings, due to the large number of different laser systems available, their varying effects on different tissues, and the lack of standard operating protocols (such as power intensity, energy, pulsed versus continuous mode, application time). In addition, each research group tends to establish its own protocol on the basis of reports or experience [31].

In contrast to previous studies, the present investigation did not demonstrate any increase in bactericidal efficacy when curcumin was combined with blue laser light. The curcumin solution with or without additional laser application did not appear to have any greater antibacterial potential than DMSO applied on its own. No statistically significant differences in the amount of bacterial reduction were observed for groups IV (SLB), III (DMSO), II (Cur), or I (C + L). This observation might be caused by using a strict wavelength of 445 nm instead of blue light with emission from 440 to 460 nm [23].

Thus, the null hypothesis was not confirmed, as the combination of curcumin and the wavelength used did not provide the expected benefit. Further research will be needed in order to identify the optimal combination of concentration and wavelength.

Already previous investigations have attempted to examine the efficacy of laser irradiation applied simultaneously with curcumin administration or independently of it [32]. The use of curcumin as a novel photoactivatable agent against oral pathogens, with antibacterial activity against suspensions of *Streptococcus mutans* [33,34], *Aggregatibacter actinomycetemcomitans* [32], *Porphyromonas gingivalis,* and

Table 1
Results of the study with regard to the total bacterial load (TBL).

Sample No.	C + L	Cur	DMSO	SLB	aPDT	aPTT	Control
1	3,59E + 06	3,83E + 06	2,04E + 06	2,36E + 06	4,99E + 06	1,26E + 05	1,84E + 07
2	3,80E + 05	1,11E + 06	3,43E + 06	4,21E + 06	3,01E + 07	1,78E + 05	8,75E + 07
3	6,50E + 05	9,88E + 05	3,99E + 06	3,61E + 06	2,56E + 06	9,06E + 05	7,94E + 07
4	2,98E + 06	1,57E + 06	3,47E + 05	6,31E + 06	6,14E + 07	4,82E + 04	1,11E + 08
5	4,11E + 06	1,08E + 06	1,18E + 06	5,54E + 06	3,02E + 07	2,78E + 05	2,29E + 07
6	8,63E + 05	6,38E + 05	2,30E + 06	5,29E + 05	6,13E + 06	1,31E + 05	5,32E + 07
7	9,63E + 04	1,94E + 06	1,51E + 06	9,00E + 04	1,21E + 06	4,39E + 05	8,38E + 07
8	2,43E + 05	1,40E + 06	1,66E + 06	1,38E + 05	5,68E + 06	1,41E + 05	4,47E + 07
9	2,98E + 06	1,56E + 06	2,44E + 06	9,63E + 05	1,37E + 07	1,52E + 05	4,74E + 07
10	2,93E + 06	5,81E + 05	4,99E + 05	3,45E + 05	1,19E + 07	2,29E + 06	3,68E + 07
11	2,22E + 06	1,20E + 06	5,44E + 05	3,33E + 06	3,82E + 04	8,63E + 05	5,96E + 07
12	1,35E + 06	1,26E + 06	2,88E + 06	7,00E + 05	7,63E + 06	3,22E + 06	3,68E + 07
13	1,54E + 05	2,33E + 06	2,84E + 06	4,14E + 06	3,30E + 07	1,75E + 05	3,16E + 07
Mean	1,7E + 06	1,5E + 06	2,0E + 06	2,5E + 06	1,6E + 07	6,9E + 05	5,5E + 07
SD	1,4E + 06	8,5E + 05	1,2E + 06	2,2E + 06	1,8E + 07	9,8E + 05	2,8E + 07
Median	1,4E + 06	1,3E + 06	2,0E + 06	2,4E + 06	7,6E + 06	1,8E + 05	4,7E + 07
Minimum	9,6E + 04	5,8E + 05	3,5E + 05	9,0E + 04	3,8E + 04	4,8E + 04	1,8E + 07
Maximum	4,1E + 06	3,8E + 06	4,0E + 06	6,3E + 06	6,1E + 07	3,2E + 06	1,1E + 08
Interquartil	2,6E + 06	4,9E + 05	1,7E + 06	3,6E + 06	2,5E + 07	7,2E + 05	4,3E + 07

SD, standard deviation.

Fusobacterium nucleatum [35] has been described. However, previous studies did not take into account the influence of the solving agents used, such as DMSO, which has been described as a pharmacologic agent, an organic solvent enhancing the penetration of solute substances, and as a radical scavenger [36]. For greater accuracy, therefore, each ingredient needs to be examined on its own in order to eliminate the possibility that single components of a treatment may have antimicrobial effects on their own. When only the reduction in the bacterial count in group I (C + L) is taken into account, the antibacterial effects are in line with the findings of previous trials. Quishida et al. observed a substantial reduction in metabolic activity and in the total biomass of multispecies biofilms when curcumin and an LED device were used, in comparison with untreated control samples [24].

Although several studies confirmed that curcumin has phototoxic effects, the mechanism involved in the photoinduced damage to biological substances remained unclear until the reaction chain was determined by Qian et al. in 2013. They found that photo-ionization requires the formation of an excited triplet state ($^3Cur^*$) via different short-lived intermediate stages. The final interaction of these with oxygen produces singlet oxygen ($^1O_2^*$) [37]; both Cur· and $^1O_2^*$ can induce photodynamic damage [38].

As the available research data showed that incoherent blue light sources have phototoxic effects on periodontal pathogens [19,39], and since endogenous porphyrin can also act as a photosensitizer [19], further investigations found that phototherapy alone leads to a significant reduction in biofilm growth. It was therefore postulated that light alone might serve as an effective adjunct to mechanical plaque control [40]. To examine the efficacy of the systems used, it has therefore become necessary to carry out comparisons with the use of each component alone.

Since previous investigations have shown that bacteria that are organized in biofilms are less accessible to antibiotics, due to protection within the polymer plaque matrix, and since the uptake of photosensitizers is limited in the same way [1], findings regarding bacterial suspensions should be treated with caution. Penetration through biofilms and their matrix may be hindered by molecular charges [41]. Effects on artificial biofilms can therefore be expected to have less potential due to aggravated photosensitizer penetration. The need to allow longer time periods for photosensitizers to penetrate biofilm structures was also demonstrated by Andrade et al. in 2013. The authors compared curcumin-mediated aPDT with planktonic and biofilm cultures of *Candida albicans*, *C. glabrata,* and *C. dubliniensis* and showed that there was reduced susceptibility to aPDT [42].

Costerton et al. evaluated the structural heterogeneity of biofilms in relation to heterogeneity in the distribution of oxygen. It was found that the oxygen concentration was high at the surface, but decreased in the lower biofilm layers, where anaerobic conditions prevail [43]. A higher level of metabolic activity is therefore found at the surface, with a lower level in the center of the biofilm. This may be one explanation for the reduced susceptibility of biofilms to antimicrobial agents [44].

The specific efficacy of oxygen-dependent processes such as aPDT may be compromised with decreasing oxygen saturation, such as that seen in deep periodontal pockets, and with increasing depth of the biofilm. In cases in which the efficacy of aPDT is compromised, it has been suggested that aPTT may be a substitute [18]. Since most highly pathogenic periodontal bacteria proliferate particularly in anaerobic conditions, aPTT may provide additional benefits. The fact that the lower layer of the biofilm may not be sufficiently exposed to light due to the turbidity of the biofilm may also be a relevant aspect [19].

The artificial biofilm models used in the present investigation may therefore provide a better reflection of clinical conditions. Since large numbers of bacterial species are involved in the etiology of oral health problems, study designs that use cultures with single microorganisms or small numbers of selected microorganisms may have significant drawbacks. Investigations of multispecies biofilms such as those used in the present study are likely to have greater validity.

The absence of environmental effects may also be mentioned as a limiting factor in study designs that use in vitro models, as the clinical effectiveness of procedures may be impaired by factors that are present in the surroundings. A number of studies have reported that the presence of organic materials such as blood or nutrient broth, as well as saliva and serum, reduces the impact of lethal photosensitization [45]. As demonstrated by Kömerik and Wilson, the composition and pH of fluids influences the effectiveness of photosensitization mediated by toluidine blue, and this limits the clinical usage of the procedure [45]. In addition, partial protection can be provided by some ROS scavengers [46].

Other limitations of in vitro designs that may be mentioned include the absence of aggravating conditions that are present on the subgingival tooth surface and poor modeling of gingival and periodontal pocket formation. The artificial periodontal pocket model used in the present study may not have fully overcome these difficulties, and may not be able to simulate the limited availability of oxygen in the deep sides of pockets at the required level of detail. As mentioned earlier, the morphological conditions on the tooth surface are not accurately imitated by the titanium bars used. Concavities, grooves, and the reduced

smoothness of actual root surfaces may make antibacterial treatment less effective.

Photodynamic therapy using diode lasers has been the topic of numerous investigations examining the effects of different photosensitization systems on various microorganisms in a variety of conditions [47–49]. Approved photosensitizers such as methylene blue, for example, have also been studied in several in vivo investigations to confirm additional benefits in periodontal treatment in clinical conditions [50,51].

Although divergent study designs make comparisons difficult, recent meta-analyses and earlier systematic reviews have suggested similar results. The meta-analysis by Sgolastra et al. published in 2013 [15] concluded that using aPDT as an adjunct to conventional treatment provides additional short-term benefits. This finding was partly in agreement with a previous meta-analysis by Atieh in 2010 [16], with results supporting a potential improvement in the clinical attachment level (CAL) and probing depth (PD) when SRP was used in combination with aPDT. A recent systematic review and meta-analysis by Smiley et al. investigated the clinical efficacy of combinations of SRP with assorted adjunctive treatment strategies, including systemic antimicrobials and aPDT. It was found that aPDT led to improvements in CAL beyond the benefits of SRP alone [31]. The latest meta-analysis by Xue et al., published in 2017, suggested short-term benefits in clinical outcome variables with a PDT when it was used as an adjunct to conventional treatment [52]. Due to the diversity of study designs, however, all of the research findings are limited and should be regarded with caution — emphasizing the need for further investigations and clinical trials in order to confirm the efficacy of aPDT in the treatment of periodontal disease.

With the advent of increasing drug resistance, side effects such as the development of microorganisms resistant to photodynamic effects are attracting particular attention — although this should be regarded as unlikely, since photosensitization is typically a multitarget process, in contrast to the mechanism of most antimicrobial drugs [53]. The expression of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase may offer protection against some reactive oxygen species (ROS), but not against singlet oxygen, the principal active ROS in aPDT [11]. The antibacterial efficacy of photosensitization is therefore not affected by bacterial drug resistance, and aPDT might therefore be regarded as a potential solution in the treatment of organisms with multidrug resistance [11].

Since singlet oxygen photosensitizers that mediate cytotoxicity are extremely chemically reactive, they have additional direct effects on noncellular biomolecules such as the polysaccharides in the extracellular polymeric substance (EPS) of bacterial biofilm. The EPS is also susceptible to photodamage, and it has been reported that aPDT causes it to break down, resulting in a dual activity that is not seen with conventional antibacterials. The ability to break down biofilms may represent a considerable advantage for photodisinfection [11].

Few data are as yet available on antimicrobial photothermal therapy. According to the manufacturers, the bactericidal effect of EmunDo® is due to its photothermal action, and a photodynamic mechanism is not mentioned [18]. Further investigations on indocyanine green by Engel et al. have shown that most of the absorbed laser light is converted into thermal heat. A local temperature increase thus appears to be the main mechanism against microorganisms with aPTT, although photodynamic activity occurs as a secondary action [54]. It has been reported that indocyanine green produces singlet oxygen as a result of laser irradiation. However, as it is itself primarily oxidized by singlet oxygen, the decomposition products of indocyanine green affect cellular integrity [54]. In addition to antibacterial efficacy, thermal damage to the surrounding tissue also needs to be taken in account [18], since preliminary not yet published findings of our own research group indicate that there is a greater increase in temperature with aPTT.

5. Conclusion

The greatest antibacterial efficacy was observed in samples treated with aPTT. Using curcumin as a photosensitizing agent for 445 nm laser irradiation did not result in any improvement in the antibacterial effect in comparison with laser application alone. As previous investigations have reported that the effectiveness of curcumin increases when it is exposed to laser light, it is possible that the wavelength used in the present study may not have been appropriate. However, this might suggest that curcumin has a narrow absorption range in the blue spectrum.

Declaration of Competing Interest

The authors declare no conflict of interest in this manuscript.

Acknowledgment

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