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Feasibility of transgingival laser irradiation for antimicrobial photodynamic therapy

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ABSTRACT

Aim: Diode lasers are commonly used for antimicrobial photodynamic therapy (aPDT). This study aimed to assess the feasibility of transgingival laser irradiation during aPDT and evaluate whether the photosensitizer can be activated.

Materials and Methods: Four diode laser settings were assessed for transgingival irradiation: 120 mW, 80 mW, 60 mW, and 40 mW. Fifteen soft-tissue pieces from a pig's lower jaw were prepared. The specimens' thickness was measured and transgingival laser irradiation was performed. A digital power meter measured laser power on the other side of the tissue. The power outcome after staining of the nonbuccal aspect of the tissue with photosensitizer dye was assessed similarly.

Results: Transgingival laser irradiation (average soft-tissue thickness: 0.84 ± 0.06 mm) resulted in different power transmission depending on the power settings and photosensitizer. The lowest values were observed with the 40 mW setting and photosensitizer (median 3.3 mW, max. 5.0 mW, min. 2.3 mW, interquartile range 1.2), and the highest at 120 mW without photosensitizer (median 41.3 mW, max. 42.7 mW, min. 38.0 mW; interquartile range 1.5).

Conclusions: This study indicates that transgingival irradiation may be suitable for aPDT, since power transmission through the gingival tissue was observed in all specimens. However, the decrease in laser power caused by both the soft tissue and the photosensitizer has to be taken into account.

1. Introduction

Antimicrobial photodynamic therapy (aPDT) is a procedure commonly used as an adjunctive treatment in periodontal therapy. It is reported to be an effective method of killing periodontopathogenic bacteria [1,2] and thus also improves clinical outcomes in patients with residual pockets [3]. The concept behind aPDT is that power applied in form of light with appropriate wavelength which correlates with the absorption maximum of the photosensitizer can activate the dye molecules, with effects on ground-state molecular oxygen, creating reactive oxygen species such as singlet oxygen that are capable of killing cells [4,5].

The power in form of light with appropriate wavelength which is used to activate the photosensitizer is generated by a laser device — specifically, a semiconductor laser. Laser systems are generally used for many purposes in dentistry, particularly in surgery, endodontology, and periodontology. Diode lasers have mainly been used since the end of the twentieth century. The working principle involved is the absorption of electromagnetic waves in the target tissue [6]. The effects of diode laser power proved to be highly suitable for killing bacteria in infected root canals [7] and reducing inflammation in periodontal pockets [8] as part of adjunctive periodontal treatment regimens.

Periodontitis generally means inflammation of the periodontal tissues, caused by a variety of periodontopathogenic bacteria [9]. Conventional treatment procedures often do not include potential adjunctive approaches to the condition. The variety of possible treatment options therefore needs to be evaluated more carefully. Particularly in view of demographic changes and the aging of the population, it is becoming evident that major challenges are developing in the field of periodontal treatment that will need to be managed [10].

Biofilm (plaque) has to be controlled and may be a focus for curative treatment options. Due to the formation of metabolic products, especially toxins from gram-negative and/or anaerobic bacteria, an immune response occurs that becomes manifest as an inflammatory reaction in the periodontal tissue. Among the commonly used periodontal treatment procedures, mechanical scaling and root planing (SRP) appears to

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be of limited benefit here due to invasion by pathogens, difficult access for scaling, furcation defects, root concavities, and deep pockets [11]. Every option in addition to mechanical cleaning of the tooth surface such as adjunctive therapy options should therefore be considered. The photomechanical effects of laser irradiation techniques such as low-level laser therapy (LLLT) and antimicrobial photodynamic therapy (aPDT) are thus becoming increasingly important. Particularly in dentistry, low-level laser therapy is an increasingly widely used method due to its biostimulatory and anti-inflammatory effects; positive effects on protein synthesis and collagen synthesis, as well as analgesia and a reduction in inflammatory effects, have been described [12]. Both in vitro and in vivo studies have shown that LLLT has bactericidal effects on microbial species in relation to both gingivitis and periodontitis therapy [8,13].

Recent studies have discussed the principles of conventional aPDT, its limitations, and potential side effects in periodontal therapy, and a novel approach involving transgingival photosensitizer activation has been suggested. The light absorption properties of soft tissues for light in the range of 600–1300 nm have been described in this context [14]. One study showed that transgingival activation of a photosensitizer based on indocyanine green–loaded nanospheres is possible, with successful use of an 810-nm diode laser [15]. The depth of penetration of different wavelengths into soft tissue appears to be the major limiting factor. It is thought that for wavelengths between 600 and 1300 nm, the optical penetration depth in human mucous tissue is in the range of 3.0–6.5 mm. The maximum has been observed in spectral ranges between 800–900 nm and 1000–1100 nm, with an optical penetration depth of 6.0–6.5 mm. For the 660-nm wavelength commonly used in antimicrobial photodynamic therapy, the optical penetration depth is about 3.0–3.5 mm [16].

In summary, aPDT, laser therapy, and low-level laser therapy appear to be treatment regimens that do not have any major side effects. These therapeutic options might therefore provide a good approach for future therapies, supplementing conventional treatment approaches. To make laser irradiation easier, particularly during periodontal treatment procedures, it might be possible to simplify the commonly used intrapocket application of laser light using transgingival irradiation.

The aim of the present study was therefore to assess the possibility of transgingival laser irradiation during an antibacterial photodynamic therapy (aPDT) procedure, testing the hypothesis that transgingival laser irradiation is possible for subgingival antimicrobial photodynamic therapy.

2. Materials and methods

2.1. Experimental design

The photodynamic system used in this study was developed by Helbo (Bredent Medical Ltd., Walldorf, Germany). It is a self-contained system suitable for different laser devices emitting a wavelength of 660 nm, with a photosensitizer dye that has an absorption maximum at exactly 664 nm according to the manufacturer instructions.

Four diode laser devices from the same model with the only difference in the laser power they emitted were assessed for their performance in transgingival irradiation. A 120-mW laser (Helbo Minilaser; Bredent Medical) which has been exclusively manufactured for this study and three devices with lower transmitted power (groups 2–4; Helbo TheraLite laser, Bredent Medical) were used. The different power settings of the laser devices were as follows: 1, 120 mW; 2, 80 mW; 3, 60 mW; and 4, 40 mW. Corresponding fiber optics were linked to each device; only the 120-mW laser was used differently as no corresponding fiber optic is available. For irradiation the laser devices with or without fiber optics were fixed at a distance of 1 mm from each specimen to make sure the resulting beam-spot diameters are closely similar for all beam powers. The actual power of each laser device was previously measured in the same geometry as for the tissue transmission measurements using a digital power meter (PM100D; Thorlabs Ltd., Dachau, Germany) and a thermal power sensor (S314C; Thorlabs) with a diameter of the pinhole aperture of approximately 25 mm. The characteristics of the different laser devices are listed in Table 1.

Table 1
The diode laser devices used in study and their energy density relative to their output power and tip diameter.

laser device	40 mW	60 mW	80 mW	120 mW
Tip diameter (mm)	0.75	0.75	0.75	2.5 (spot diameter)
Output power (mW)	40.9	55.0	80.4	117.6
Energy density (mW/cm^2)	9257	12449	18198	1395

mW, milliwatts; mm, millimeter; cm^2, square centimeter.

Fifteen slices of gingival soft tissue from the lower jaw of a domestic pig were prepared and had their mean thickness verified (average soft-tissue thickness 0.84 ± 0.06 mm). The thickness of each specimen was measured in three areas of the marginal gingiva. Each specimen was separately stored in numbered polypropylene tubes containing 0.9% isotonic saline solution with 0.001% of sodium azide added (B. Braun Melsungen AG, Melsungen, Germany). A schematic side-view diagram showing the experimental setup can be seen in Fig. 1.

2.2. Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed in this study.

2.3. Test series no.1 (standard series)

Fifteen pieces of soft tissue were successively fixed into a pinhole aperture after they had been rinsed with a sodium chloride solution. Laser irradiation was then performed at three points from the buccal aspect, using the fiber optics belonging to each laser; only the 120-mW laser was used differently as no corresponding fiber optic is available. The laser devices with or without fiber optics were therefore fixed at a distance of 1 mm from each specimen. The respective irradiated areas were located within a distance of 2 mm parallel to the tissue margin of the marginal gingiva. The laser power transmitted was measured on the opposite side of the soft tissue using a digital power meter (PM100D; Thorlabs, Dachau, Germany). The pinhole aperture was thus used to exclude scattered light from the environment and ensure that almost all of the laser power could be detected by the power meter. This procedure was repeated with each diode laser device. Each specimen was then placed in a single tube containing a 0.9% isotonic saline solution with an addition of 0.001% of sodium azide and stored at 14 °C.

2.4. Test series no.2 (photosensitizer series)

The same study protocol as described above was used to assess the power outcome after staining of the nonbuccal aspect of the soft tissue with a photosensitizer dye based on phenothiazine chloride (Helbo*Blue* Photosensitizer; Bredent Medical) which has an absorption maximum at exactly 664 nm according to the manufacturer and can therefore be activated by laser devices emitting a wavelength of 660 nm.

Each specimen was rinsed with sodium chloride, and the photosensitizer was then applied with a cannula (Sterican 0.50 × 25 mm BL / LB size 17/23; B. Braun Melsungen). After an application time of 1 min, the photosensitizer dye was rinsed with sodium chloride solution. Each of the soft-tissue specimens was fixed into the pinhole aperture and measured again with all four laser settings at each of the three initially measured points. The distance between the laser fibers and the specimen and the distance between the three individual points were maintained. A computer-generated random number table was used to

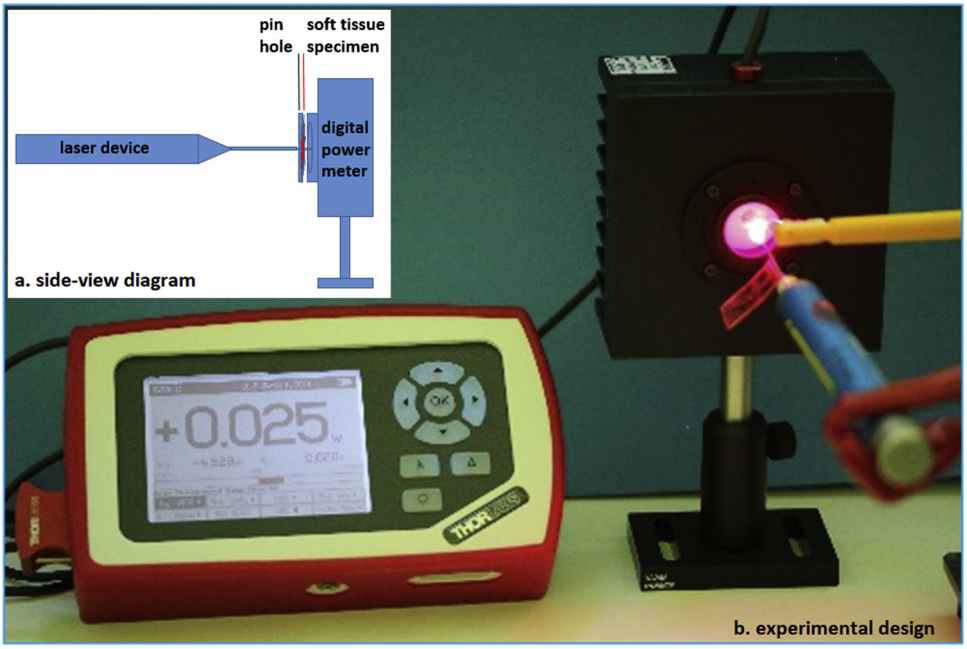

Fig. 1. a. side-view diagram and b. experimental design used in the study: digital power meter (S314C, Thorlabs, Dachau, Germany), laser device (Helbo TheraLite Laser, Bredent Medical, Walldorf, Germany), and a mounted soft-tissue specimen.

determine the sequence of the four laser settings used in each specimen.

2.5. Statistical analysis

A power analysis was performed prior to the study, with the Cohen effect size set at 0.5 [17]. For an alpha error of 0.05 and a power of 0.8, a sample size of at least 13 specimens in each group was calculated. The normal distribution of the values was assessed using the Shapiro–Wilk test. Since not all of the data were normally distributed, values were analyzed statistically using a nonparametric test (Friedman) and Wilcoxon pairwise comparison. Differences were considered statistically significant at $p < 0.05$. Box plot diagrams show the median, first and third quartiles, minimum and maximum values (whiskers). Values of more than 1.5 to three times the interquartile range were specified as outliers and marked as data points. Values higher than three times the interquartile range were specified as far outliers and marked as asterisks.

3. Results

Transgingivally applied laser irradiation resulted in different transmitted power depending on the power settings and photosensitizer being investigated (Table 2, Fig. 2).

The highest values were measured with the 120-mW setting (median 41.3 mW, max. 42.7 mW, min. 38.0 mW, interquartile range [IQR] 1.5) in the group without photosensitizer applied. The lowest values in the nonphotosensitizer group were measured for the 40-mW laser (median 8.7 mW, max. 9.7 mW, min. 6.0 mW, IQR 1.5). The 60-mW and 80-mW groups showed transmitted power in between those of the previous two groups (Table 2, Fig. 2).

Table 2
Transmitted power with the different lasers, without photosensitizer.

laser device	120 mW	80 mW	60 mW	40 mW
n	15	15	15	15
Mean (in mW)	40.8	22.4	13.8	8.1
Median (in mW)	41.3	22.7	13.3	8.7
Minimum (in mW)	38.0	20.7	11.3	6.0
Maximum (in mW)	42.7	24.0	16.7	9.7
Interquartile (in m W)	1.5	0.7	2.5	1.5

mW, milliwatts.

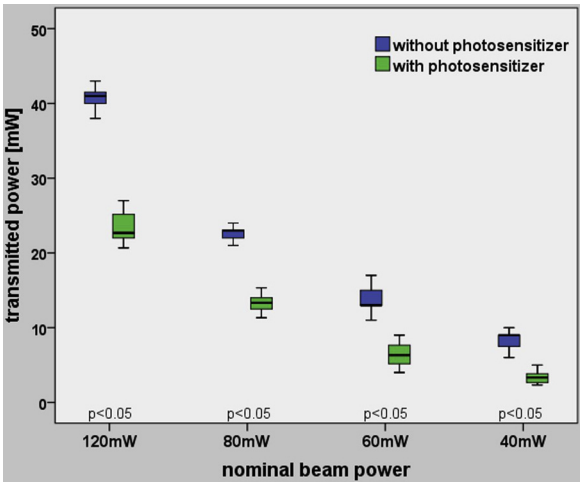

Fig. 2. Box plot diagram for the transmitted laser power in the photosensitizer and nonphotosensitizer groups (n = 15 in each subgroup).

In the photosensitizer group, the highest values were observed using the 120-mW laser (median 22.7 mW, max. 27.0 mW, min. 20.7 mW, IQR 3.2). The lowest values were measured for the 40-mW setting and with photosensitizer applied (median 3.3 mW, max. 5.0 mW, min. 2.3 mW, IQR 1.2). As in the nonphotosensitizer test series, the 60-mW and 80-mW laser devices showed transmitted power in between these two groups (Table 3, Fig. 2).

The laser values given by the manufacturer (ME) were checked using a power meter to evaluate the actually measured energy values (AE). The overall mean power losses in the photosensitizer and nonphotosensitizer groups showed power reductions of approximately 86% and 73%, respectively, calculated relative to the actual measured energy values (Table 4).

Table 3
Transmitted power with the different lasers, with photosensitizer.

laser device	120 mW PS	80 mW PS	60 mW PS	40 mW PS
n	15	15	15	15
Mean (in mW)	23.4	13.2	6.4	3.4
Median (in mW)	22.7	13.3	6.3	3.3
Minimum (in mW)	20.7	11.3	4.0	2.3
Maximum (in mW)	27.0	15.3	9.0	5.0
Interquartile (in mW)	3.2	1.5	2.5	1.2

mW, milliwatts; PS, photosensitizer.

4. Discussion

All previous publications about aPDT have described the results after subgingival laser irradiation [1,4]. These studies reported a spot size at the target surface of 0.25 cm^2 and an application time of 30 s with an effective power transmission of 40 mW (energy density 4.87 J/cm^2; power density 0.16 W/cm^2), as defined by the company that developed the technique, mentioned above [18]. It was therefore hypothesized that an effective power of at least 40 mW also needs to be achieved transgingivally in order to activate the photosensitizer dye. None of the laser devices up to the 80-mW laser provided sufficient transgingival power to activate the photosensitizer on the inside of the marginal gingiva. Absorption by the marginal gingiva proved to be so great that it only allowed the 120-mW laser in the nonphotosensitizer group to achieve 40 mW or more of effective power transmission transgingivally. Activation of only small amounts of the photosensitizer can therefore be expected. The results of the present study thus show the limitations of transgingival activation during an aPDT procedure.

Both the set-up and the sequence of the experiments were clearly structured, and the effective transmitted powers were verified. The values given by the manufacturer were checked using a power meter. The results showed a certain discrepancy between the nominal and actual power transmission. In the present study, only the actual power values at the end of each working tip were recorded. The manufacturer's instructions were strictly followed in the photosensitizer group and in the use of the photosensitizer dye. Each specimen received the same amount of photosensitizer solution, and the exposure time was measured with a microchronometer.

Another problem, the scattering of environmental light and radiation, was prevented by using a pinhole aperture trying to exclude environmental disturbing light. Since the 120-mW laser is not supported by a fiber optic, the influence of the scattered radiation on the power outcome had to be excluded by the use of the pinhole aperture as described above. At the same time, it needs to be considered that all relevant scattered light cannot be excluded. Furthermore, the tissue samples will reflect and also scatter the light of the incoming laser beam. It needs to be taken into account that lateral light loss in the samples as well as light scattered to large angle, which may not be detected by the power meter, may lead to the overestimation of the light absorption of the tissue and therefore to underestimation of the light fluence through the tissue samples [16].

The distance between the laser, the specimen, and the digital power meter was the same in all procedures (Fig. 1). The fiber optics for the 40-mW and 80-mW lasers were of the same length and were cleaned after each measurement, to prevent contamination at the laser fibers.

The sequence of application of the laser systems within each specimen was based on a computer-generated random-number table, so that adjustment to previously measured values was not possible. For each specimen, values were also measured at three different points on the marginal gingiva and a mean value was calculated, reducing the effects of potential variation in the gingival thickness.

Several studies have compared the effectiveness of SRP and aPDT, and some have reported critically on the effects of aPDT. One study conducted in 2002 did not show any additional microbiological and clinical benefits of aPDT in comparison with conventional SRP [19]. However, the results of a meta-analysis by Sgolastra et al. from 2013 demonstrated that there are short-term benefits in relation to gains in the clinical attachment level (CAL) and reductions in pocket depth (PD) when PDT is used as an adjunct to conventional therapy. There is as yet no evidence supporting the use of aPDT as an alternative to SRP [20]. It should therefore be emphasized that applying the photosensitizer dye without laser irradiation will not result in any change in the numbers of live bacteria; so the study confirmed that laser irradiation is essential for an antimicrobial effect to occur [21].

To date, only the photosensitizer benefits with modulated aPDT have been discussed. However, even if the whole of the photosensitizer dye solution cannot be activated using transgingival laser irradiation, there is another effect that should be mentioned — the low-level laser effect. Low-level laser irradiation results in an increase in mitochondrial ATP production and therefore a revitalization of cells [22,23]. Initial in-vivo tests of diode laser irradiation as an adjuvant therapy in conjunction with conventional periodontal therapy approaches proved to be quite successful with regard to bacterial elimination and wound healing [9]. Clinical attachment, bleeding, and pocket depth have also shown significantly better results [24]. Phototherapy has also been shown to be effective in periodontology, with a reduction in the probing pocket depth, plaque index, gingival index, gingival crevicular fluid, and matrix metalloproteinase-8 (MMP-8) [25]. It might therefore be assumed that LLLT alone could be used as a successful adjuvant method.

However, some studies have not identified any benefits when investigating lasers for nonsurgical periodontal treatment [26]. The discrepancies between various studies might be due to different power settings in relation to fluency and density, for example [27]. The effects of laser-induced temperature increase also need to be considered critically, since the vital pulp may suffer permanent damage [28]. In contrast to other laser systems such as the Er:YAG laser, it is not possible to remove mineralized deposits from the root surface using diode lasers [29].

5. Conclusion

The present study indicates that transgingival irradiation may be suitable for antimicrobial photodynamic therapy, since power transmission through the gingival tissue was observed in all specimens. However, the decrease in laser power caused by both the soft tissue and

Table 4
Overall mean power losses with the different lasers, without photosensitizer and with photosensitizer (PS).

ME AE (mW)	120 mW (117.6)	80 mW (80.4)	60 mW (55.0)	40 mW (40.9)	Overall mean power loss
n	15	15	15	15	
AME without PS	40.8 mW / 34.7%	22.4 mW / 27.9%	13.8 mW / 25.1%	8.1 mW / 19.8%	73%
AME with PS	23.4 mW / 19.9%	13.2 mW / 16.4%	6.4 mW / 11.6%	3.4 mW / 8.3%	86%

mW, milliwatts; mm, millimeter; cm^2, square centimeter.
AE, actually measured energy value; AME, average only value of the irradiation throughout soft tissue (mW/%); ME, manufacturer's stated energy value; PS, photosensitizer.

the photosensitizer needs to be taken into account. Laser power, the thickness of the soft tissue, application time, blood circulation, pigmentation of mucosa and blood, secretion and dye residue may be important in assessing the impact of transgingival laser irradiation and activation of the photosensitizer dye.

Declaration of Competing Interest

The authors declare no conflict of interest in this manuscript.

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