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Cavity Disinfection With a 445 nm Diode Laser Within the Scope of Restorative Therapy – A Pilot Study

Inés Lusche[1], Cornelius Dirk[2], Matthias Frentzen[1,3], Jörg Meister[3,4,5*]

[1]Department of Operative and Preventive Dentistry, Bonn University, Welschnonnenstrasse 17, D-53111 Bonn, Germany
[2]Oral Technology, Bonn University, Wilhelmsplatz 5, 53111 Bonn, Germany
[3]Center of Dento-Maxillo-Facial Medicine, Bonn University, Welschnonnenstrasse 17, D-53111 Bonn, Germany
[4]Center of Applied Medical Laser Research and Biomedical Optics (AMLaReBO), Bonn University, Welschnonnenstrasse 17, D-53111 Bonn, Germany
[5]Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

***Correspondence to**
Jörg Meister, Center of Dento-Maxillo-Facial Medicine, Bonn University, Welschnonnenstrasse 17, D-53111 Bonn, Germany
Tel: +49 228 287 22268;
Fax: +49 228 287 22694;
Email: jmeister@uni-bonn.de

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Abstract

Introduction: Cavity disinfection is necessary to prevent a progressive infection of the crown dentin and pulp. Increasing intolerance and resistance to antiseptics and antibiotics as well as the controversy over the effects of those on the dental hard tissue and composite have prompted the investigation of alternative treatment options. The objective of this pilot study is to evaluate the antibacterial potential of a diode laser with a wavelength of 445 nm in the cavity preparation using the bacterium *Streptococcus salivarius* associated with caries in conjunction with the characteristics and influences of dentin on light transmission.

Methods: The bactericidal effect of the laser irradiation was determined in culture experiments by using caries-free human dentin samples on bacteria-inoculated agar. For this, dentin discs (horizontally cut coronal dentin) of 500 µm and 1000 µm thicknesses were produced and irradiated with the laser with irradiation parameters of 0.7-1 W in a cw-mode and exposure times of between 5-30 s. Based on the different sample thicknesses, the penetration depth effect of the irradiation was ascertained after the subsequent incubation of the bacteria-inoculated agar. Additional influential parameters on the irradiation transmission were investigated, including surface moisture, tooth color as well as the presence of a smear layer on the dentin surface.

Results: The optical transmission values of the laser radiation for dentin were significantly dependent on the sample thickness ($P = 0.006$) as well as its moisture content ($P = 0.013$) and were independent of the presence of a smear layer. There was a 40% reduction in bacteria after the radiography of the 500-µm-thick dentin samples, which was shown as the lowest laser dose (443 J/cm^2).

Conclusion: These findings indicate that the diode laser with light emission at a wavelength of 445 nm is interesting for the supportive cavity disinfection within the scope of caries therapy and show potential for clinical applications.

Keywords: Diode lasers; 445 nm; Cavity disinfection; Dentin disinfection; Bacteria reduction.

Introduction

The treatment of caries, as a therapy of one of the most frequent bacterial diseases of the oral cavity, has evolved throughout the years. Thereby the growing understanding of caries as a chemical-parasitic process has strongly affected excavation techniques.[1] Whether the complete excavation is the preferred option over an incomplete excavation will continue to be a controversial topic.[2-4] Even so, to ensure the long-term vitality of the tooth, irrespective of the excavation depth, it is crucial to actively reduce the cavity bacteria remaining in the tubules.[5] Conventional liquid antiseptics such as chlorohexidine (CHX) or hydrogen peroxide (H_2O_2) have shown variously strong disinfection potencies, side effect potentials, possible interactions with the filling material as well as structural changes of the dental hard tissue itself.[6-10] Independently of this, the action of liquid antiseptics is restricted because they cannot penetrate deeply into the dentin due to inherent pressure gradients of the fluid-filled dentin tubules.[11,12] Consequently, for modern tooth preservation, it is important to evaluate disinfection alternatives which are depth-effective and show minimal side effects without forming bacterial resistance.

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Besides being established in human medicine (dermatology, oncology, hospital hygiene),[13-15] photonic technologies for microbial decontamination have also made headway into dentistry.[16] The antimicrobial potency of light of various wavelengths has manifested itself in combination with wavelength-specific sensitizers which destroy the opsonized bacterial membranes via various photo-oxidative processes through radical formation (antimicrobial photodynamic therapy, aPDT) as well as by direct action on bacteria.[17] The aPDT acts specifically through exogenic sensitizers,[18] that due to their optical absorption maximum, take up photonic energy (of lasers, for example) which directly leads to heat generation and/or radical- or singlet oxygen formation.[19] The presence of oxygen[20] as well as sensitizers which fit to the bacteria structure (gram(+) or gram(-)) and the light dose are therapy prerequisites. Possible intolerances and side effects on the patient and the theoretically possible development of resistance of a few bacterial strains to various sensitizers make the further development of photonic therapy approaches without sensitizers interesting.[21-25]

With its low photon energy, the aPDT- related wavelength in the red to infrared spectral range is sufficient for activating photosensitizers. Without sensitizers, an antibacterial effect could correlate with the dose increase which is harmful to people. Thus, blue light in the spectral range of 400-470 nm, with its closeness to the strongly antibacterially effective UV-light, but without its possibly damaging effect, has garnered great scientific interest because of its corresponding high photon energy.[26-29]

There are already *in vitro* and *in vivo* successes in the fight against gram(+) as well as gram(-) pathogens, whereby various authors disagree with one another about the question which bacteria form reacts more sensitively to blue light disinfection.[26] *Propionibacterium acnes*, *Helicobacter pylori*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*[13,26] have been shown to be just as photosensitive as *P. intermedia* and *P. gingivalis,*[25,30] which could be significantly reduced, depending on the power, through irradiation with blue laser light (405 nm). Using 405 nm irradiation, Rhodes et al[31] could demonstrate a significant reduction in the beta-lactam-resistant bacterium *E. coli* as well as a light sensitivity of antibiotic-resistant gram(-) bacteria. Meanwhile, in clear differentiation from the red spectrum, Lipovsky et al[32] identified the wavelength of 415 nm within the blue spectrum of light as the wavelength with the strongest bactericidal potential on various constructed germs (gram(+) and gram(-)). Despite its stable matrix formation, the caries-associated bacterium *S. mutans* successfully reacted to the blue light irradiation in studies.[22,33]

To date, no uniform conclusion can be drawn because of the heterogeneous experimental designs (test medium, direct or indirect irradiation, gram(+) or gram(-) bacteria) with diverse photonic parameters (wavelength, application type, emission power, irradiation time, repeats, dose) as well as the inhomogeneous bacteria spectrum of dental infections. The understanding of the effect of blue laser light (445 nm) is additionally complicated if studies on photonic disinfection are combined with the transmission properties of human samples of dentin (dentin type, cut direction, height, thickness). For the first time, this current pilot study investigates the possible use of a diode laser source (445 nm) as an antiseptic within the scope of the filling therapy and evaluates the material-specific influences of dentin on the light transmission.

Materials and Methods

Laser Source

A diode laser with a wavelength of 445 nm was used as the radiation source (SIROLaser Blue®, Sirona, Bensheim, Germany). The power range was 0.7-1 W in a continuous wave (cw) mode. The samples were irradiated using a quartz glass fiber with an outer diameter of $\varnothing_{outer}$ = 320 µm.

Dentin Sample Preparation

Using a band saw (Leitz, Sägemikrotom 1600®, Oberkochen, Germany), the horizontal cut samples of thicknesses of 500 µm and 1000 µm were prepared from caries-free coronal dentin of permanent teeth. Corresponding to the clinical analogy, the dentin discs were stored, upon leaving the smear layer alone, in a sterile rinsing solution (0.9% physiological saline solution, 0.01‰ sodium azide, Merck, Darmstadt, Germany).

Culture Medium

Petri dishes (∅ = 9 cm) (Greiner Bio-One GmbH, Frickenhausen) were filled with Caso Underlay-Agar (DPBS with 10 mL/L Caso Bouillon, 10 g/L agarose, 200 µL/L Tween-20) at a filling height of 1 mm and were inoculated with the test bacterium *S. salivarius* subsp. *salivarius*. The inoculated underlay-agar of the control petri dishes were drizzled with 5 µL of antibiotic ampicillin (25 mg/mL) and 5 µL SDS (sodium dodecyl sulfate) (10%) as the positive control.

After laser irradiation, the petri dishes (including the controls) were covered with a transparent nutritive Caso Overlay-Agar (doubly-concentrated Caso Bouillon with 10 g/L agarose) and were incubated at 37°C for 24 hours. Subsequently, the clean cultivation of the bacteria (homogenous growth) as well as the inhibition zones of the control petri dishes and irradiation fields could be evaluated.

Laser Treatment

To avoid foreign contamination, the dentin discs were sterilized before the laser treatment at 121°C for 20 minutes. Subsequently, the petri dishes filled with the underlay-agar were each given 4 dentin discs lying on cover-plate bridges according to a compass method

(Figure 1), and the dentin discs were irradiated in a clockwise direction (from 'South' to 'West' to 'North') with increasing exposure times of between 5 s to 30 s (1-2 irradiations per dentin disc). The 'East' disc stayed unirradiated as the control area.

The dentin discs were irradiated perpendicularly, whereby the laser fiber was adaptively kept at a distance of 1 mm to the surface, generating a diameter of approximately 1 mm. In the first trial, each petri dish was irradiated with light of a fixed power value (0.7, 0.8, 0.9 and 1 W) to enable a clear, unambiguous assignment of later inhibition zones. This was followed by irradiations with 0.7 and 1 W for 5, 20 and 30 s to determine possible trends and maximum values ($n_{total\ @\ 500\ \mu m}$ = 127). The acquisition of the laser transmission values (for dentin and/or agar) was carried out with a power and energy measurement instrument (LabMax-Top®, Coherent, Santa Clara, USA) as well as the corresponding detector unit (PM10, Coherent).

The applied energy between 3.5 and 30 J generates, with respect to the irradiated area of 0.0079 cm^2, doses in the range of 443 and 3797 J/cm^2 (Table 1).

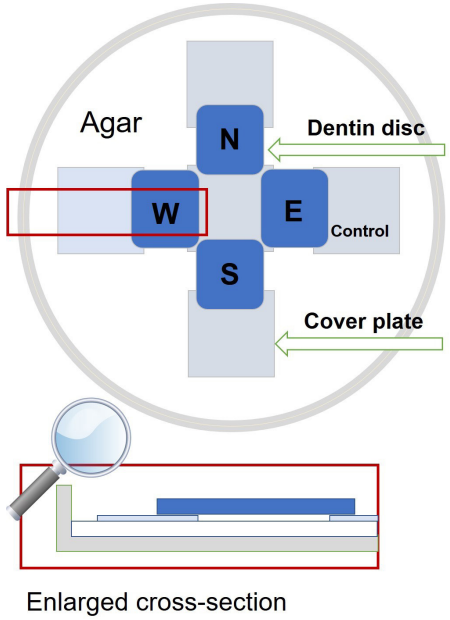

Figure 1. Test design compass; for every compass direction, there is a dentin disc which lies on a cover-plate bridge over a bacterially inoculated agar. From the direction "South" to "West" to "North", each dentin disc is irradiated once to twice in irradiation increments of 5-30 s, whereby the "East" disc is not irradiated.

Influencing Variables

A parameter screening was performed for evaluating possible material influences of the dentin discs on the laser light transmission. Here, the considered influencing parameters were: 1. Tooth color or brightness, 2. Smear layer, and 3. Moisture.

1. Tooth Color/Brightness

Dentin discs of 500 µm thickness were assigned, corresponding to the ascertained tooth color (Vita®, Bad Säckingen, Germany), to four brightness groups (n = 103):

- Group 1: Tooth color A1, B1, C1, D1 = Brightness 1
- Group 2: Tooth color A2, B2, C2, D2 and intermediate tones >1 = Brightness 2
- Group 3: Tooth color A3, B3, C3, D3 and intermediate tones >2 = Brightness 3
- Group 4: Tooth color A4, B4, C4, D4 and intermediate tones >3 = Brightness 4.

2. Smear Layer

Dentin discs (500 µm thickness) were irradiated using a laser output power of 0.7 W and 1 W with an exposure time of 20 s (n = 107), first with the smear layer and then without the smear layer by using two different demineralization substances (phosphoric acid or Calcinase®, lege artis, Dettenhausen, Germany) with the respective working time of 20 s (based on the clinical analogy of dentin conditioning).

3. Moisture

The laser light transmission of dentin discs of 500 µm thickness was measured at the power of 0.7 W for 20 s (n = 44) for the corresponding material states of "dry", "moist" and "moist with applied water drop".

Statistical Evaluation

The statistical calculations took place by means of OriginPro and SPSS. The significance level was set with an error probability of $\alpha < 0.05$. Various statistical test methods were used to analyze the influences of the parameters under investigation. T tests were used to investigate the influencing variables as smear layer, sample thickness and output power on light transmission. For group comparisons and influences, the data were analyzed by one-way ANOVA, Tukey HSD and Bonferroni. To analyze correlations between laser settings and bacteria

Table 1. Applied Dose in Ws/cm^2 of the Irradiated Sample Area Per Irradiation Energy Depending on the Irradiation Power (0.7-1 W) and Irradiation Time (5-30 s)

	Applied Doses in Ws/cm^2					
	5 s	**10 s**	**15 s**	**20 s**	**25 s**	**30 s**
0.7 W	443	886	1329	1772	2215	2658
0.8 W	506	1012	1518	2025	2531	3037
0.9 W	569	1139	1708	2278	2848	3417
1.0 W	632	1265	1898	2531	3164	3797

inhibition chi-square tests were used.

Results

Investigation of Light Transmission: Influencing Variables

1. Brightness

There was a comparable transmission behavior of the medians with normal distribution within a brightness tone (tooth colors). Moreover, no significant influence of the tooth brightness on the transmission could be detected for altogether non-normally distributed sample groups (1-4) ($P = 0.202$, Figure 2).

2.Smear Layer

There were no smear layer effects on the light transmission detected, neither in direct comparison of each dentin disc before and after smear-layer removal (t test: $P_{Calcinase} = 0.45$, $P_{Phosphoric\ acid} = 0.14$) nor between the sample groups before and after removal (one factor analysis of variance: $P_{Calcinase} > 0.28$, $P_{Phosphoric\ acid} > 0.06$). Likewise, there was no statistical significance found in the comparison of the transmission values of both related demineralization substances (ANOVA: $P > 0.97$, Figure 3).

3. Moisture

The light transmission decreased significantly with the degree of drying (moist with applied water drop → moist dry). Within the widely scattered transmission values of each group, a normal distribution of the random samples could be determined ($P > 0.2$) using the Kolmogorov-Smirnov test. The significant change in the transmission values of the material states of "dry" and "moist with applied water drop" was statistically confirmed via Tukey-HSD ($P = 0.013$) and Bonferroni ($P = 0.014$) (Figure 4).

Investigation of Light Transmission: Bacteria Inhibition

By means of the current parameter screening regarding dentin light transmission the moisture of the dentin discs was determined to be the main influencing factor. Neither the presence of a smear layer nor the color/brightness of the dentin discs showed discernible effects on the laser light transmission. Thus, the tests on photonic disinfection were performed on moist dentin discs with an existing smear layer in a random distribution of tooth color/brightness.

Of 127 irradiations on 500-µm-thick dentin discs, 52 irradiations caused the formation of a sufficient inhibition zone in the underlying bacteria-inoculated underlay-agar (Figure 5).

Inhibition zones were exhibited over all irradiation times and power values and occurred already at irradiations lasting only 5 s with laser power of 0.7 W, relating to an applied dose of 443 J/cm^2.

After the penetration of 1000-µm-thick dentin discs (n = 24), no formation of inhibition zones could be observed even at a maximum irradiation dose of 3797 J/cm^2.

Influence of Power and Exposure Time

Increasing the applied doses caused an increase in the

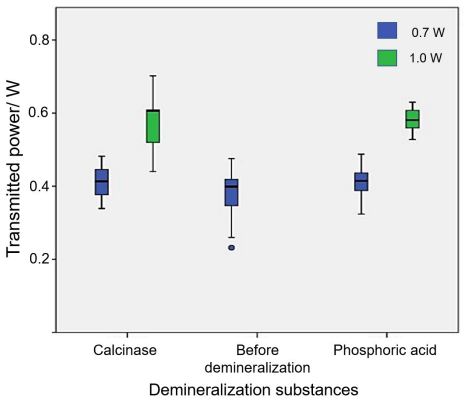

Figure 3. Dentin (500 µm-thick) Transmission Before (0.7 W) and After Demineralization With 2 Different Substances (Irradiation: 0.7 W and 1 W for 20 s). There was no difference before and after the treatment nor between the two substances with 0.7 W and 1 W; n = 103.

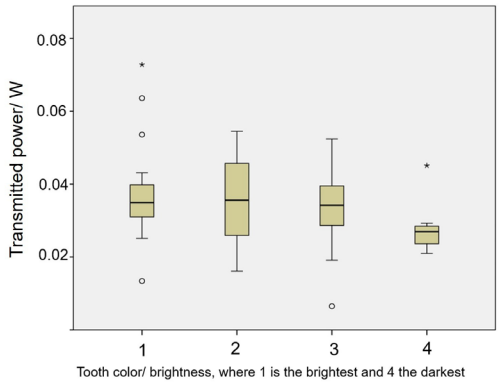

Figure 2. Presentation of the Transmission Values (Irradiations at 0.7 W for 20 s) of the 4 Possible Tooth Brightness Groups (1-4). There was no discernible influence of tooth brightness on the transmission. Group 4 was non-representative because of the small sample number; transmission through dentin (500 µm-thick) and agar (1000 µm-thick), n = 103.

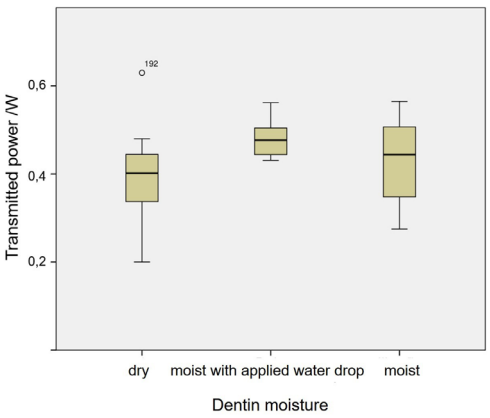

Figure 4. Dentin (500 µm) Transmittance (0.7 W for 20 s) of 3 Different Conditions. Transmittance changes significantly from the condition "moist with applied water drop" to "dry"; n = 44.

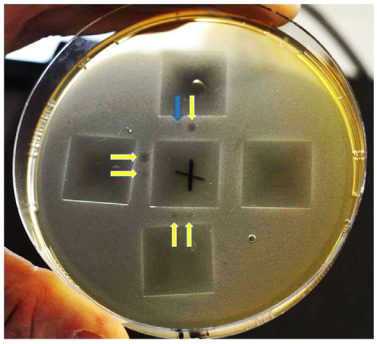

Figure 5. Exemplary Display Of Bacterial Inhibition Zones (Yellow Arrows) in a Homogeneous Bacterial Lawn (With Some Air Bubbles) After Laser Exposure. The compass model exhibits inhibition for exposure times from 5 s ("South") to 30 s ("North"). No visible inhibition was discerned after exposure for 25 s (blue arrow).

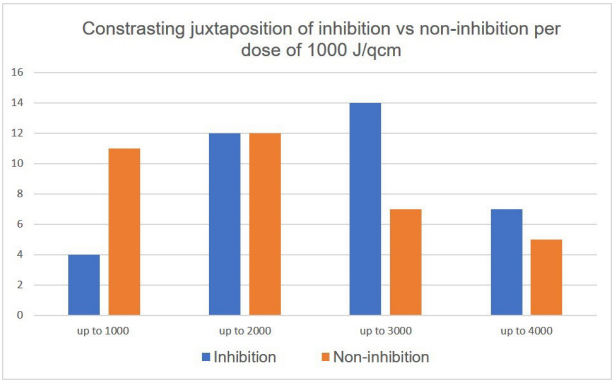

Figure 6. Increasing Doses of Laser Energy Led to Increasing Numbers of Inhibition Zones in Agar, Indicating a Trend; n = 127.

number of inhibition zones (Figure 6).

The maximum formation of inhibition zones was observed for all power settings at an irradiation time of 30 s, and with irradiation power of 1 W, this formation was observed over all time settings. The statistical crossing of both variables using various chi-square tests resulted in a significant correlation between the occurrence of inhibition zones and the emission power of the laser (P = 0.003) as well as the irradiation time (Pearson-Chi: P = 0.006, likelihood-quotient: P = 0.004).

A specific influence of the irradiation time or applied laser power on the formation of inhibition zones could not be determined here. Nonetheless, the distribution of the inhibition zones implies a possible optimal window of action.

Discussion

This current work aims to fundamentally investigate the possible use of blue laser light (445 nm) as an antiseptic within the scope of caries therapy. Isolated bacterial studies under idealized *in vitro* conditions (agar or bacterial suspensions) have already shown successful antibacterial results within the blue light spectrum.[21,26,34] Moreover, clinically relevant *in vitro* studies have already been performed on extracted teeth or tissue samples[35] as well as *in vivo* clinical tests on mice showing a significant reduction in the strongly resistant bacterium *Acinetobacter baumannii* in surface biofilm structures.[36]

Due to the lack of corresponding studies, this current investigation was compared to studies with similar designs but differences in method, wavelength, irradiation angle, dentin type and thickness or germs. The literature showed power-dependent bactericidal effects through dentin using 810 nm diode lasers with 0.5 to 7 W for 30 s and dentin slices from 500 µm up to 2000 µm,[37] with 1.5 W and irradiation cycles of 5 s (in pulsed mode) in root canals,[38] with 1 W and 1.5 W and irradiation cycles of 5 s and 1000 µm vertical dentin slices or using a 980 nm diode laser with up to 2.8 W for 32 s and root canal dentin slices from 100 to 500 µm.[39] Gutknecht et al[40] observed a power-dependent bactericidal effect of 445 nm diode laser irradiations (0.4, 0.6 and 1.2 W) through vertical slices of root canal dentin (300, 500 and 1000 µm) with an irradiation angle of 5° up to 40 s irradiation time.

Accordingly, the required broad range of our laser settings was adjusted to a power output maximum of 1 W and irradiation time maximum of 30 s. The extreme light attenuating the characteristics of dentin in combination with the highly scattering blue wavelength was also factored in. Considering the clinical aspect, a short application time (<1 minute) was deemed appropriate.

The design of this current pilot study regarding the bactericidal potential of the blue light laser was oriented as closely as possible to the clinical situation of the dentist's daily routine of filling therapy. Horizontal dentin discs of human dental crown showed perpendicular dentin tubules in the center (corresponding to a cavity oriented tangentially to the pulp cavity). Analogous to the anatomical tooth structure, these tubules were kept moist via the agar from below. The bacterially inoculated agar underneath the dentin discs (underlay-agar) simulated the state in matrices of bound bacteria in deeper layers of the dentin. The investigations on slightly moist dentin should approximate the state of the necessary collagen stabilization within the scope of dentin conditioning or the state of the dentin in the oral cavity. In addition, for the photonic disinfection per laser light, the smear layer was left alone in order to evaluate the disinfecting capacity prior to the application of filling materials without necessary dentin conditioning.

Various possible material effects on the optical transmission of dentin (as a prerequisite for potential photonic disinfection) were studied by means of a special parameter screening. The influences of tooth brightness, the smear layer, the degree of moisture, irradiation time and power, dentin strength and possible carbonization on the photonic disinfection capacity should specify the clinical application field of the investigated wavelength.

Investigation of Light Transmission: Influencing Variables

No influences of tooth color/brightness or the smear layer on the light transmission could be detected by taking into account the thickness of the dentin discs (500 µm) and the use of the presented test design. The results of the study on the influence of light transmission by the smear layer vary from significant to irrelevant due to noncomparable study designs such as those involving various disc thicknesses, cut directions/heights on the tooth, type and action time of the applied demineralization agents, wavelengths as well as the basic type and age of the tooth.[41,42]

The degree of dryness of the dentin discs correlates congruently with other studies, whereby a significant decrease in the optical transmission was found with increasing dryness.[41] In tests on wet- or dry-stored dentin samples, Kienle and Hibst[43] found rising scattering coefficients with an increase in the degree of dryness. Furthermore, Masic et al showed that water stabilizes the collagen structure, whose dehydration led to massive inhomogeneous changes in shape.[44]

In the present case, the orientation of the dentin tubules parallel to the light application favors the transmission of the radiation and thus increases the luminous efficiency.[45-48] Nevertheless, the layer thickness remains a strongly limiting factor of transmission. As could be observed in this study, dentin (500 µm) attenuated the laser power by approximately 30%, whereas a thickness of 1000 µm reduced the power to only a third.

Investigation of Light Transmission: Bacteria Inhibition

Using the present study design, a sufficient germ reduction of over 40% of the agar-bound bacterium *S. salivarius* subsp. *salivarius* could be detected after the transmission of the laser light through the dentin discs (500 µm-thick).

The increase in the bactericidal effect correlates accordingly with the applied dose (power and irradiation time). This principally concurs with the results of other authors.[37]

Carbonizations were observed at about 30 irradiations (of $n_{total\ @\ 500\ \mu m} = 127$), 23 of which simultaneously showed bacterial inhibition zones in the agar. This means that from a total of 52 occurring bacterial inhibition zones in the agar, carbonizations in the overlying dentin accordingly occurred during 23 irradiations. For the remaining 29 inhibition zones, the dentin discs remained undamaged. A tendency towards more incidences of carbonization was observed with the increase in the emission power. A specific influence of the irradiation time could not be established.

To determine the threshold of penetration depth, three power measurements were performed on the dentin discs of double thickness (1000 µm) under the same test conditions. As Figure 7 illustrates, there is a parallel shift of the conceived measurement curves towards significantly lower transmission values (*t* test, $P = 0.006$). In the bacteriological evaluation, there were no detected

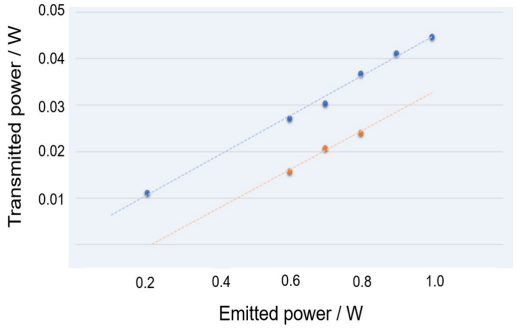

Figure 7. Average Transmission Values for Dentin Samples (500 µm-thick, Blue, n = 139, and 1000 µm-thick, Red, n = 16) With Agar (1000 µm-thick). The linear fit shows a parallel shift towards significantly lower values for thicker dentin samples; n = 155.

inhibitory effects on the agar-bound bacteria. These relationships are in line with the results of other studies, according to which an increase in the dentin thickness led to a strong intensity reduction of light in the blue spectral range.[41,49]

Since all test parameters in the current study were the same, presumingly an intrinsic influence of the dentin causes the intensity reduction. The extinction coefficient ε of the Lambert-Beer law for the material with respect to the specific loss of light intensity of a wavelength is composed of various attenuation factors such as absorption μ_a und scattering μ_s. With $\varepsilon = \mu_a + \mu_s$, it is certainly feasible that now, at a layer thickness of 1000 µm, the tooth color as well as the increasing scattering of the photon over the longer pathway in the dentin may adversely affect the transmission behavior.

Mode of Action

The antibacterial effect of laser light on tissue is affected by light-conducting structures, the penetration depth, the tissue interaction of the corresponding wavelength and the laser parameters.[37,49] The current study supports the other authors' reports, according to which the conduction of light in dentin does not originate from the long predominant assumption of waveguide theory, but rather from the forward scattering (g = 0.93) on the outer tubule walls within the peritubular dentin.[43,50] This is confirmed by the hardly changing optical transmission of dentin after the removal of the smear layer (open tubules).

The scattering behavior of light in tissue increases with a decrease in wavelength. This presumed disadvantage of the undesired light distribution of short-wave blue light as opposed to long-wave infrared light is balanced by the higher photon energy (445 nm: 2.8 eV, 810 nm: 1.53 eV, 980 nm: 1.27 eV), which acts as a driving force of direct antibacterial processes. Likewise, a photothermally active complex is possible, whereby the energy transport might occur directly to the bacterium or indirectly via the carbonized dentin areas, altered collagen components or thermally induced alteration of chemical components

of dentin. The tendential increase in the formation of inhibition zones in bacterially-infected agar with an increase in the irradiation dose speaks for photothermal effects, whereas the wide distribution of the inhibition events over all irradiation times and powers rules out a purely thermal cause. This was confirmed through studies in which diode laser irradiations (<5 W) of bacterial solutions or bacteria-agar compounds as well as of dentin samples (1000 µm) *ex vivo* had not caused any temperature increases of ΔT >6.5°C.[30,37]

Moreover, an indirect photothermal component through an increased absorption behavior of the drying dentin is feasible, whereby water, as a heat conductor, is no longer available. Indeed, dentin possesses a strong temperature damping, a low-temperature conductivity (0.0015 $Wcm^{-1}K^{-1}$ to 0.006 $Wcm^{-1}K^{-1}$) as well as supporting defense mechanisms of the pulpo-dentinal complex which can actively react to temperature increases.[11,51-53] This potential, however, is diminished with a decrease in the residual dentin thickness over the pulp [11,37,54] and the exceeding of critical temperature ranges.[55.56] Nevertheless, the combined factors of strongly scattering blue light and immensely light attenuating dentin could be considered essential in keeping the pulp safe. It could be shown that tooth irradiations with a diode laser of 445 nm generated half the temperature of 980 nm irradiations.[57] Furthermore, studies on photobiomodulation of pulp tissue showed the lowest cell stimulation on odontoblast-like cells after 450 nm irradiations through dentin of only 200 µm thickness in contrast to the compared wavelengths of 630 and 840 nm.[58] To date, the short- and long-term effects of blue light transmission through dentin on the bordering pulp *in vivo* have not been examined in the context of disinfection. However, the correlation of a significant reduction of the optical transmission with increasing dentin thickness (Figure 7) down to the complete loss of light intensity up to a dentin thickness of 4 mm[41] minimizes probable pulp damage caused solely by laser intensity (applying an appropriate distance). However, considering the known absorption maximum of the blue wavelength for hemoglobin, it is essential that a possible direct interaction between the laser light and the pulp tissue be investigated further.

Photochemical processes, in the sense of the activation of the cell's own sensitizers, possibly stand at the beginning of a phototoxic cascade which eventually results in the destruction of bacterial cell walls. Corresponding cell-own chromophores such as porphyrins in pigmented bacteria (for example, families of the *Prevotella* and *Porphyromona),*[13] which may be responsible for the light-induced inner-bacterial formation of reactive oxygen species (ROS), were already identified.[33] On the other hand, in *in vivo* studies, *Porphyromonas gingivalis* and *P. intermedia* reacted clearly more strongly to the blue light irradiation[59] than, for example, *P. nigrescens* und *P. melaninogenica*, whose porphyrin content is twenty times greater.[35] The phototoxic effect on non-pigmented bacteria is rather attributed to flavins and cytochromes, which assume the rule of the sensitizer and, similar to the aPDT, create toxic radicals in the presence of oxygen.[30,55] Makdoumi et al[60] could prove that the addition of riboflavin significantly increases the bactericidal potential of blue light (412 nm and 450 nm) on an examined MRSA-strain.

Even an indirect effect of blue light is possible. For instance, a reduction of the difficult-to-treat bacteria *Acinetobacter baumannii* and *Pseudomonas aeruginosa* could be attained through a bacterial change of the biofilm composition.[61] Furthermore, a combination of photothermally and photochemically acting bactericidal processes is feasible. On the one hand, in the actual chemotropic bacterium *A. baumannii,* Mussi et al[62] discovered a photoreceptor protein which is expressed by a gene (blue light-sensing A, blsA) and enables the bacterium to perceive blue light. On the other hand, the authors found an additional temperature effect on the ability to perceive light via the photoreceptor protein and to react to it.

Fixed study designs, which allow comparability and a synergistic gain of knowledge, would be helpful for understanding the mode of action of blue light as a total complex.

Clinical Relevance

Established through the present pilot study design, the independence of the light transmission from the smear layer showed that blue light irradiation (445 nm), unaffected by the presence of this layer, can eliminate bacteria in the dentin. Since the smear layer presents a sufficient barrier to liquid antiseptics,[63,64] the diode laser, thus, is interesting for the cavity disinfection prior to the filling therapy of all plastic fillings, independent of whether the acid-etching technique is necessary for it or the retention of the smear layer even is explicitly desired.

The evaluated penetration depth of light of the examined wavelength in dentin shows the potential to reach bacteria in a depth of 500 µm, that have evolved a dense inflammation.[5] This makes the diode laser interesting not only as a supplement but also as a possible replacement for liquid antiseptics.[65]

The significant influence of dentin moisture on light transmission is fundamentally interesting for dental practice. On the one hand, the required basic moisture of collagen within the scope of dentin conditioning offers the ideal prerequisite for a maximum laser light transmission (water window, index matching) and thus maximum disinfection of bordering dentin areas. On the other hand, the drying of the dentin can be used to actively reduce the light transmission of dentin layers close to the pulp.

By means of the current pilot study and parameter screening, one might presume possible increasing effectiveness with increasing laser doses for the investigated

blue wavelength with respect to its bactericidal effect (Figure 6). At doses from values of 2000 J/cm^2 upwards the inhibition outweighed the non-inhibition. However, the optimal laser dose for the bacterial spectrum to be studied, the influences of irradiation repeats, increasing irradiation distances with increasing areas and correspondingly decreasing applied doses, the influences of a moving laser application, additional water cooling, as well as possible clinical effects or side effects (reduced power parameter due to increased photon energy) all need to be investigated further.

Conclusion

Using the current study design, it could be shown that diode laser irradiations (445 nm) can eliminate bacteria in deep-layer dentin of the dental crown without the involvement of an exogenic sensitizer. Whereas the optical transmission of dentin (500 µm-thick) remains unaffected by the smear layer and by the tooth color, the photonic bactericidal activity depends on the laser dose as well as on the dentin layer thickness and moisture. The results of this pilot study provide basic data to extend clinical application possibilities of a therapeutic blue light source to include supporting cavity disinfection within the scope of the filling therapy and show the influences of dentin characteristics for clinically relevant application.

A fixed wavelength of light with broad therapeutic use is interesting from an economic perspective and likewise enhances its applicability in a dentist's everyday routine improving the overall safety of photonic treatment.

Conflict of Interests

The authors declare no conflict of interest.

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