Antimicrobial Efficacy of Photodynamic Therapy and Light-Activated Disinfection Against Bacterial Species on Titanium Dental Implants

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Purpose: *The aim of this study was to evaluate the efficacy of photodynamic therapy (PDT) and light-activated disinfection (LAD) against a 3-day-old bacterial suspension prepared from three different bacterial species present on titanium dental implants, and to analyze the possible alterations of the implant surfaces as a result of the PDT and LAD.* ***Materials and Methods:*** *The study was conducted on 72 titanium dental implants contaminated with a bacterial suspension prepared from three bacterial species:* Prevotella intermedia, Aggregatibacter actinomycetemcomitans, *and* Porphyromonas gingivalis. *The contaminated implants were incubated under anaerobic conditions for 72 hours and then were randomly divided into four experimental groups and two control groups (n = 12 each), according to the following treatment protocols: group 1 (PDT1): PDT (660 nm, 100 mW, 60 seconds) with toluidine blue; group 2 (PDT2): PDT (660 nm, 100 mW, 60 seconds) with phenothiazine chloride dye; group 3 (LAD): light-emitting diode (LED) with toluidine blue; group 4 (toluidine blue): treatment with only toluidine blue for 60 seconds. In the positive control group, the implants were treated with a 0.2% chlorhexidine-based solution for 60 seconds, and in the negative control group, no treatment was used.* ***Results:*** *The highest bacterial reduction was recorded in the PDT1 (98.3%) and PDT2 (97.8%) groups. The results of this study showed that there was a statistically significant reduction of bacteria in the PDT1 and PDT2 groups compared with the negative control group ($P < .05$), individually for each bacterial species as well as for all three species together. LAD was less effective than PDT1 and PDT2, and did not show a statistically significant difference compared with the negative control or any other treatment group. Toluidine blue was the least effective treatment in terms of both the total bacterial count and the individual count for each bacterial species.* ***Conclusion:*** *Both PDT1 and PDT2 protocols showed a high efficacy against a 3-day-old bacterial biofilm on dental implants and were more effective compared with LAD.* Int J Oral Maxillofac Implants 2018;33:831–837. doi: 10.11607/jomi.6423

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Peri-implantitis is an inflammatory process affecting the soft and hard tissue around an osseointegrated implant, resulting in the loss of supporting bone.[1] Microorganisms living on the implant surface are considered to be the initial cause of peri-implantitis.[2] The bacteria associated with peri-implantitis are very similar to advanced periodontitis, with most of them being spirochetes and nonmotile gram-negative bacteria such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola*, etc.[3] They adhere easily to the rough micro- and macrostructure of dental implants, a property that makes debridement and decontamination of the implant surface difficult.[4–7]

The treatment of peri-implantitis is based on arresting the inflammatory process and the bone loss that occurs as a result of the disease. Since peri-implantitis is initiated and exacerbated by bacteria, the removal of

these microbiota and their byproducts is thus essential for the treatment of peri-implantitis.[4] Decontamination of the implant surfaces can be performed by mechanical methods (plastic curettes, ultrasonic scalers, air-powder abrasives, and ablative lasers) and chemical methods (citric acid, H_2O_2, chlorhexidine digluconate, and ethylenediaminetetraacetic acid [EDTA]), which are also associated with the use of local and systemic antibiotics.[8] However, according to some studies, the total resolution of peri-implantitis could not be achieved using the aforementioned methods,[9,10] and the treatment approach can sometimes cause damage to the implant surface or promote bacterial resistance.[11,12] Due to these reasons, the attention has been focused on the effects of other treatment options.

Antimicrobial photodynamic therapy (PDT) has been proposed for bacterial elimination in the treatment of peri-implantitis, based on its successful application in the treatment of periodontitis.[13] PDT is a photo-chemical decontamination procedure based on the activation of a photosensitive dye by laser light, leading to the generation of cytotoxic reactive oxygen species[14,15] that are toxic for the bacterial cells.[13] The light needed to activate the photosensitizing agent must be compatible with the dye and with a specific wavelength in order to cause the transition of the photosensitizer from a low-energy ground state to a high-energy singlet state. Past in vitro, animal, and clinical studies reported various effects of PDT when used as an adjunct to the treatment of peri-implantitis.[16–18] In addition, PDT is a noninvasive and safe method that does not damage the implant surface.[13]

Recently, light-activated disinfection (LAD) has emerged as an alternative treatment option, and use of light-emitting diodes (LEDs) as a light source for LAD has been suggested. They are a low-cost alternative to lasers and have the potential of achieving similar results.[17,19,20]

The aim of this study was to evaluate and compare the efficacy of PDT and LAD against a bacterial suspension prepared from three different bacteria present on titanium dental implants, and to analyze the possible alterations of the implant surfaces as a result of PDT and LAD.

MATERIALS AND METHODS

Study Sample

The study sample consisted of 72 sterile titanium dental implants (BlueSky, Bredent) with a diameter of 4.0 mm and length of 12 mm.

Bacterial Contamination of Dental Implants

All microbiologic procedures were performed at the laboratory of the Department of Clinical and Molecular Microbiology, University Hospital Centre Zagreb.

A bacterial suspension was prepared from *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, and *Porphyromonas gingivalis*. The strain of *Prevotella intermedia* was isolated from a clinical sample at the Clinical Hospital Centre in Zagreb. *Aggregatibacter actinomycetemcomitans* (ATCC 33384) and *Porphyromonas gingivalis* (ATCC 33277) were purchased from The Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Germany, in frozen cultures. The bacteria were grown separately in Columbia Agar for 72 hours, and then, using thioglycolate broth, a bacterial suspension was prepared for each of the bacteria and mixed together in a joint suspension. A density of 600 nm equivalent of 1×10^8 colony-forming units (CFU)/mL was set by optical densitometer (Densimat, Biomerieux).

Each implant was placed in sterile Eppendorf tubes (Eppendorf) containing 300 µL of the prepared bacterial suspension and incubated under anaerobic conditions for 72 hours using the GasPak anaerobic system (Becton, Dickinson and Co). The bacterial suspension covered the entire lengths of the implants in the Eppendorf tubes.

Antimicrobial Protocols

After the incubation period, the implants were taken out of the anaerobic chamber conditions and randomly divided into four groups (n = 12 implants per group) and two control groups (12 implants each).

Group 1: Photodynamic Therapy (PDT1)

The implants were treated with a diode laser (660 nm, Laser HF, Hager Werken) with a 320-µm optical flat fiber tip and a toluidine blue–based dye (155 µg/mL, LaserHF Paro-PDT solution). The laser parameters were as follows: power output, 100 mW; power density, 124.3 W/cm^2, continuous mode of irradiation; and time of irradiation, 60 seconds.

Group 2: Photodynamic Therapy (PDT2)

The implants were treated with a diode laser (660 nm, Helbo Therapielaser, Helbo Photodynamic Systems) and a three-dimensional (3D) fiber optic tip with a spot size of 0.06 cm in diameter (HELBO 3D Pocket Probe, Helbo Photodynamic Systems), with phenothiazine chloride dye (10 mg/mL, Helbo Blue photosensitizer). The laser parameters were as follows: power output, 100 mW; power density, 35.37 W/cm^2; continuous mode of working; and time of irradiation, 60 seconds.

Group 3: Light-Activated Disinfection (LAD)

The implants were treated with LED curing light (Optilight Ld, Gnatus). The curing light was modified with a red LED light, (660 nm, LZ1-00R205, Ledengin). A toluidine blue solution (Biognost) was used as a photosensitive dye. The diameter of the light source tip was 6 mm. The

parameters were: power output of 200 mW; power density, 0.71 W/cm^2; continuous mode of working; and time of irradiation, 60 seconds of the treatment time.

The implants in the previous three groups (PDT1, PDT2, and LAD) were coated with the photosensitive dye for 60 seconds. They were then rinsed with a sterile saline solution in order to remove the excess photosensitive dye. In order to standardize the irradiation treatment protocols for all implants, the implants were placed in a rotational electric motor (Shenzhen Powerful Electronics), with a power of 12 V, 120 mA with a rotating speed of 10 rounds per minute. An insertion drill (SKY TK Mounter long) was fixed to the electric motor, and then, the implants were placed on the insertion drill. The light source was placed 5 mm away from the surface of the rotating implant, and the treatment time was 60 seconds (Fig 1).

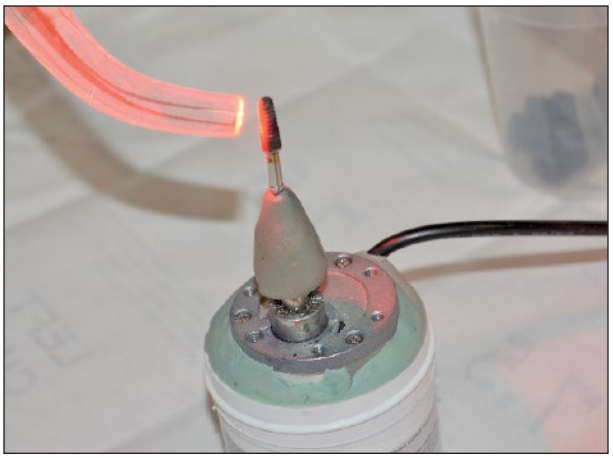


Fig 1 The implant placed on the rotational electric motor and treated with the light source (LAD), 5 mm away from the implant.

Group 4: Toluidine Blue Treatment

The implants were immersed in a photosensitive dye (toluidine blue, Biognost) solution (1 mg/mL) for 60 seconds, and then, they were rinsed with sterile saline solution to remove the excess dye.

In the negative control group, the implants did not receive any treatment, and after their removal from the bacterial suspension, the implants were kept in room conditions for 60 seconds before microbiologic analysis.

In the positive control group, the implants were immersed in 0.2% chlorhexidine gluconate solution (Curasept ADS Curaden International) for 60 seconds. After removal from the chlorhexidine solution, the implants were rinsed with sterile saline to remove the remaining solution.

Microbiologic Analysis

Immediately after the treatment procedures, every implant was placed in 1.5 mL Eppendorf test tubes containing 500 µL of phosphate-buffered saline (PBS) and vortexed for 60 seconds to remove the remaining bacterial cells from their surfaces. From each tube, 100 µL were transferred to 100 µL of Mueller Hinton broth, and a volume of 20 µL of PBS was also transferred to a microplate well containing 180 µL of broth creating a 10-fold dilution. Ten-fold serial dilution was performed using 96-well microtiter plates; 30 µL of suspension from each well was then inoculated to Brucella agar plates. The plates were incubated in anaerobic conditions for 72 hours, and the CFUs were counted. Macroscopically distinctive colonies were confirmed with MALDI Biotyper (Bruker Daltonics).

Scanning Electron Microscopy Analysis

After microbiologic analysis, one random implant was chosen from each of the treatment groups, and one sterile nontreated implant was chosen for scanning electron microscopy (SEM). The implants for SEM were stored in paraformaldehyde 2% for 2 hours. Then, the implants were dehydrated in increasing concentrations of ethanol (60%, 75%, 95%), for 30 minutes in each and left for drying all night. The surfaces of the implants were observed using SEM (Vega TS5136MM, Tescan). The SEM images were taken at 1:250 magnifications, and all the images were taken between the fourth and the fifth thread.

Statistical Analysis

To determine the difference between the groups for each bacterial species separately and for the total count of bacteria, the obtained data were compared by analysis of variance test (ANOVA). Multiple comparisons among the groups were performed using the Tukey test. The level of significance was set at 5%.

Due to the large differences in the standard deviations among the groups, the data were transformed according to the following equation:

$$L = \log_{10}(N + 1)$$

In order to calculate the bacterial reduction in percentage, as compared with the negative control group, the statistical data were not transformed into logarithmic form. The following formula was used instead:

$$1 - \frac{T}{C} = 100\left(1 - \frac{T}{C}\right)\%.$$

Here, *T* stands for the treatment group and *C* stands for the negative control group.

All calculations were performed using the statistical package SAS system for Windows (Release 8.02, SAS Institute).

Table 1 Mean and SD for Each Bacteria Separately and Total Bacterial Count Presented in Logarithmic Form

	A actinomycetem			*P gingivalis*			*P intermedia*			Total		
Group	Mean	SD	*P**	Mean	SD	*P**	Mean	SD	*P**	Mean	SD	*P**
PDT1	3.3^{b}	2.2	< .0001	3.7^{bc}	2.5	.0003	4.3^{ab}	2.4	.0096	4.7^{bc}	2.3	.0022
PDT2	3.1^{b}	2		2.8^{c}	2.4		3.6^{b}	2.4		3.9^{c}	2.3	
LAD	5.4^{ab}	2.3		5.2^{abc}	2.2		5.4^{ab}	3.1		6.1^{abc}	2.5	
TB	6.2^{a}	2.3		6.2^{ab}	2.0		6.7^{a}	2.4		7.0^{ab}	2.2	
PC	4.7^{ab}	2.7		4.7^{abc}	2.3		4.9^{ab}	2.7		5.4^{abc}	2.6	
NC	6.5^{a}	1.7		6.8^{a}	1.9		7.0^{a}	2.2		7.4^{a}	1.8	

**P* value for ANOVA test.
Statistical analysis with ANOVA test and comparisons between every group with Tukey post hoc test. The letters in superscript (a,b,c) are the results of post hoc comparison (Tukey test). When groups are compared, the presence of the same letter in superscript means that there is no statistically significant difference among them.
LAD = light-activated disinfection; TB = toluidine blue; PC = positive control; NC = negative control; ANOVA = analysis of variance.

Table 2 Mean and SD for Each Bacteria and Total Count (in cfu/mL)

	A actinomycetemcomitans			*P gingivalis*			*P intermedia*			Total		
Group	Mean	SD	R (%)	Mean	SD	R (%)	Mean	SD	R (%)	Mean	SD	R (%)
PDT1	2.08E+5	(3.31E+5)	99.8	6.64E+6	(2.00E+7)	98.0	6.68E+6	(1.23E+7)	98.0	1.35E+7	(2.49E+7)	98.3
PDT2	1.92E+5	(5.71E+5)	99.8	2.55E+5	(5.88E+5)	99.9	1.68E+7	(5.77E+7)	95.1	1.72E+7	(5.82E+7)	97.8
LAD	6.05E+7	(1.71E+8)	50.1	2.83E+7	(6.13E+7)	91.4	1.59E+8	(2.67E+8)	53.5	2.48E+8	(4.66E+8)	68.7
TB	8.43E+7	(1.18E+8)	30.4	3.43E+7	(4.33E+7)	89.6	1.79E+8	(2.70E+8)	47.6	2.98E+8	(3.53E+8)	62.4
PC	4.42E+7	(8.76E+7)	63.6	1.67E+7	(3.17E+7)	94.9	7.50E+7	(1.22E+8)	78.1	1.36E+8	(2.02E+8)	82.8
NC	1.21E+8	(2.90E+8)		3.28E+8	(6.78E+8)		3.42E+8	(5.54E+8)		7.91E+8	(1.50E+9)	

R = reduction.
Bacterial reduction presented in percentages compared with NC.

RESULTS

The results showed that there were statistically significant differences between the groups for each bacterial species separately and also for the total number of bacteria ($P = .002$). These data are presented in logarithmic form in Table 1.

The bacterial reduction compared with the negative control group, expressed in percentage and log reduction, is shown in Table 2. The largest bacterial reduction in terms of the total count of bacteria was recorded in the PDT1 (98.3%) and PDT2 (97.8%) groups. These two groups were significantly superior compared with the negative control group ($P < .05$). The LAD group caused a 68.7% bacterial reduction and did not have significant differences when compared with the negative control group.

The PDT1 and PDT2 groups showed the largest bacterial reduction when compared with each of the bacteria separately. Compared with the negative control group, the PDT1 group was significantly more effective in the eradication of *A actinomycetemcomitans* and *P gingivalis*, however without a significant difference in the eradication of *P intermedia*. The PDT2 group was significantly more effective in the eradication of each of the bacteria when compared with the negative control group ($P < .05$).

The toluidine blue group was the least effective compared to the other study groups with only 62.4% bacterial reduction; moreover, it did not differ significantly compared with the negative control group in terms of the total number of bacteria or for each of the bacteria separately.

The positive control group caused 82.8% bacterial reduction, and none of the study groups (PDT1, PDT2, LAD, and toluidine blue) were significantly different in terms of the total number of bacteria.

The SEM images obtained from the PDT1, PDT2, and LAD groups visually did not show any surface alterations, cracks, or damage, when compared with the images obtained for the sterile implants, and their surfaces appeared to be very similar to the surface of the sterile implant (Figs 2 to 5).

Fig 2 *(Left)* Sterile implant; magnification 1:250.

Fig 3 *(Right)* Implant treated with PDT1; magnification 1:250.

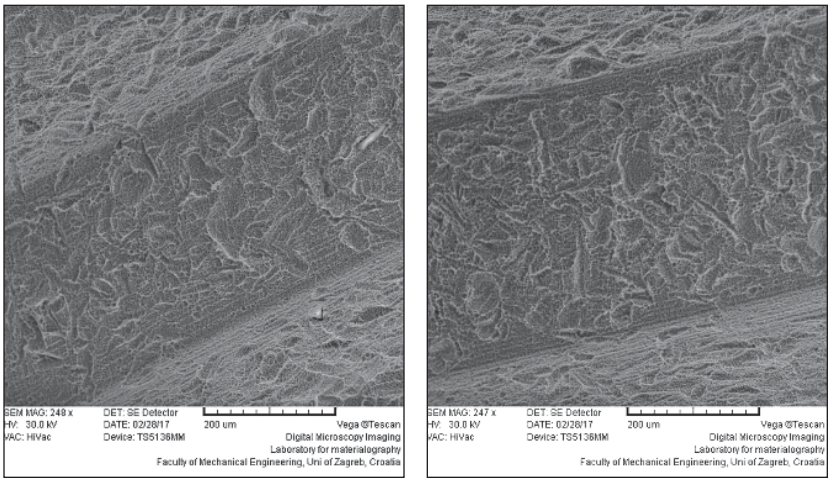
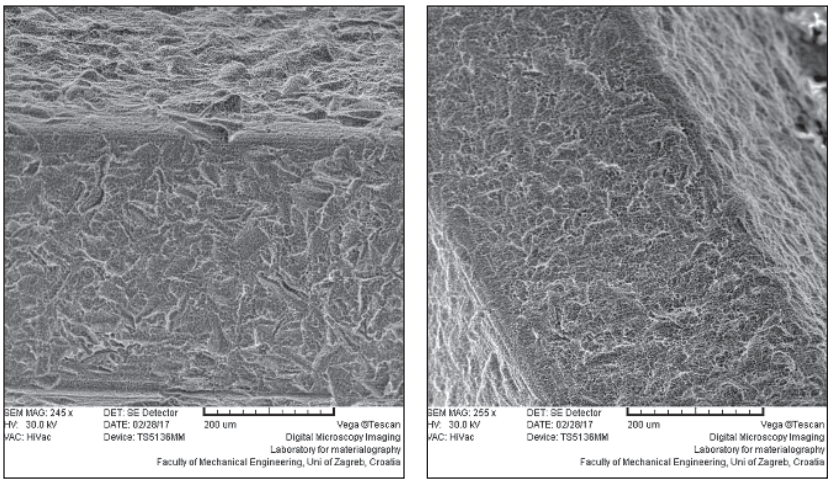


Fig 4 *(Left)* Implant treated with PDT2; magnification 1:250.

Fig 5 *(Right)* Implant treated with LAD; magnification 1:250.



DISCUSSION

The lack of a clear protocol for treating peri-implantitis has increased the focus of the scientific community toward the use of PDT as a treatment option or an adjuvant treatment for peri-implantitis in recent years.[16,21,22]

In the present experimental study, the effect of PDT was evaluated on artificially contaminated dental implants under in vitro conditions. The deliberate contamination of the implants was performed in order to reproduce the adhesion stage of biofilm formation. A similar methodology has been used in many other studies that deal with in vitro contamination and decontamination of titanium implants.[13,21,22]

The main focus of this study was to determine if PDT is efficient as compared to the negative control group and to the conventional disinfection with chlorhexidine solution (positive control group). Furthermore, the focus was to investigate if different types of devices and photosensitizers affect the results of PDT and whether different bacteria react differently to PDT.

Similar to previous studies,[21,23] the results of the present study showed that PDT causes great bacterial reduction compared with the negative control group, which, as expected, had the greatest bacterial count.

PDT1 and PDT2 did differ significantly from the negative control group in terms of each bacterial species separately and also in terms of the total count of bacteria. When compared with the positive control group, there was no statistically significant difference even though there was greater bacterial reduction in PDT1 and PDT2.

LAD was the least effective treatment group among the PDT groups and did not differ significantly compared with the negative control or positive control groups, having even less bacterial reduction than the positive control group. However, it must be pointed out that the implants belonging to the LAD group were treated using a modified dental LED curing light and not with a laser light source. This was done to test the LED light as an alternative light source to lasers. Many recent studies have tested the efficacy of LED lights as

a photodynamic light source. The results of these studies are dependent on the study design, power output, irradiation time, and the photosensitizer used. In their study, Nielsen et al[24] concluded that the combination of toluidine/red light has an excellent antimicrobial effect compared with riboflavin/blue light. Similarly, Umeda et al[25] reported a good bactericidal effect when using LED in combination with methylene blue or toluidine blue. In contrast, the results of the present study showed a reduction of only 68.7% for the total count of bacteria. The authors assume that the difference in power density between the LAD and the other two study groups (PDT1 and PDT2) might be the reason that LAD was less effective in reducing the bacterial count. Power density is dependent on the power output of the device and the light beam diameter. Since the device used for this research was an LED curing light, the light beam diameter was larger than that in PDT1 and PDT2, which led to lower power density.

Regarding the photosensitizers used, the most common photosensitizers used for PDT treatments are phenothiazine derivatives. They are also the most effective photosensitizers for eradicating oral microorganisms.[24] However, comparing photosensitizers in in vitro conditions is very difficult due to the differences in absorption by the photosensitizer and bacteria.[26] Moreover, some bacteria have the capability of producing endogenous photosensitizers (eg, *Porphyromonas gingivalis*), a property that further proves the difficulty in comparing photosensitizers in in vitro conditions.[27] However, the present study did not find any difference between the groups (PDT1, PDT2, and LAD) that were treated using different photosensitizers in combination with a light source.

In another study group, a photosensitizer was used as the sole treatment option without the application of light (toluidine blue group). This group was the least effective of all treatment groups investigated in this study. There was no significant difference between this group and the negative control group, in terms of the total bacterial count or in terms of each bacterial species, separately. As in many other studies, this further proves that in order to have an effective PDT, there must be an interaction between the light source and the photosensitizer. The use of a photosensitizer or light alone is not effective and is not recommended as a treatment option.[21,28,29]

In addition to the antimicrobial effect of PDT, the aim of the present study was to examine if PDT causes physical alterations on implant surfaces. The authors did not observe any structural changes on the implant surfaces. Similar findings have also been reported by Haas et al,[17] who similarly examined the implant surfaces after treatment with PDT and compared their findings with sterile implants. This proves that PDT can be safely used for the decontamination of implant surfaces without concerns regarding potential damage to the implant surfaces.

CONCLUSIONS

Within the limited scope (in vitro contamination and decontamination of the implant surfaces) of this study, it can be concluded that PDT is a successful treatment option for decontaminating the surfaces of dental implants.

For understanding the effect of PDT further, in vitro and clinical studies should be performed to evaluate PDT. Treatment time, type of photosensitizer, and power of the light source should be further investigated so that a proper and effective treatment protocol can be established.

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