Evaluation of the Antimicrobial Efficacy of Different Types of Photodynamic Therapy on the Main Pathogenic Bacteria of Peri-Implantitis

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Chapter

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Dragana Gabrić, Ana Budimir, Ivona Bago, Luka Marković, Verica Pavlić and Bleron Azizi

Abstract

Every year, with the increasing number of dental implants placed, there is an increase in the incidence of peri-implantitis. The treatment of peri-implantitis is very complex and among other things includes mechanical and chemical decontamination of the implant surfaces, which is very challenging and often not predictable due to the surface properties of the implants. Photodynamic therapy recently has emerged as a potential treatment alternative or adjuvant treatment to peri-implantitis. Its potential to decontaminate implant surfaces without damaging the surface and the implants surrounding tissues has generated much interest in the scientific community. The possibilities of photodynamic therapy in treatment of peri-implantitis are opening new challenges in establishing optimal conditions for the clinical application of aPDT. Due to its non-invasiveness and ease of use this method can be effective when applied alone or as an adjunct therapy to conventional methods for treating peri-implantitis.

Keywords: photodynamic therapy, implants, peri-implanitits, titanium, zirconia

1. Introduction

Antimicrobial photodynamic therapy (aPDT) is becoming a treatment option in dental medicine in different areas such as the diagnosis of malignant transformation of oral lesions, the treatment of head and neck cancer, as well as the treatment of bacterial and fungal infections [1, 2].

In periodontology, the conventional therapeutic approach for treating periodontal diseases consists of mechanical cleaning combined with chemical decontamination, or the use of antimicrobial therapy which can be applied systemically or locally. The mechanical debridement has its own limitations in removing all the infections, such as the difficulties in reaching deep pockets and, as a result, the etiological factors continue to damage the periodontal ligament. Also, when mechanical debridement is used frequently, it can cause damage of the root surface [3].

The limitations of the conventional periodontal therapy have shifted the focus towards aPDT, as an effective alternative treatment for periodontal diseases [4–8]. aPDT is having many advantages over conventional therapy. The main advantage is the fact that photosensitizer can be placed directly into the periodontal pocket and then activated with an optical fiber tip in order to kill microbial cells, without damaging the host tissues. This makes aPDT a safe procedure against periodontal microbiota [9, 10].

Many studies have demonstrated potential improvements after the use of aPDT in conjunction with mechanical debridement [11–13]. However, there are several studies that report different results [5, 14–16]. Atieh suggested as a result of his meta-analysis, potential improvements after aPDT combined with scaling and root planning in probing periodontal pocket depth (PPD) reduction and greater clinical attachment level (CAL) gain [13]. Similarly, in their study Sgolastra et al. reported that the combination of aPDT and conventional treatment provides additional benefits by reducing the PPD and increasing the CAL [11].

In endodontics, aPDT is used for the disinfection of the root canal. Conventional endodontic treatment consists of a combination of mechanical cleaning and shaping of the canals, the use of disinfecting solutions for irrigation and the placement of medicaments in between appointments. Sometimes, due to the root canal anatomy it is difficult to completely disinfect the canals by using only mechanical and chemical decontamination methods [17, 18]. aPDT demonstrated promising results as an adjunct therapy for the root canal disinfection in many studies. Raymond et al. [17] evaluated the efficacy of the combination of conventional treatment with photodynamic therapy *in vitro*. Their results showed that the combination of both therapies is more effective than the use of traditional treatment alone. Rios et al. [19] in their study used a combination of light-emitting diode (LED) as a light source and toluidine blue O dye as a photosensitizer. They suggested that photodynamic therapy can be used as an adjunctive antimicrobial procedure in endodontics. Similarly in their clinical study, Bago et al. [20] demonstrated that aPDT when used as an addition to the conventional mechanical and chemical root canal cleaning, can lead to significantly more reduction of the bacteria and in some samples the total elimination of the bacteria.

Photodynamic therapy is used also in oral and maxillofacial surgery due to its potential to be used as an anti-cancer treatment and its antimicrobial potential. Oral squamous cell carcinomas (SCC) are the most frequent tumors in the oral cavity [21]. Up to date the traditional methods for treating SCC have not been very successful in increasing the 5-year survival rate. Furthermore they cause different side effects such as mouth sore, jaw pain and difficulties in chewing or swallowing [22].

One of the developing factors of oral SCC are considered to be the pre-malignant lesions such as erythroplakias and dysplastic leukoplakias. Around half of oral SCC cases are associated with leukoplakias [23]. The potential therapeutic possibilities of photodynamic therapy are not limited only for oral SCC and other head and neck cancers, but also against pre-malignant, primary, recurrent and metastatic lesions [24, 25]. PDT when compared to conventional treatments of these lesions, has an advantage due to its selective tumor destruction and minimal invasiveness without affecting the healthy tissues. In addition, it can combined with conventional therapy to increase the overall treatment success [26].

The PDT antimicrobial potential in oral and maxillofacial surgery, is mostly used for the disinfection of soft tissue or bone during surgical interventions, as a preventive measure. In a study done by Neugebauer et al. [27] it was demonstrated that use of aPDT caused significantly lower incidence of alveolar ostitis. In a another study it was concluded that the effect of photodynamic therapy is almost the same as the effect of 2.5% NaOCl without causing adverse effects on surrounding tissues on periapical lesion model *in vitro* [28]. Batinjan et al. [29] showed that

aPDT causes reduced postoperative wound swelling and decreased wound temperature after the removal of the impacted third mandibular molar.

PDT has recently also been used as an adjuvant therapy for the treatment of medication-related osteonecrosis of jaws (MRONJ), that is highly related to bisphosphonate-related osteonecrosis of the jaw (BRONJ). In a study done by Minamisako et al. [30], it was suggested that both low level laser therapy (LLLT) and PDT are beneficial in the clinical management of the MRONJ. Similarly, Rugani et al. [31] concluded that photodynamic therapy can be used as treatment option in the early stages of BRONJ or as an adjunct therapy when surgical intervention is indicated.

1.1 Peri-implant diseases and aPDT

In 1978. Brånemark presented two-stage threaded titanium implants in a root-form [32]. The concept of osseointegration of the implants was first brought during the 1950s and 1960s after observing bone growth in contact with titanium. Brånemark defined osseointegration as: "A direct connection between living bone and a load-carrying endosseous implant at the light microscopic level." [33]. Since then, dental implants have become a long-term reliable treatment option for replacing missing teeth [34]. An ideal implant should have the following properties: biocompatibility, adequate toughness, strength, corrosion resistance, facture and wear resistance [35–37].

The "gold standard" of dental implants are considered to be the implants produced from titanium and its alloys. Titanium has excellent biocompatibility and it was shown that long term surgical rates of titanium implants are excellent [38, 39]. However, due to their dark gray color sometimes the implants can reflect through the peri-implant soft tissue. This poses an esthetic challenge especially when a thin biotype of gingiva is present or when there is a resorption of the buccal lamina [39, 40]. Due to these reasons, many scientists have shifted their focus into producing ceramic implants [41].

The infection around dental implants can be presented as peri-implant mucositis or peri-implantitis. Peri-implant mucositis is a reversible inflammatory process and it affects only the soft tissues around the dental implant. This is followed by reddening, swelling and bleeding on probing [42]. Peri-implantitis on the other hand affects both soft and hard tissues around the implant and as a result loss of supporting bone occurs [43]. The microbial etiology of peri-implantitis is well documented in literature [44]. The microorganisms found in peri-implantits are very similar to those found in advanced periodontitis [45, 46]. Most of them are spirochetes and non-motile anaerobic Gram-negative bacteria such as: *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans,, Prevotella intermedia, Tannarella forsythia, Treponema denticola* etc. [47]. In the oral cavity the implant surfaces are colonized very rapidly by the bacteria, which leads to the formation of a bacterial biofilm on the implant surface. When peri-implanitits is in its early stages, there are no significant symptoms and most of the time it is diagnosed during routine dental check-up. It is of great importance to diagnose peri-implanitits in its early stages in order to prevent the progression of the disease and increase the chances for good osseointegration [48].

According to Teughels et al. [49], the quantity and quality of plaque formation and bacterial adhesion on implant surfaces is influenced by the chemical composition, and the surface roughness of the implant. Rough surfaces and those with greater surface free energy, accumulate more plaque. Furthermore, initial bacterial adhesion is attracted more to surfaces with high wettability and pits and grooves in the roughened surfaces. The formation of bacterial plaque in these surfaces is difficult to remove.

To date, many treatment methods have been proposed for treating peri-implantitis. They can be grouped in two categories: resective and regenerative therapies [50].

The main goal of resective treatments is to eliminate the etiological factors of peri-implanittis and maintain optimal conditions. These treatments are mainly done by cleaning and decontaminating implant surfaces. Regenerative treatments aim to reconstruct the pre-existing hard and soft tissues by using bone substitute grafts, membranes and growth factors [50, 51]. Resective treatment of peri-implanitits is similar to the treatment of periodontitis and it consists of mechanical cleaning of the biofilm from the implant surface. This is of the utmost importance when treating peri-implanitits. During resective treatment, plastic curettes, air-powder abrasive or ablative lasers and ultrasonic scalers are used [52]. The main objective is to clean the surface which can stop the progression of the disease and increase the chances for re-osseointegration of the implant. However, due to the implant surface roughness, the bacterial adhesion and colonization is very difficult to remove and sometimes mechanical debridement alone is not very effective [53]. It has been suggested by some authors that the mechanical elimination of the implant threads and then smoothing the implant surface (implantoplasty) should be done, in order to improve the decontamination of the implant surface. In addition this procedure allows better maintenance and oral hygiene when threads are exposed to the oral environment [54]. When decontaminating the implant surface, the use of metallic curettes is not recommended due to the fact that they can alter the surface roughness of the implant and favor bacterial colonization. As an alternative, plastic curettes should be used because they do very little damage or none at all [55, 56].

Recently, as a treatment alternative, many scientists have shifted their focus towards the laser decontamination of the implant surfaces. In a study done by Kreisler et al. [57] the mechanical effects of Nd:YAG (Neodymium: yttrium-aluminum-garnet), Ho:YAG (Holmium: yttrium-aluminum-garnet), Er:YAG (Erbium: yttrium-aluminum-garnet), CO2 (Carbon dioxide) and GaAlAs (Gallium-Aluminum-Arsenide) lasers were evaluated, on different types of implant surfaces.. According to their results, Nd:YAG and Ho:YAG lasers cause significant damage to the implant surfaces, while CO2 and Er:YAG lasers when used in specific power settings do not cause any damage. GaAlAs laser did not damage the implant surface in any power settings. As an adjunct therapy to mechanical methods for treating peri-implantitis, the use of chemical decontamination and antibiotic therapy are being used with the aim of improving the treatment outcome. The most commonly used antimicrobial solutions are chlorhexidine, hydrogen peroxide, tetracycline or minocycline, citric acid, and phosphoric acid [58].

Recently aPDT has emerged as a new treatment option or adjuvant treatment to the conventional treatment of peri-implantitis. Its potential to decontaminate the implant surfaces without any damage to the implant or the surrounding tissues has generated a lot of interest in the scientific community. In addition aPDT is more effective than the use of lasers alone [53]. In their study, Hayek et al. [59] demonstrated that aPDT is both effective and non-invasive method when compared to traditional therapy during surgical treatment of peri-implantitis with elevated mucoperiosteal mucosal flaps. These beneficial characteristics of aPDT make it as promising novel and non-invasive method which can be used alone or as an adjunct therapy of peri-implantitis. [2].

2. The efficacy of photodynamic therapy in *in vitro* conditions

There are many *in vitro* studies evaluating the efficacy of photodynamic therapy against causative bacteria of peri-implantitis. The aim of our research was to evaluate the efficacy of aPDT on titanium and zirconia dental implants. For this purpose three different devices in combination with photosensitive dye were used.

In addition, our aim was to evaluate if aPDT causes damage and alteration to the implant surfaces which would interfere with the re-osseoinegration of the implants in the clinical conditions.

The study sample consisted of 144 sterile dental implants (72 titanium dental implants and 72 zirconia dental implants) (Bredent®, Senden, Germany). Both, titanium and zirconia dental implants were with a diameter of 4.0 mm and 12 mm of length, with sandblasted and acid etched surface. Each of the implants was in an unopened sterile packaging.

2.1 Bacterial contamination of dental implants

A bacterial suspension was prepared from three bacteria species: *Prevotella intermedia, Aggregatibacter actinomycetemcomitans*, and *Porphyromonas gingivalis*. These bacteria are commonly found in peri-implant diseases.

The bacteria were cultivated separately in Columbia Agar for 72 hours and then, using thioglycolate broth, a bacterial suspension was prepared for each of the bacteria. The suspension of each of the bacteria was then mixed together in a joint suspension.

In a single use tubes 300 μl of the bacterial suspension was put and then each implant was put separately in single use tubes (**Figure 1**). The tubes were incubated in anaerobic conditions for 72 hours.

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

2.2 Group 1. LaserHF (PDT1)

The implants were treated with a diode laser (Laser HF®, Hager Werken, Duisburg, Germany) and a toluidine blue-based dye (155 μg/ml, LaserHF® Paro-PDT solution). The laser parameters are presented in 1.

2.3 Group 2. Helbo laser (PDT2)

A combination of a diode laser (Helbo® Therapielaser, Helbo Photodynamic Systems GmbH & Co KG, Grieskirchen, Austria) and a phenothiazine chloride dye (Helbo® Blue photosensitizer) was used for the treatment of the implants belonging to this group. The laser parameters are presented in **Table 2**.

Figure 1.
Implants placed in Eppendorf tubes containing bacterial suspension. Implants covered in their entire length by the bacterial suspension.

Wavelength: 660 nm
Fiber tip: 320 μm optical fiber tip
Power output: 100 mW
Power density: 124.3 W/cm^2
Irradiation Time: 60 seconds
Distance from the implant: 5 mm

Table 1.
PDT1 treatment parameters.

Wavelength: 660 nm
Fiber tip: 3D pocket probe
Power output: 100 mW
Power density: 35.37 W/cm^2
Irradiation Time: 60 seconds
Distance from the implant: 5 mm

Table 2.
PDT2 treatment parameters.

2.4 Group 3. Light-emitting diode treatment group (PDT3)

The implants belonging to this group, were treated with LED curing light (Optilight Ld®, Gnatus, Brazil). A red LED light, (Ledengin,Inc.®, San Jose, USA) was used in combination with a toluidine blue solution (Biognost®, Zagreb, Croatia). The laser parameters are presented in **Table 3**.

2.5 PDT1, PDT2 and PDT3 decontamination steps

The first step was coating the implants with the respective photosensitive dye according to the treatment group. After 60 seconds the implants were rinsed with sterile saline solution. For standardization of the treatment protocols for every treatment group, the implants, were placed in a rotating electric motor (Shenzhen Powerful Electronics, Shajing, China), with a rotating speed of 10 rpm.

The treatment time was 60 seconds for every group from a distance of 5 mm from the implant. The treatment procedures for titanium and zirconia implants are shown in **Figures 2** and **3**.

2.6 Group 4. Treatment with toluidine blue only (TB)

The implants belonging to this group were placed in photosensitive dye (toluidine blue; Biognost®, Zagreb, Croatia) solution (1 mg/ml) for 60 seconds. Then they were rinsed with sterile saline solution.

2.7 Control groups

Two control groups were included. The negative control group (NC) did not receive any treatment. After removing the implants from the bacterial suspension and keeping them in room conditions for 60 seconds, microbiologycal analysis followed.

Wavelength: 660 nm
Fiber tip: 6 mm LED composite curing tip
Power output: 200 mW
Power density: 0.71 W/cm^2
Irradiation Time: 60 seconds
Distance from the implant: 5 mm

Table 3.
PDT3 treatment parameters.

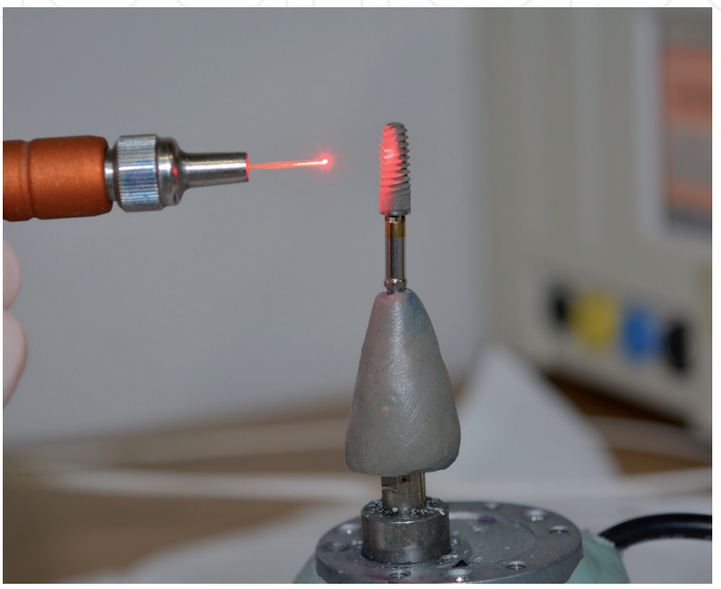

Figure 2.
A titanium implant treated from a distance of 5 mm for 60 seconds while rotating on the electric motor.

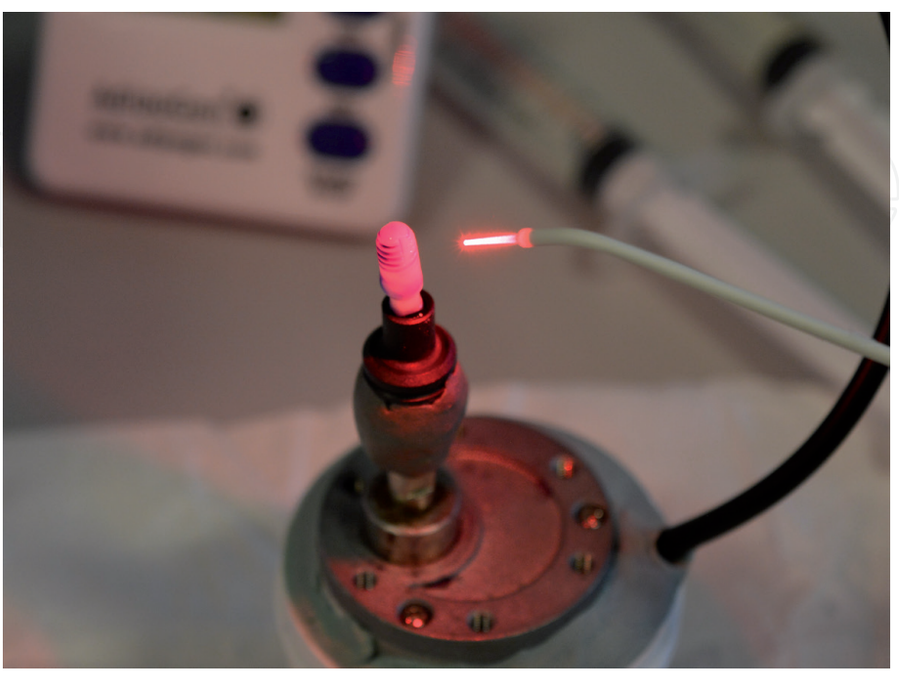

Figure 3.
A zirconia dental implant placed in a rotational motor and treated with PDT2 for 60 seconds.

The implants belonging to the positive control group (PC) were put in 0.2% chlorhexidine gluconate solution (Curasept ADS® Curaden International AG, Kriens, Switzerland) for a duration of 60 seconds. After their removal from the chlorhexidine solution, the implants were only rinsed with sterile saline to remove the remaining solution.

2.8 Microbiological analysis

After treatment procedures each implant was placed in a tube containing 500 μl of phosphate buffered saline (PBS). The tubes were vortexed for 60 seconds (Vortex, Genius 3, IKA, Germany). This was done to remove the remaining bacterial cells from the implant surfaces.

From each tube, 100 μl were taken and using a 96-well microtiter plates a ten-fold dilution was performed and 30 μl of suspension from each well was then inoculated to Brucella agar plates.

The plates were placed in anaerobic conditions and after 72 hours and the colony forming units per milliliter (CFU/ml) were counted (**Figure 4**). MALDI Biotyper (Bruker Daltonics, Germany) was used to macroscopically differentiate distinctive colonies.

2.9 Scanning electron microscopy analysis

Scanning electron microscopy (SEM) was performed on one randomly selected implant from each of the treatment groups and one sterile non-treated implant. The implants for SEM analysis were stored for 2 hours in 2% paraformaldehyde and, later on dehydrated in increasing concentrations of ethanol (60%, 75% and 95%), for 30 minutes in each and dried overnight. The surfaces of the prepared implants

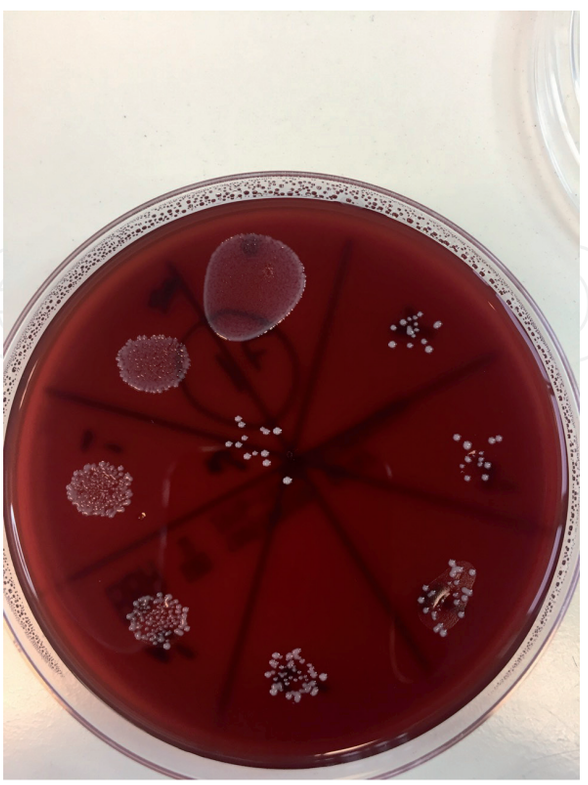

Figure 4.
Visible colonies of bacteria on Brucella agar plate.

were analyzed by SEM (Vega TS5136MM, Tescan, Brno, Czech Republic). The SEM images were taken at 1:250 magnifications under high vacuum (HiVac) with a high voltage (HV) of 30 kV. All the images were taken between the fourth and the fifth thread of the implants. As for the zirconia implants, they are non-conducting material and in order to make the samples conductive and avoid charging of the sample surface, the implants were coated with gold and palladium sputter (SC7620 Mini Sputter Coater, Quorum Technologies Ltd., UK).

2.10 Statistical analysis

The differences between the groups for each bacterial species separately and for the total count of bacteria, were compared by analysis of variance test (ANOVA) and Tukey test, as a post hoc. The level of significance was set at 5%. The statistical package SAS system for Windows (Release 8.02, SAS Institute Inc., Cary, NC, USA) was used.

3. Results

To determine the difference among the groups and between the two types of implants, multivariate analysis of variance test was applied.

The comparison between the two types of implants: titanium and zirconia, regardless of the study groups, showed that there was a significantly lower number of bacteria on zirconia implants for all three types of bacteria separately, as well as for the total number of bacteria (**Table 4**).

For the comparison among the study groups regardless of the type of implant, Tukey test was applied. Regarding the total number of bacteria, the least bacteria were found in PDT1 and PDT2. These two groups were followed by PDT3 and PC without significant difference among them. The negative control group (NC) as expected, had the largest number of bacteria compared to the other groups. The same results were obtained for the number of each bacteria separately.

The total number of bacteria for every group and both implant types are shown in **Figure 5** in schematic form. The difference between zirconia implants and titanium implants was not the same for all groups. The smallest difference between both types of implants in the number of bacteria was for the control group. The impact was almost the same for PDT1, PDT2, PC and TB, while the largest difference between titanium and zirconia implants were in the PDT3 group. The results for each of the bacteria separately are shown in **Figures 6**–**8**.

3.1 Titanium implants

There were statistically significant differences among the groups for each of the bacteria separately and also for the total number of bacteria ($p = 0.0022$). These results are presented in **Table 5** in logarithmic form. Regarding the total number of bacteria, the largest reduction was observed in the PDT1 (98.3%) and PDT2 (97.8%) groups. These two groups had statistically significant difference when compared to NC ($p < 0.05$). In the PDT3 group there was a 68.7% bacterial reduction, without statistically significant difference when compared to NC (**Table 5**).

When each bacteria was compared separately, the PDT1 and PDT2 groups also showed the largest bacterial reduction. PDT1 group, was significantly more effective in the eliminating *A. actinomycetemcomitans* and *P. gingivalis* ($p < 0.05$).

	Aggregatibacter actynomycetemcomitans					Porphyromonas gingivalis				
Factor	N	mean	st.d.		p*	N	mean	st.d.		p*
Implants										
Zirconia	72	1.9	(2.1)		**<0.0001**	72	1.6	(2.1)		**<0.0001**
Titanium	72	4.9	(2.5)			72	4.9	(2.6)		
Group										
PDT1	24	1.9	(2.2)	b	**<0.0001**	24	2.0	(2.5)	b	**<0.0001**
PDT2	24	1.8	(2.0)	b		24	1.6	(2.1)	b	
PDT3	24	3.1	(2.9)	ab		24	2.9	(2.8)	ab	
TB	24	4.3	(2.7)	a		24	4.0	(2.7)	a	
PC	24	2.9	(2.7)	ab		24	2.8	(2.6)	ab	
NC	24	6.2	(1.3)			24	6.2	(1.6)		
	Prevotella intermedia					**Total**				
Factor	N	mean	st.d.		p*	N	mean	st.d.		p*
Implants										
Zirconia	72	2.0	(2.1)		**<0.0001**	72	2.3	(2.3)		**<0.0001**
Titanium	72	5.3	(2.7)			72	5.8	(2.5)		
Group										
PDT1	24	2.6	(2.5)	b	**<0.0001**	24	2.8	(2.6)	b	**<0.0001**
PDT2	24	2.0	(2.3)	b		24	2.3	(2.4)	b	
PDT3	24	3.1	(3.2)	ab		24	3.6	(3.2)	ab	
TB	24	4.5	(2.9)	a		24	5.0	(2.7)	a	
PC	24	3.2	(2.7)	ab		24	3.6	(2.7)	ab	
NC	24	6.4	(1.8)			24	7.1	(1.5)		

**p-value for MANOVA test.*
abc - result of post-hoc comparison (Tukey test). Having the same letter means that there is no statistically significant difference.

Table 4.
Number of bacteria by implant type and treatment groups in logarithmic form.

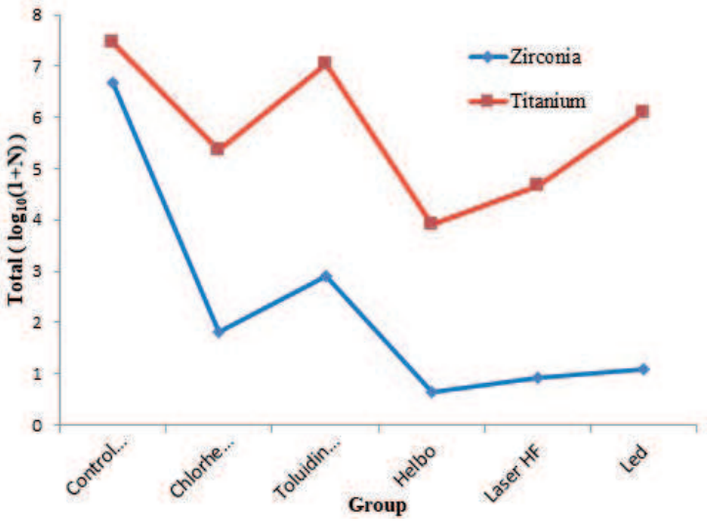

Figure 5.
The total number of bacteria in logarithmic form, for both types of implants and for each study groups.

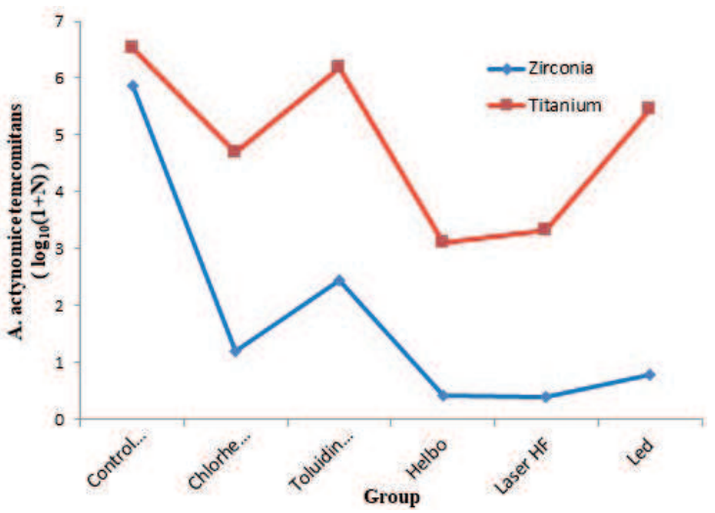

Figure 6.
The number of A. actinymycetemcomitans in logarithmic form for both types of implants and the study groups.

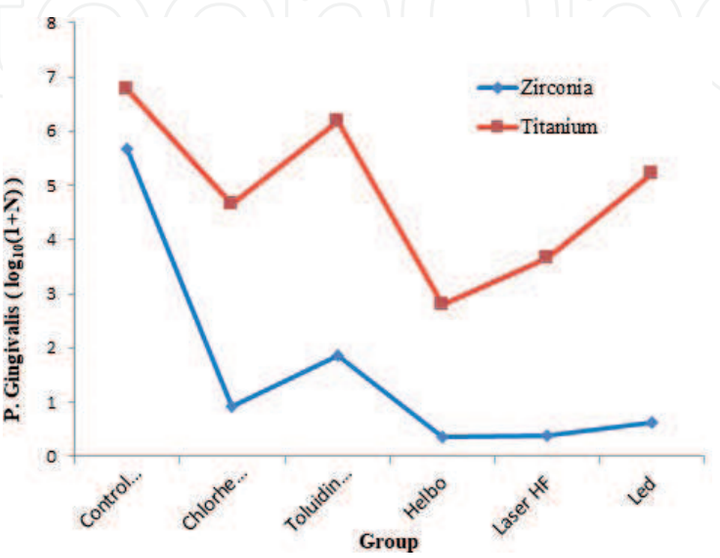

Figure 7.
The number of P. gingivalis in logarithmic form for both types of implants and the study groups.

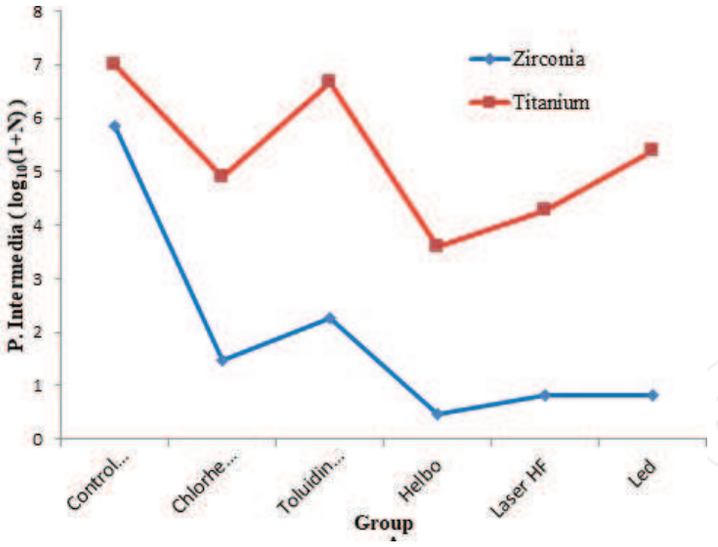

Figure 8.
The number of P. intermedia in logarithmic form for both types of implants and the study groups.

As for *P.intermedia* the PDT1 group showed no significant difference compared to NC group. On the other hand the PDT2 group was significantly more effective in the elimination of each of the bacteria separately when compared to the NC group ($p < 0.05$).

The least effective among the groups, when compared to the NC group, was the TB group (62.4%). Compared to NC there was no significant difference neither for the total number nor for each bacteria separately.

3.2 Zirconia implants

Statistically significant difference was observed among the groups. This was the case for both the total number of bacteria and each bacteria separately ($p < 0.0001$). Every group showed vast bacterial reduction with statistically significant difference when compared to the negative control (NC). These results are shown in **Table 6** in logarithmic form.

The PDT1, PDT2 and PDT3 had the largest bacterial reduction for each bacterium separately, as well as for the total count of bacteria. There was a reduction of more than 99% in comparison to NC. However, between these three groups the differences in bacterial reduction were not statistically significant difference neither for each of the bacteria separately nor for the total number of bacteria ($p > 0.05$).

The lowest bacterial reduction for each bacteria separately and also for the total number of bacteria was observed in the TB group. The PC group had lower bacterial reduction compared to PDT1, PDT2 and PDT3 without statistically significant differences among them. It also did not differ significantly compared to the TB in terms of the total bacterial count, *P. gingivalis* and *P. intermedia*. It had a significant difference compared to the TB only for *A. actinomycetemcomitans*.

3.3 Scanning electron microscope analysis

The SEM images from the PDT1, PDT2, and PDT3 groups did not show any surface alterations, cracks, or damage when compared to the images obtained for the sterile implants. Visually, their surface appeared to be very similar to the surface of the sterile implant, for both titanium and zirconia implants (**Figures 9–12**).

	Aggregatibacter actynomycetemcomitans					**Porphyromonas gingivalis**					**Wilks' lambda**
Group	N	mean	st.d.		p*	N	mean	st.d.		p*	p
PDT1	12	3.3	2.2	b	**0.0006**	12	3.7	2.5	bc	**0.0003**	**0.0026**
PDT2	12	3.1	2.0	b		12	2.8	2.4	c		
PDT3	12	5.4	2.3	ab		12	5.2	2.2	abc		
TB	12	6.2	2.3	a		12	6.2	2.0	ab		
PC	12	4.7	2.7	ab		12	4.7	2.3	abc		
NC	12	6.5	1.7	a		12	6.8	1.9	a		
	Prevotella intermedia					**Total number of bacteria**					Wilks' lambda
Group	N	mean	st.d.		p*	N	mean	st.d.		p*	p
PDT1	12	4.3	2.4	ab	**0.0096**	12	4.7	2.3	bc	**0.0022**	**0.0026**
PDT2	12	3.6	2.4	b		12	3.9	2.3	c		
PDT3	12	5.4	3.1	ab		12	6.1	2.5	abc		
TB	12	6.7	2.4	a		12	7.0	2.2	ab		
PC	12	4.9	2.7	ab		12	5.4	2.6	abc		
NC	12	7.0	2.2	a		12	7.4	1.8	a		

**p-value for ANOVA test.*
abc - result of post-hoc comparison (Tukey test). Having the same letter means that there is no statistically significant difference.

Table 5.
Results of ANOVA and Tukey's post hoc test for the titanium implants.

	Aggregatibacter actynomycetemcomitans					Porphyromonas gingivalis					Wilks' lambda
Group	N	mean	st.d.		p*	N	mean	st.d.		p*	p
PDT1	12	0.4	0.8	a	**0.0001**	12	0.4	0.8	b	**0.0001**	**0.0001**
PDT2	12	0.4	0.6	a		12	0.3	0.5	b		
PDT3	12	0.8	1.1	a		12	0.6	0.7	b		
TB	12	2.4	1.3			12	1.9	1.1	a		
PC	12	1.2	1.0	a		12	0.9	1.2	ab		
NC	12	5.9	0.7			12	5.7	1.0			
	Prevotella intermedia					**Total number of bacteria**					Wilks' lambda
Group	N	mean	st.d.		p*	N	mean	st.d.		p*	p
PDT1	12	0.8	0.9	b	**0.0001**	12	0.9	1.0	b	**0.0001**	**0.0001**
PDT2	12	0.5	0.7	b		12	0.7	0.8	b		
PDT3	12	0.8	0.9	b		12	1.1	1.2	b		
TB	12	2.3	1.2	a		12	2.9	1.2	a		
PC	12	1.5	1.5	ab		12	1.8	1.5	ab		
NC	12	5.9	1.3			12	6.7	0.9			

**p-value for ANOVA test.*
abc - result of post-hoc comparison (Tukey test). Having the same letter means that there is no statistically significant difference.

Table 6.
Results of ANOVA and Tukey's post hoc test for the zirconia implants.

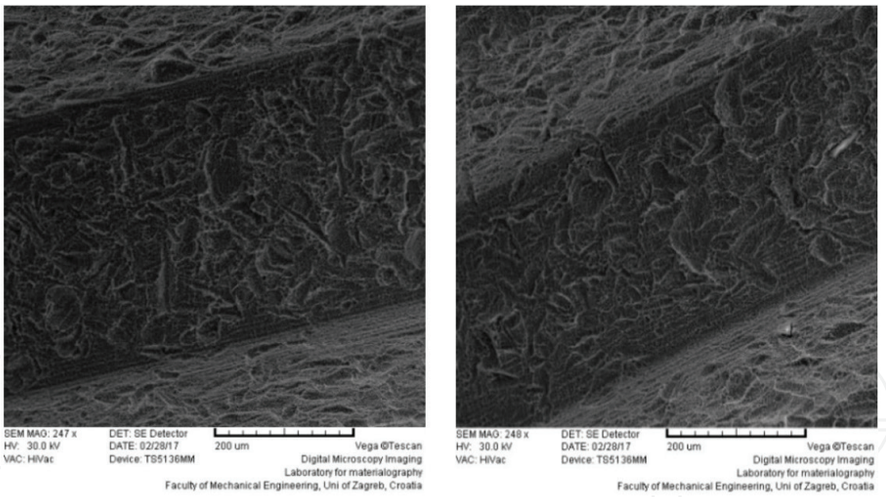

Figure 9.
Sterile titanium implant; magnification 1:250 (left). Titanium implant treated with PDT1; magnification 1:250 (right).

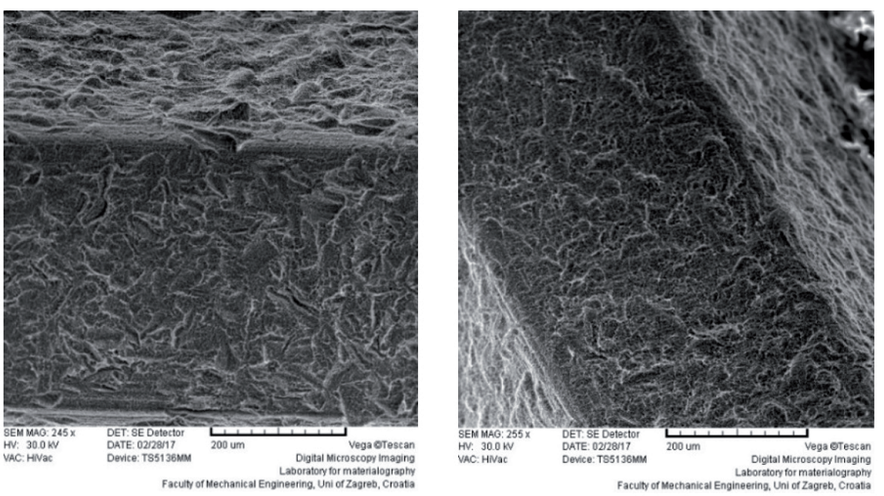

Figure 10.
Titanium implant treated with PDT2; magnification 1:250 (left). Titanium implant treated with PDT3; magnification 1:250 (right).

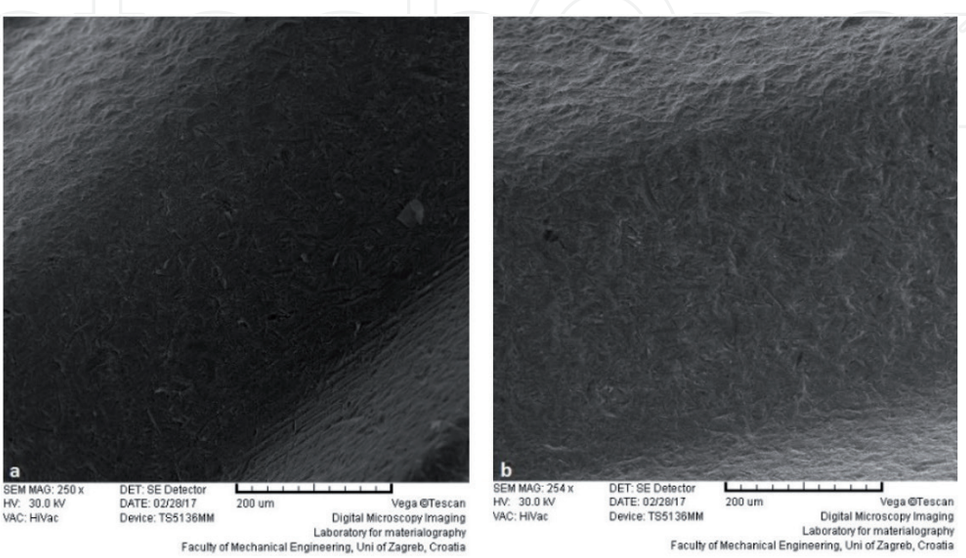

Figure 11.
Sterile zirconia implant; magnification 1:250 (left). Zirconia implant treated with PDT1; magnification 1:250 (right).

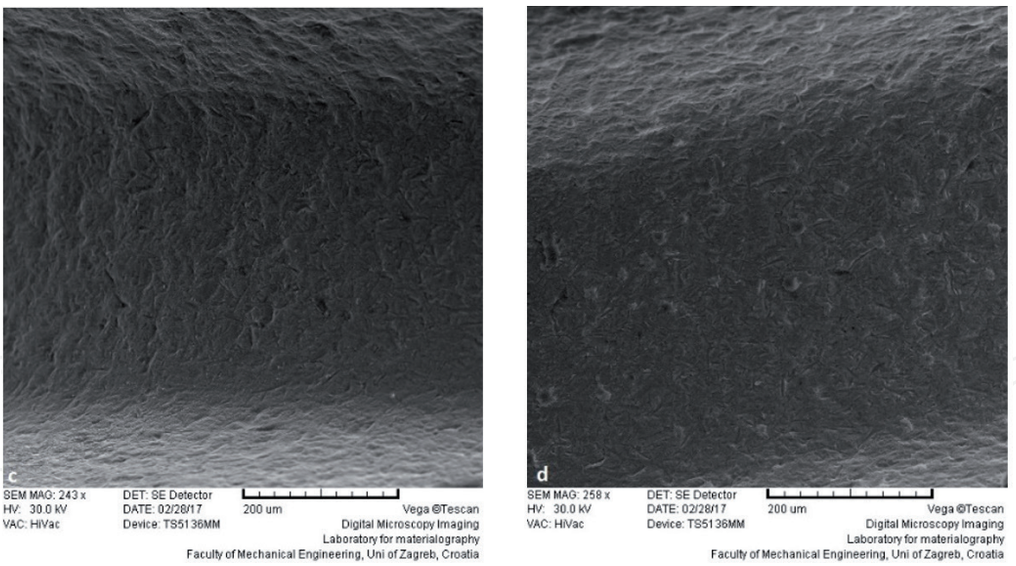

Figure 12.
Zirconia implant treated with PDT2; magnification 1:250 (left). Zirconia implant treated with PDT3; magnification 1:250 (right).

4. Clinical application of photodynamic therapy

The clinical efficacy of PDT against peri-implantitis has been demonstrated in several clinical studies. In a randomized controlled trial study by Wang et al. [60] it was shown that PDT combined with mechanical debridement significantly improved pocket depth, clinical attachment loss, plaque index and sulcus bleeding index compared with baseline and the control groups in participants with peri-implantitis. Similar results were obtained in a 3 months randomized clinical trial done by Rakasevic et al. [61].

Since the main goal when treating peri-implantitis is to eliminate the bacteria from the soft tissues and the implant surface, in order to create conditions for grafting and re-osseointegration, the use of photodynamic therapy is mostly used as an adjunct therapy during the treatment of peri-implanitits with the purpose of eliminating bacteria from the rough surfaces of the implants. The treatment of peri-implantitis can be non-surgical and surgical. During the non-surgical treatment of peri-implantitis the photosensitive dye is applied on the pocket around the infected implant and the light source is applied. This procedure is shown in **Figure 13**.

However, photodynamic therapy is mostly used in conjunction with surgical treatment of peri-implantitis as an adjunct therapy after implantoplasty, mechanical debridement and chemical decontamination of the implant surface. The surgical approach is presented in **Figures 14–17**.

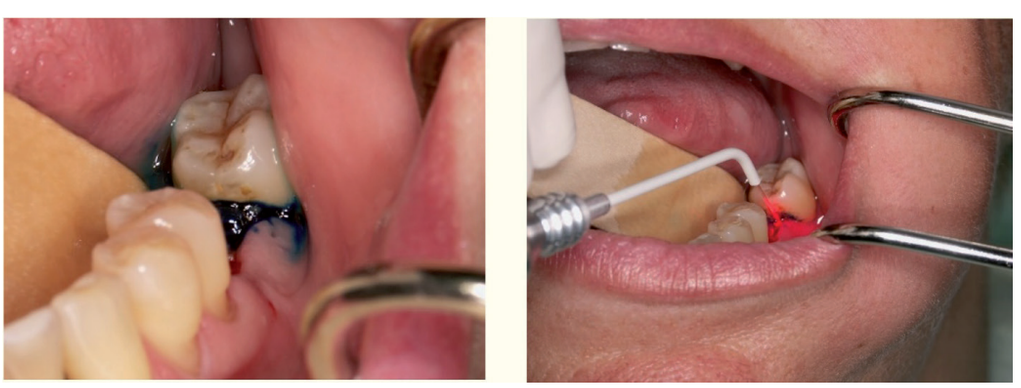

Figure 13.
(Left) application of the dye. (Right) application of the light source.

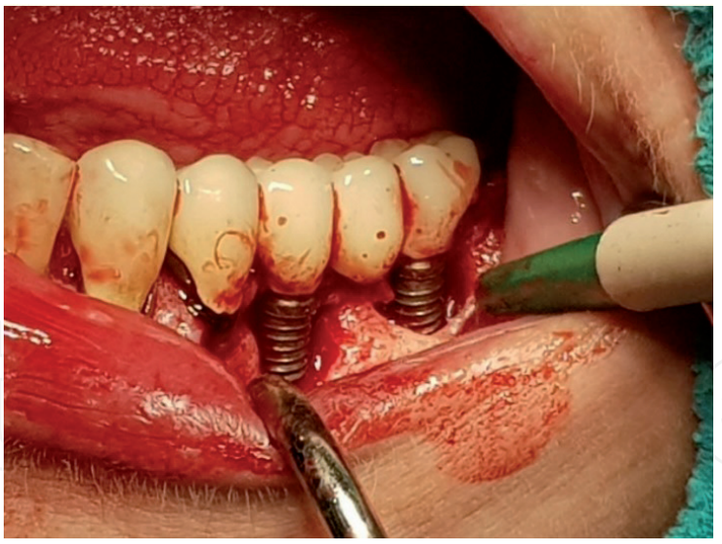

Figure 14.
Surgical treatment of peri-implantits. Visible bone resorption around the implants.

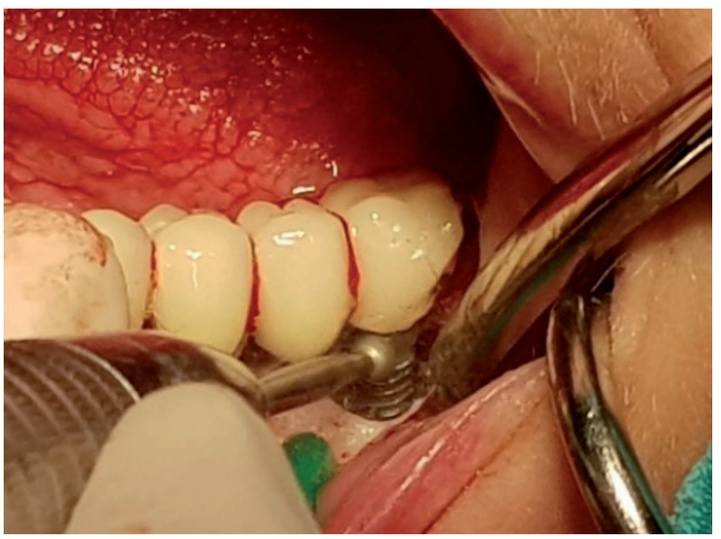

Figure 15.
Implantoplasty procedure.

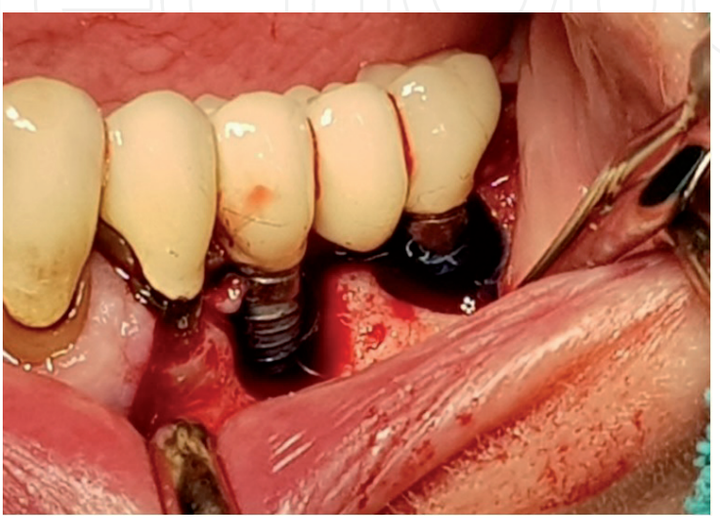

Figure 16.
Application of the photosensitive dye.

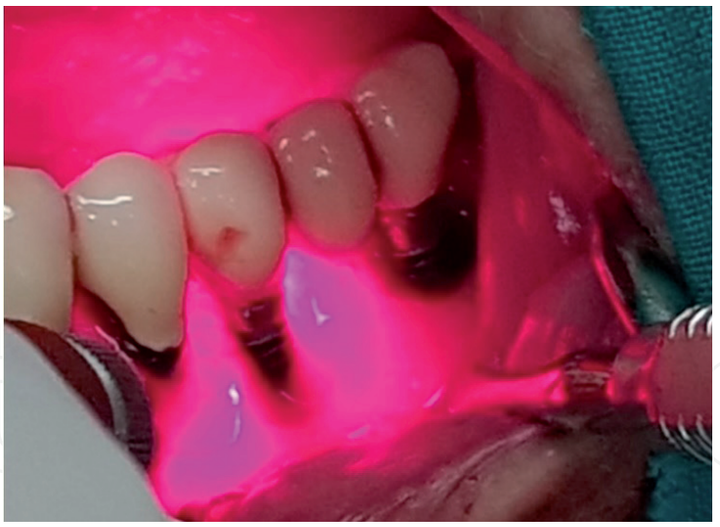

Figure 17.
Application of the light source.

5. Discussion

One of the main reasons of implant failure is peri-implantitis. The prevalence rates of peri-implantitis differs among different studies and this is due to the different reporting methods and characteristics [62–64]. Van Velzen et al. [65] in their 10 years prospective cohort study reported a prevalence of 7%. Meijer et al. [66] reported that after 10 years 29.7% of patients were affected by peri-implantitis. Fardal et al. [67] report a rate of 53.5% at the patient level and 31.1% at the implant level, which is much higher than the data from other studies.

The treatment of peri-implantitis is complex and it often includes combination of conventional therapy with the addition of antimicrobials. However, use of antimicrobials does not have a long term effects and it can lead to antimicrobial resistance and development of superinfections [68]. Therefore, alternative antimicrobial approaches for achieving implant disinfection have been sought.

Photodynamic therapy is a promising alternative when treating periodontal diseases and peri- implant diseases. Up to date there have been many *in vitro* [69–71] and clinical studies [60, 61] evaluating the effect of photodynamic therapy in treating peri-implantitis.

Regarding the *in vitro* evaluation, the present study aimed to evaluate the efficacy of photodynamic therapy on dental implants contaminated under *in vitro* conditions. The implants were contaminated in order to try to recreate the adhesion stage of biofilm formation on the implant surface. Many *in vitro* studies have used similar methodology to achieve titanium implant contamination [62, 63, 71].

The main focus of our study was to evaluate if photodynamic therapy is efficient in eradicating the bacteria from the implant surface when compared to the negative control group (NC) and to the conventional treatment with chlorhexidine solution (PC). Furthermore, the focus was to evaluate different types of devices and with different parameters and photosensitizers and the reaction of different bacteria to aPDT.

The results from our study showed that PDT1 and PDT2 groups were more eliminated 98.3% and 97.8% of the total number of bacteria when compared to NC group. These groups were the most effective among the study groups. Both PDT1 and PDT2 groups were a combination of a diode laser with a wavelength of 660 nm and a photosensitizer.

The results of this study are similar to other *in vitro*, *in vivo* and clinical studies [64, 71, 72]. Marotti et al. [71] in their study demonstrated that aPDT is effective against the

bacteria present in peri-implantitis. The irradiation time did not influence the results. Similar results were obtained from both the groups (3 minute and 5 minutes irradiation time) and there was no significant difference between them. The effect of aPDT did not differ significantly from the disinfection with 0.12% chlorhexidine solution. Our results were similar to this study and even though we used a higher concentration of chlorhexidine (0.2%), both PDT1 and PDT2 had no significant difference when compared to PC.

In a study done by Haas et al. [64] it was demonstrated that 60 seconds of light exposure in combination with photosensitizer can effectively eradicate *A. actinomycetemcomitans, P. gingivalis* and *P. intermedia*. One of the goals of the present study was to evaluate aPDT against each bacteria separately. The results obtained for *A. actinomycetemcomitans* and *P. gingivalis* were similar to the results obtained for the total bacterial count. Both PDT1 and PDT2 had significant difference when compared to the NC. However, the results of *P. intermedia* showed that PDT2 was more effective against this bacteria when compared to the other groups.

The least effective treatment group was PDT3 without statistically significant difference compared to NC or PC groups regarding the total bacterial count. It must be noted that for the PDT3 group we used a modified dental LED light and not a diode laser. This was done to evaluate and compare the LED light against diode lasers as a light source.

The efficacy of LED lights as a light source in photodynamic therapy has been tested in many studies however, only a few studies have tested its efficacy on titanium implant surfaces. The results from these studies are conflicting since the study design and light source parameters differ greatly. In a study conducted by Cho et al. [73] the efficacy of a green LED light was tested. The LED light was combined with erythrosine dye and was evaluated against *A. actinomycetemcomitans*. Their results showed that this combination is effective and reduces the bacteria attached titanium surfaces up to 92.4%. The irradiation time in this study was 60 seconds and the treatment was done on only one surface of titanium discs. This provides uniform distribution of the light source. In contrast, in the present study we applied the light source in a rotating motion in order to emulate clinical application of aPDT around a contaminated implant. This might be the reason why our results showed differ with the aforementioned study [73].

In contrast to the *in vitro* study by Cho et al. [73], in a clinical study done by De Angelis et al. [74] the use of LED light showed no significant difference after 4 months of follow up when compared to mechanical debridement and scaling.

In our study we evaluated the efficacy of aPDT on two types of implants: titanium and zirconia dental implants. The efficacy of aPDT on zirconia implant surfaces has not been evaluated in other studies up to date.

The results obtained from our study showed that each test group was very effective in eliminating the bacteria from the zirconia surface and all had significantly lower bacterial count when compared to NC. However, in between the groups there was no significant difference. The higher efficacy of aPDT against zirconia surfaces when compared to titanium surfaces might be due to the surface properties of zirconia which might lead to a lower affinity of the bacteria to be attached to zirconia surfaces. Zirconia surfaces are smoother, have a lower surface roughness and lower surface free energy [75, 76]. In a study done by Scarano et al. [75] titanium and zirconia oxide discs were placed in the mouths of patients in order to evaluate in which surface the bacteria adhere less. After 24 h it was shown that there were significantly less bacteria on the zirconium oxide surfaces. Al-Radha et al. [76] showed similar results. In their study titanium blasted with zirconia and the zirconia material showed better results when compared to the titanium surface regarding the adhesion of bacteria after coating the surfaces with saliva pellicle.

PDT1, PDT2 and PDT3 in addition to the significant difference compared to NC, they also had significant difference from the TB group. The results of the

PDT3 group for the zirconia dental implants were comparable to PDT1 and PDT2. This can suggest that LED light with additional improvements in light distribution and parameters can have an antimicrobial effect. As mentioned before there are conflicting results regarding the antimicrobial effect of using LED. Several studies reported beneficial results following use of LED lights, as a light source [77, 78]. On the other hand, several studies reported insignificant improvement in the treatment outcomes using LED light for PDT [74]. However, it is difficult to compare the results of present study with the previous ones, mainly due to the differences in the study protocols and lack of studies conducted on zirconia implant surfaces.

The use of diode lasers in many studies has been shown to be safe in regard to the implant surface, compared to Nd:YAG, Er:YAG, CO2 and Ho:YAG lasers, which can damage the implant surfaces [57]. Castro et al. [79] concluded that 980 nm diode laser irradiation does not damage titanium implant surfaces and seems to be safe irrespective of power output used.

In the present research, no structural changes on the implant surfaces following therapy was observed. PDT1, PDT2 and PDT3, did not cause visible damage on titanium or zirconia implant surface at a magnification of 1:250.

Regarding the clinical use of PDT, several studies reported conflicting results. Many studies demonstrated improvement in clinical outcomes of patients with peri-implantitis when aPDT was combined with mechanical debridement [60, 80, 81]. Romeo et al. [82] suggested that PDT is a useful adjunct therapy but it could not replace the mechanical and surgical treatment of peri-implantitis. Similarly other studies suggest that the PDT improves the outcomes of peri-implantitis [60, 83, 84].

On the other hand, there are several studies that report no added benefit from using PDT when compared to conventional treatment modalities for peri-implantitis [85, 86].

The results of this *in vitro* study should be considered preliminary, since it cannot be generalized to *in vivo* and clinical conditions. The biggest concern related to future *in vivo* and clinical applications is stability of achieved *in vitro* results (short term beneficial effects in reducing the number of periopathogens). Also, the presence of plaque formation on implants, degree of salivation and host-immune response is very important.

6. Conclusion

It is of utmost importance that further clinical trials be conducted in order to clarify the potential efficacy of PDT as an adjunct therapy to peri-implantitis and clear and effective treatment protocols should be established in order to benefit the most from the properties of PDT.

Conflict of interest

The authors declare no conflicts of interest.

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