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Review

Antimicrobial efficacy of photodynamic therapy on dental implant surfaces: A systematic review of in vitro studies

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ABSTRACT

Background: To systematically review the literature regarding the antimicrobial effects of photodynamic therapy (PDT) on multi-bacterial species and the possible surface alterations of dental implants as a result of PDT.

Methods: The addressed focused question was: "Does PDT show antimicrobial efficacy against multi-bacterial species colonization and result in surface alteration on dental implants?" Electronic databases including MEDLINE and EMBASE up to and including December 2018 were searched.

Results: Seven studies were included. Two studies used a total of 110 titanium dental implants, while 1 study included a total of 72 zirconia dental implants. Three studies investigated the antimicrobial PDT effects on titanium discs, while 1 study used titanium plates with germanium prisms. All in-vitro studies used diode laser. Energy fluence was reported only in 2 studies. Power output and density were 100 mW (mW) and 150 mW cm^{-2}, respectively. All in-vitro studies reported the multibacterial species outcomes after the application of antimicrobial PDT. All studies showed a significant reduction in the bacterial load. Only two studies reported the outcomes of microstructural changes on the titanium surface, in which both studies did not report any significant alterations on the titanium implants or discs with the application of PDT.

Conclusion: This systematic review demonstrated significant reduction in the bacterial load but inconclusive findings regarding structural alterations on the titanium surface with the use of PDT. The results of this review should be considered preliminary and further in-vitro studies with standardized laser parameters are needed to obtain strong conclusions.

1. Introduction

Dental implant therapy is the mainstream therapy for restoring missing teeth and is commonly practised by dental practitioners. Osseointegration is one of the crucial determining factors for dental implant survival and success [1]. The engineering and biology of dental implants is widely researched and scientists are still exploring the clinical, immunological, and microbiological parameters around dental implants. However, there are certain local and systemic risk factors such as tobacco smoking and diabetes mellitus that may modify the overall success of the treatment [2–7]. The primary hallmark for dental implant failure is the dental plaque and the quality of bacterial species present in it [8]. Such bacterial microflora causes inflammation of the soft peri-implant structures that may lead to erythema and bleeding, and if left for longer period of time around dental implants, may eventually lead to implant failure and loss [9,10].

Non-surgical manual debridement which is not only the contemporary treatment choice, but regarded as a gold standard therapy in the treatment of peri-implant inflammation [11,12]. Other than physical methods, techniques of implant surface decontamination can be chemical too including chlorhexidine, antimicrobials, hydrogen peroxide, etc. [13]. In modern dentistry, certain adjunctive therapies including low level laser and antimicrobial photodynamic therapy (PDT) are being extensively explored that may add additional benefits for reducing bacterial niche [14]. Several clinical studies have investigated the reduction of inflammation around dental implants through mechanical debridement and application of PDT using various laser lights and photosensitizers [15].

With the use of PDT that involves a laser light of specific wavelength with photosensitizer application which further stimulates photosensitizer dye molecules. This causes changes from ground singlet state to excited triplet state in the dye molecule which oxidizes to form highly reactive and cytotoxic singlet oxygen resulting bacterial cell death [16].

Since the detoxification of dental implants may require different methods and no universally acknowledged treatment guidelines have been established for the surface treatment of dental implants, this gray area still remains an area of further exploration. The current systematic

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review systematically reviewed the literature regarding the effect of antimicrobial PDT on the multi-bacterial species and the possible surface alterations of the dental implants as a result of PDT.

2. Materials and methods

2.1. Focused question

A general protocol using the 'Preferred Reporting Items for Systematic Review and Meta-Analysis' (PRISMA) guidelines was followed [17]. A focused question was devised which adhered to the general selection criteria: "Does PDT show antimicrobial efficacy against multi-bacterial species colonization and result in surface alteration on dental implants?"

2.2. Selection criteria

Independent screening of the titles for potential articles was assessed. The studies were included based on the following eligibility criteria;

- **Population:** Study sample consisting of dental implants;
- **Interventions:** Treatment with antimicrobial PDT;
- **Comparisons:** Either with laser therapy, chemical detoxification or no treatment;
- **Outcomes:** bacterial load and outcomes related to surface alterations; and
- **Study design:** The review only contained in-vitro studies published in English language.

Animal studies, letters to the editor, review articles, and unpublished abstracts were excluded.

2.3. Search strategy

Electronic databases including MEDLINE and EMBASE up to and including December 2018 were searched. The literature search was conducted using the combinations of the following Medical Subject Heading (MeSH) and text words: ((Photochemotherapy OR photosensitizing agents OR photodynamic therapy) AND (bacteria OR infection) AND (dental implants OR surface). Manual searching of the *Photodiagn Photodyn Ther, Plos One, Int J Oral Maxillofac Implants, Clin Oral Implants Res* and *Lasers Med Sci* was performed.

2.4. Screening methods and data abstraction

Titles and abstracts of studies that fulfill the inclusion criteria were screened and assessed. Data were extracted from the included studies as per following parameters: author/country, study sample and groups, types of bacterial species assessed, laboratory analysis, study outcome, and laser parameters.

3. Results

3.1. Study selection

Based on titles and abstracts search, initially 70 studies were identified. After removing duplicates (n = 5) and screening of abstracts, a total of 52 articles were not relevant to the objective of the review, hence excluded. Thirteen full-text studies were selected for screening of which, 6 studies were eliminated because they did not match the inclusion criteria. The final selection resulted in the inclusion of 7 studies [18–24]. All included studies [18–24] were conducted at either health care centers or university hospital. Fig. 1 shows flow diagram of study selection process and results of the literature search according to PRISMA guidelines [17].

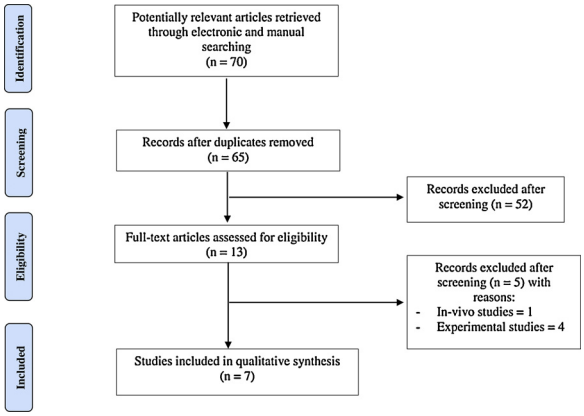

Fig. 1. Study selection process and results of the literature search (PRISMA flow diagram).

3.2. General characteristics of included studies

All included in-vitro studies originated from Croatia [18], Brazil [24], Italy [20], Iran [19], Switzerland [21], United Kingdom [22], and United States [23]. Two studies used a total of 110 titanium dental implants [19,24], while 1 study included a total of 72 zirconia dental implants [18]. Three studies investigated the antimicrobial PDT effects on titanium discs [20–22], while 1 study used titanium plates with germanium prisms [23]. All studies employed antimicrobial PDT as test groups while control groups among the studies included the use of chlorhexidine [19,22,24], photosensitizer alone [18], laser treatments [18–21,24], titanium brushes [22], curettes [21], saline solution [19], and the use of phosphate-buffered saline [22]. A variety of bacterial species were assessed that includes *Staphylococcus aureus, Prevotella intermedia, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Parvimonas micra, Tannerella forsythia, Treponema denticola, Streptococcus gordonii, Actinomyces naeslundii, Fusobacterium nucleatum, Campylobacter rectus, Filifactor alocis, Eikenella corrodens,* and *Porphyromonas gingivalis.* Out of 7 in-vitro studies, 4 studies [18,20,22,23] employed scanning electron microscopy, 4 studies evaluated microbial culture analysis in colony forming units (CFU) [19,19,20,21,24], while 1 study each evaluated the outcomes using multiple attenuated internal reflection infrared spectroscopy [23], fluorescence analysis, LPS inactivation [20], attachment of epithelial cells, gingival fibroblasts and osteoblast-like cells, respectively [21] (Table 1).

No meta-analysis could be performed due to the methodological heterogeneity in the included studies, for example, study groups, laser/photosensitizer parameters, and a variation in the outcomes. Therefore, the outcomes are reported as a narrative review.

3.3. Photochemotherapy related parameters

All in-vitro studies used diode laser [18–24]. Two studies used separate photochemotherapy protocols [18,19], while 1 study [19] used LED lamp in addition to the diode laser. The wavelengths of diode lasers used in the included PDT studies ranged from 625 nm to 810 nm. Energy fluence was reported only in 2 studies [20,23]. Power output and density were 100 mW (mW) and 150 mW cm^{-2}, respectively. The duration of irradiation was reported in 6 studies which ranged from 60 to 300 s [18–22,24]. Only 3 PDT protocols reported optic fibre diameter [18,19,24]. Different photosensitizers were reported by different studies. Methylene blue were used in 3 studies [20,22,24] whereas toluidine blue (TBO) [18,19] and phenothiazine chloride (PTC) [18,21] were used as photosensitizers in 2 studies, respectively. Only 1 study reported the use of indocyanin green [19]. In all included studies [18–24], photosensitizer was applied with the time duration that ranged between 1 and 5 min. Concentration of photosensitizer was

Table 1
General description of included studies.

Investigators	Country	Sample size; implant type; diameter; length	Groups	Bacterial species	Laboratory analysis	Main outcomes
Azizi et al. [18]	Croatia	72 zirconia dental implants; 4 mm; 12mm	• PDT 1 • PDT 2 • LT • Toluidine blue • Positive control • No treatment	Prevotella intermedia, Aggregatibacter actinomycetemcomitans, and Porphyromonas gingivalis	Scanning electron microscopy	PDT showed a significant reduction in the bacterial load without causing damage to the surface microstructures compared with other groups
Saffarpour et al. [19]	Iran	50 titanium dental implants; 4.3 mm; 10mm	• PDT 1 • PDT 2 • LT • CHX • Saline solution	Aggregatibacter actinomycetemcomitans	Number of CFU per implant	PDT showed a significant reduction in the bacterial load as compared to control groups
Giannelli et al. [20]	Italy	Titanium discs; 6 mm; NA	• PDT • LED	Staphylococcus aureus	Viable cell count, Scanning electron microscopy, fluorescence analysis, LPS inactivation	Comparable reduction in the bacterial load without causing damage to the surface microstructures were seen in both groups
Eick et al. [21]	Switzerland	Titanium discs; NA; NA	• CUR • LT • PDT • CUR + PDT	Streptococcus gordonii, Actinomyces naeslundii, Fusobacterium nucleatum, Campylobacter rectus, Filifactor alocis, Eikenella corrodens, Prevota intermedia, Porphyromonas gingivalis, Parvimonas micra, Tannerella forsythia, Treponema denticola, Aggregatibacter actinomycetemcomitans	CFU of the biofilms, attachment of epithelial cells, gingival fibroblasts and osteoblast-like cells	Laser group showed a significant reduction in the bacterial load as compared to PDT group
Widodo et al. [22]	United Kingdom	114 titanium discs; 15 mm NA	• PDT • TiB • PDT + TiB • PBS • CHX • CP	Staphylococcus aureus	Microbial culture analysis, scanning electron microscopy	PDT + TiB showed a significant reduction in the bacterial load as compared to other groups
Mang et al. [23]	United States	12 titanium plates/ 3 germanium prisms; NA; NA	PDT	Fusobacterium nucleatum	Multiple attenuated internal reflection infrared spectroscopy, scanning electron microscopy	PDT showed a significant reduction in the bacterial load
Marotti et al. [24]	Brazil	60 titanium dental implants; 4 mm; 12mm	• PDT • LT • CHX • No decontamination	NA	Number of CFU per implant	PDT showed a significant reduction in the bacterial load as compared to other groups

CHX; chlorhexidine, LT; Laser therapy, LPS; lipopolysaccharide, CP; cotton pellet, CFU; colony forming unit, CUR; curette, NA; not available, PDT; Photodynamic therapy, TiB; titanium brush, LED; light emitting diode, PBS; phosphate-buffered saline.

Table 2
Laser parameters of included studies.

Investigators	Type of laser	Wavelength (nm)	Energy fluence (J cm^{-2})	Power output (mW)	Power density (mW cm^{-2})	Duration of irradiation (seconds)	Optic fibre diameter (mm)	Types of PS	Pre-irradiation time (minutes)	Concentration of PS (mg/mL)	Number of laser sessions
Azizi et al. [18]	PDT 1: Diode laser	660	NA	100	124.3	60	0.32	TBO	1	0.155	NA
	PDT 2: Diode laser	660	NA	100	35.37	60	NA	PTC	1	10	
Saffarpour et al. [19]	PDT 1: LED lamp	625–635	NA	NA	2.000–4.000	60	4	TBO	1	NA	NA
	PDT 2: Diode laser	810	NA	300	2.38	60	NA	ICG	5	NA	
Giannelli et al. [20]	Diode laser	635	21	100	0.35	60	NA	MB	5	NA	NA
Eick et al. [21]	Diode laser	660	NA	100	NA	10	NA	PTC	3	NA	NA
Widodo et al. [22]	Diode laser	665	NA	11	NA	60	NA	MB	1	NA	NA
Mang et al. [23]	Diode laser	630	25–100	NA	150	NA	NA	NA	NA	NA	NA
Marotti et al. [24]	GaAlAs diode laser	660	NA	30	NA	180-300	0.5	MB	5	0.01%	NA

PS; photosensitizer, PTC; phenothiazine chloride, TBO; toluidine blue, ICG; indocyanin green, MB; methylene blue, NA; not available, LED; light emitting diode, PDT; photodynamic therapy, nm; nanometers, J cm^{-2}; joules per square centimeters, mW; milliwatts, mW cm^{-2}; milliwatts per square centimeters, mg/mL; milligram per milliliter.

reported only in 2 studies [18,24]. None of the included studies reported about the number of laser sessions fired (Table 2).

3.4. Main outcomes of the studies

All in-vitro studies reported the multibacterial species outcomes after the application of antimicrobial PDT [18–24]. All studies showed a significant reduction in the bacterial load. Only two studies reported the outcomes of microstructural changes on the titanium surface [18,20], both studies did not report any significant alterations on the titanium implants or discs with the application of PDT (Table 1).

4. Discussion

This is the first systematic review that investigated the antimicrobial effects of PDT in the reduction of multi-bacterial species and if PDT could produce any surface alterations on the titanium surface. Overall, all studies showed a significant reduction in the bacterial load. Although, only two studies reported the outcomes of microstructural changes on the titanium surface [18,20], both studies did not report any significant alterations on the titanium implants or discs with the application of PDT.

The improvement in the inflammatory response around dental implants through PDT can be explained by its mechanism of action against the bacterial load. The accumulation of microbial plaque bacteria in the peri-implant sulcular area is succeeded by the onset of immune inflammatory cells, for example, macrophages, lymphocytes, and neutrophils. If not cleaned, this creates an exalted response by which these immune cells along with local peri-implant tissues direct various inflammatory proteins which can lead to tissue degradation and bone resorption. Thus, it may be assumed that the increased bleeding on probing and peri-implant plaque index could be the direct impact of the reduction in perio-pathogenic microbes in peri-implant lesions following the application of PDT.

There was a significant methodological heterogeneity and incomplete information about laser and photosensitizer parameters in the included studies [18–24]. A previous study indicated the impact of frequency of laser application on the overall effect of laser treatment. In the present review, none of the studies described the number of laser applications. It might be hypothesized that a single application of laser in PDT alone might be difficult to maintain anti-bacterial effect for short follow-up period. Other factors, for example, fiber diameter could influence power density and energy output in the application of laser during PDT and could alter the certain amount of energy released during the process, likely affecting the anti-bacterial and consequently anti-inflammatory effect of photodynamic therapy. In this review, all included studies for PDT showed a significant reduction in the number of bacteria. Therefore, it appears that PDT could play a vital role in reducing bacterial load around dental implants.

Literature suggests that the gram-negative bacteria are more resistant to PDT compared with gram positive bacteria because of the presence of outer membrane in gram-negative bacteria, which hampers the uptake of the photosensitizer [25]. However, because of the cationic charge of TBO, it binds to the outer membrane of the gram-negative bacteria and interacts with the lipopolysaccharide [26]. It is noted that 2 studies used TBO in their studies and showed significantly more substantial results than others. Thus, it may be proposed that TBO is a suitable photosensitizer for destroying the bacteria causing periodontal disease and peri-implantitis, particularly the black pigmented bacteria [27].

The main limitation of the present study was the number of included studies in the systematic review and no statistical analysis of the data in the form of meta-analysis. Due to significant heterogeneity in the outcomes of data presented, the author was unable to perform the meta-analysis. Although, the authors were contacted to obtain the laboratory data in the form of mean scores and standard deviations, however, only

2 authors responded affirming that they could not share due to unavailability of the data. In addition, this review showed inconclusive findings regarding structural alterations on the titanium surface with the use of PDT. Further studies should focus on critical surface alterations that may provide further in depth research on how PDT ma change surface microtopography of dental implants that could lead to significant increase in bacterial accumulation around dental implants.

However, based on the results of included studies, it is suggested that the role of PDT in reducing bacterial load around dental implants is beneficial. Additionally, further in-vitro analyses should be undertaken in order to appreciate the treatment outcome. Therefore, studies with standardized control and experimental (PDT and LT) groups are suggested to validate the conclusive effect of PDT and lasers in the reduction of bacterial load. Lastly, the outcomes of the current systematic review that contains only in vitro studies surely may not be generalized to in vivo conditions. The distinctive environmental factors, such as adjustable plaque formation and accumulation, variable salivation, host-immune system, and limited accessibility cannot be established in in vitro studies.

5. Conclusion

This systematic review demonstrated significant reduction in the bacterial load with the application of PDT. In addition, this review showed inconclusive findings regarding structural alterations on the titanium surface with the use of PDT. The results of this review should be considered preliminary and further in-vitro studies with standardized laser parameters are needed to obtain strong conclusions.

Conflict of interest statement

The author declare no conflict of interest.

References

[1] U. Daood, N. Banday, Z. Akram, J.K. Tsoi, P. Neelakantan, H. Omar, T. Abduljabbar, F. Vohra, N. Al-Hamoudi, A.S. Fawzy, Mechanical and spectroscopic analysis of retrieved/failed dental implants, Coatings 7 (2017) 201.

[2] Z.H. Al-Sowygh, S.M. Ghani, K. Sergis, F. Vohra, Z. Akram, Peri-implant conditions and levels of advanced glycation end products among patients with different glycemic control, Clin. Implant Dent. Relat. Res. 20 (2018) 345–351.

[3] Z. Akram, F. Vohra, I.A. Bukhari, S.A. Sheikh, F. Javed, Clinical and radiographic peri-implant parameters and proinflammatory cytokine levels among cigarette smokers, smokeless tobacco users, and nontobacco users, Clin. Implant Dent. Relat. Res. 20 (2018) 76–81.

[4] M.A. AlQahtani, A.S. Alayad, A. Alshihri, F.O. Correa, Z. Akram, Clinical peri-implant parameters and inflammatory cytokine profile among smokers of cigarette, e-cigarette, and waterpipe, Clin. Implant Dent. Relat. Res. 20 (2018) 1016–1021.

[5] Z.H. Al-Sowygh, M.K. Aldamkh, A.M. Binmahfooz, K.A. Al-Aali, Z. Akram, O.A. Qutub, et al., Assessment of matrix metalloproteinase-8 and-9 levels in the peri-implant sulcular fluid among waterpipe (narghile) smokers and never-smokers with peri-implantitis, Inhal. Toxicol. 30 (2018) 72–77.

[6] M.S. BinShabaib, A. Mehmood, Z. Akram, S.S. ALHarthi, Peri-implant clinical and radiographic status and whole salivary cotinine levels among cigarette and waterpipe smokers and never-smokers, J. Oral Sci. 60 (2018) 247–252.

[7] T. Abduljabbar, Z. Akram, F. Vohra, S. Warnakulasuriya, F. Javed, Assessment of interleukin-1β, interleukin-6, and tumor necrosis factor-a levels in the peri-implant sulcular fluid among waterpipe (narghile) smokers and never-smokers with peri-implantitis, Clin. Implant Dent. Relat. Res. 20 (2018) 144–150.

[8] H. Tenenbaum, O. Bogen, F. Séverac, R. Elkaim, J.L. Davideau, O. Huck, Long-term prospective cohort study on dental implants: clinical and microbiological parameters, Clin. Oral Implants Res. 28 (2017) 86–94.

[9] T. Berglundh, G. Armitage, M.G. Araujo, G. Avila-Ortiz, J. Blanco, P.M. Camargo, et al., Peri-implant diseases and conditions: consensus report of workgroup 4 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions, J. Clin. Periodontol. 45 (2018) S286–S291.

[10] S. Renvert, C. Lindahl, G.R. Persson, Occurrence of cases with peri-implant mucositis or peri-implantitis in a 21–26 years follow-up study, J. Clin. Periodontol. 45 (2018) 233–240.

[11] S.M. Lupi, M. Granati, A. Butera, V. Collesano, Y. Rodriguez, R. Baena, Air-abrasive debridement with glycine powder versus manual debridement and chlorhexidine administration for the maintenance of peri-implant health status: a six-month randomized clinical trial, Int. J. Dent. Hygiene. 15 (2017) 287–294.

[12] F.S. del Amo, S.H. Yu, H.L. Wang, Non-surgical therapy for peri-Implant diseases: a systematic review, J. Oral Maxillofac. Res. 7 (2016) e13.

[13] A. Mellado-Valero, P. Buitrago-Vera, M.F. Solá-Ruiz, J.C. Ferrer-García, Decontamination of dental implant surface in peri-implantitis treatment: a literature review, Med. Oral Patol. Oral Cir. Bucal 18 (2013), https://doi.org/10.4317/medoral.19420.

[14] Z. Akram, S.A. Al-Shareef, U. Daood, F.Y. Asiri, A.H. Shah, M.A. AlQahtani, et al., Bactericidal efficacy of photodynamic therapy against periodontal pathogens in periodontal disease: a systematic review, Photomed. Laser Surg. 34 (2016) 137–149.

[15] F. Vohra, M.Q. Al-Rifaiy, G. Lillywhite, M.I. Hassan, F. Javed, Efficacy of mechanical debridement with adjunct antimicrobial photodynamic therapy for the management of peri-implant diseases: a systematic review, Photochem. Photobiol. Sci. 13 (2014) 1160–1168.

[16] F. Cieplik, D. Deng, W. Crielaard, W. Buchalla, E. Hellwig, A. Al-Ahmad, et al., Antimicrobial photodynamic therapy–what we know and what we don't, Crit. Rev. Microbiol. 44 (2018) 571–589.

[17] D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman, Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement, Ann. Internal Med. 151 (2009) 264–269.

[18] B. Azizi, A. Budimir, I. Bago, B. Mehmeti, S. Jakovljević, J. Kelmendi, et al., Antimicrobial efficacy of photodynamic therapy and light-activated disinfection on contaminated zirconia implants: an in vitro study, Photodiagn. Photodyn. Ther. 21 (2018) 328–333.

[19] A. Saffarpour, R. Fekrazad, M.N. Heibati, A. Bahador, A. Saffarpour, A.R. Rokn, et al., Bactericidal effect of erbium-doped yttrium aluminum garnet laser and photodynamic therapy on Aggregatibacter actinomycetemcomitans biofilm on implant surface, Int. J. Oral Maxillofac. Implants 31 (2016) 71–78.

[20] M. Giannelli, G. Landini, F. Materassi, F. Chellini, A. Antonelli, A. Tani, et al., Effects of photodynamic laser and violet-blue led irradiation on Staphylococcus aureus biofilm and Escherichia coli lipopolysaccharide attached to moderately rough titanium surface: in vitro study, Lasers Med. Sci. 32 (2017) 857–864.

[21] S. Eick, I. Meier, F. Spoerlé, P. Bender, A. Aoki, Y. Izumi, et al., In vitro-activity of Er: YAG laser in comparison with other treatment modalities on biofilm ablation from implant and tooth surfaces, PLoS One 12 (2017) e0171086.

[22] A. Widodo, D. Spratt, V. Sousa, A. Petrie, N. Donos, An in vitro study on disinfection of titanium surfaces, Clin. Oral Implants Res. 27 (2016) 1227–1232.

[23] T. Mang, S. Rogers, D. Keinan, K. Honma, R. Baier, Antimicrobial photodynamic therapy (aPDT) induction of biofilm matrix architectural and bioadhesive modifications, Photodiagn. Photodyn. Ther. 13 (2016) 22–28.

[24] J. Marotti, P. Tortamano, S. Cai, M.S. Ribeiro, J.E. Franco, T.T. de Campos, Decontamination of dental implant surfaces by means of photodynamic therapy, Lasers Med. Sci. 28 (2013) 303–309.

[25] C.R. Rovaldi, A. Pievsky, N.A. Sole, et al., Photoactive porphyrin derivative with broad-spectrum activity against oral pathogens in vitro, Antimicrob. Agents Chemother. 44 (2000) 3364–3367.

[26] A.A. Takasaki, A. Aoki, K. Mizutani, et al., Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases, Periodontol. 2000 (51) (2009) 109–140.

[27] M.N. Usacheva, M.C. Teichert, C.E. Sievert, M.A. Biel, Effect of Ca+ on the photobactericidal efficacy of methylene blue and toluidine blue against gram-negative bacteria and the dye affinity for lipopolysaccharides, Lasers Surg. Med. 38 (2006) 946–954.