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Biofilm Inactivation using Photodynamic Therapy in Dentistry: a review of literature

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Abstract

Introduction: Photodynamic therapy (PDT) is a therapy involving light and a photosensitising chemical substance, used in conjunction with molecular oxygen in order to elicit cell death (photo-toxicity) and thus ability to kill microbial cells, including bacteria, fungi and viruses. Photodynamic therapy is an alternative method of biofilm disruption and it is considered a new way of microorganism inactivation. It is also an additional procedure to reduce the infection rate in patients, caused by the increasing antimicrobials resistance of bacteria. *The aim* of this literature review was to evaluate the specific effects and the antibacterial effectiveness of photodynamic therapy using different types of photosensitizers (Erythrosine, Rose Bengal, Toluidine blue, Methylene blue, Ozone, Riboflavin, Curcumin, Chlorhexidine, SAPYR) and a visible light of a specific wavelength for each photosensitizer and to reveal the applications of PDT in periodontics, endodontics, prosthodontics and dental caries. **Methods:** A research of literature was performed in an attempt to find all the articles published on this topic in the last 10 years. The articles was searched by using a certain combination of different keywords (*photodynamic therapy) and (diode laser) and (teeth)* in PubMed database. **Results:** A total number of 83 articles were found. After applying inclusion and exclusion criteria, 35 articles were taken into consideration for our study and among them 4 were a manuscript, 3 was a review of literature, 1 was an *in vivo* evaluation and 27 were *in vitro* studies. **Conclusion:** Considering that none of the disinfection methods can completely remove the biofilm, PDT is a therapeutic tool complementary to conventional disinfection, with great applicability in dentistry. PDT showed significantly efficacy in reduction of biofilms. Exposure to light in the presence of a photosensitizing chemical substance helps in the reduction of microbes and the protocols could be recommended for clinical usage, but only together with ‘classic ‘ disinfection.

Key words: *Photodynamic, PDT, biofilm, photosensitizer, oral, antimicrobial therapy, light,*

Introduction

Microbial infections are a serious issue in public health and it was shown that biofilm-growing microorganisms are the main etiological factor in the development of oral disease. Thus there is a pressing need for development of new unconventional methods to remove the resistant bacteria and to prevent and control the increasing numbers of antibiotic and antimicrobial-resistant pathogens (1).

The application of PDT in the field of dentistry is still viewed as an unconventional method of treatment, a biologically therapeutic modality and an adjunct method of the traditional therapies, with one of its benefits being the fact that it can be repeated several times without severe side effects. Studies show it does not lead to cumulative toxicity (2). Moreover, it can be used also with immunocompromised people, mainly due to the low risks involved.

The application of the photodynamic therapy in dentistry is worth attention, as the method is noninvasive, painless, and the results of the published studies seem promising. PDT also represents a novel therapeutic approach in the management of oral biofilms. It has been seen that various combination of photosensitizers and light with different wavelength are effective against a broad spectrum of microorganism.

Methods

2.1. Search strategy. For our research we used the PubMed database and we selected and evaluated all the articles that resulted from a systematic search. The search aimed at finding articles published in the last 10 years, from 2011 until 2020 and by using a certain combination of words: (photodynamic therapy) and (diode laser) and (teeth).

Selection criteria. We used several inclusion criteria : a) articles written in English; b) studies of PDT antibacterial effects on biofilms and planktonic cells; c) studies in which PDT was used as a complementary therapy; d) studies in which PDT was used in combination with photosensitizers. We used also exclusion criteria: a). articles that did not report data; b). studies performed on animals.

2.3. Data extraction. All the authors involved in this study performed a screening of the titles found using the search strategy, with no difference in their findings. The articles relevant to the topic of the present study were selected using the inclusion and exclusion criteria and the full text version of these articles was obtained. At the same time, various aspects related to the utility of PDT in dentistry (the applicability of this method, the photosensitizers used and the microorganisms on which this combines therapy is effective) were evaluated.

Results

A total number of 83 articles were found. After applying both the inclusion and exclusion criteria, a total of 48 articles were excluded because 1 study was performed on animals and 47 were not relevant for our topic. The other 35 articles were analyzed and after reading, all 35 were taken into consideration for the present study. Several trials have shown that the use of photo activated therapy is a highly effective adjunct process used in the treatment of decays. The major effects of PDT were summarized in Table 1.

Discussion

The aim of this review was to create an update regarding the use of PDT in different fields of dentistry. We also indented to identify and analyze the efficacy of different types of photosensitizers and their possible adverse effects on dental tissues. All the authors evaluated the selected articles and organized their findings by answering to some relevant, based on topic questions.

Which is PDT's mechanism of action?

Etymologically, photodynamic therapy refers to the study of activating effects of light on living organisms. Based on this principle, PDT can be used to inactivate cell functions by interacting with a photosensitizer, irradiated with light of appropriate wavelengths. By achieving a certain level of irradiation, the molecular energy then transfers lead to the production of cytotoxic products (free radicals, singlet oxygen), which in turn are capable of modifying metabolic activities to an irreversible extent or of damaging essential components of the cell (3,4). The mechanism of action was summarized in Figure 1.

Which is the mechanism of action of photosensitizers?

A photosensitizer is a photo-activated chemical substance that can be activated upon exposure to the appropriate wavelength and light source.

SAPYR is a new generation photosensitizer which contains a positive charge for an appropriate adherence to cell walls of pathogens. Against biofilms, this photosensitizer has a dual mechanism of action: it inactivates pathogens in a polymicrobial biofilm with high efficacy and it is capable of disrupting the biofilm structure even without illumination. SAPYR has a good efficacy of bacteria killing, also against monospecies and polyspecies biofilm, therefore photodynamic bacterial inactivation using this type of photosensitizer offers an efficient approach to destroying all kind of oral key pathogens such as *Staphylococcus aureus* (5).

Methylene blue (MB) is proved to have an increased photodynamic action against *Aggregatibacter actinomycetemcomitans* organized on biofilm if it is used combined with red laser. Mainly due to its hydrophilic capacity and low molecular weight it can positively increase its effectiveness in reducing Gram-negative bacteria. MB acts by forming either hydroxyl radicals (type I) or singlet oxygen (type II), which are both cytotoxic products. The process of bacteria inactivation by MB involves a combination of type I and type II processes, and its efficiency is still under relative circumstances (6).

Curcumin is a yellow pigment, and also the predominant ingredient of turmeric powder, and it also can be used as a photosensitizer. To be effective it requires a very short light exposure. It has a wide spectrum of anti-bacterial action and reduced affinity for binding to mammalian cells. Its mechanism of action mostly focuses on anti-plaque effects that can also inhibit bacterial reproduction (7).

Rose Bengal is an anionic xanthene capable of high absorption bands in the green wavelength range (480-550 nm). Its singlet oxygen quantum yields are between 0.6-0.8, therefore mainly acting according to type II mechanism (3).

Erythrosine is a red dye, having a similar acting mechanism with Rose Bengal.

Riboflavin is a highly biocompatible photosensitizer whose action can be triggered using LED lamps in the dental office. The antimicrobial effect of photoactivated disinfection using riboflavin is yet to be widely studied and observations of its mechanism of action up to this point concluded in minor reduction of CFU counts. Even though riboflavin's use in PDT resulted in a complete eradication of *P. gingivalis* and *P. intermedia*, it can not be yet recommended in disinfection process of periodontal and endodontic infections (8).

Toluidine Blue is another photosensitizer which proved to be more effective than Riboflavin. It has proven to have a mildly moderate yet still more significant effect on *A. actinomicetemcomitans* and *P. gingivalis.* It is catalogued as a cationic photosensitizer and displayed an improved interaction with the Gram-negative cell membrane, which resulted in a strong antimicrobial effect (8). This photosensitizer is more efficient when used in PDT, in comparison with Radachlorin on S.mutans's viability (9). Common photosensitizers such as Methylene blue, Malachite green and Toluidine blue had shown in time their antimicrobial effects but their main disadvantage was teeth staining when applied on dental tissue (10).

For that reason, herbal extracts have been getting attention lately. *Curcuma longa*, *Hamamelis virginiana*, *Citrus lemon* and *Hypericum perforatum* can be used as photosensitizers in PDT, having an antimicrobial effect on planktonic and biofilm-organized bacteria. More than that, they present advantages like reducing the risk of teeth staining and have low toxicity (11).

Which are the applications of PDT in dentistry?

In the field of dentistry, the using of photodynamic therapy has been an adjunct antimicrobial strategy to conventional techniques for the treatment of many diseases in the dental context (1,12).

Taking into account the relevance of preventing periodontal disease and the reduced number of alternatives of treatment procedures which can fully control this disease, researchers started focusing on finding alternate methods for periodontal therapy. The main cause of periodontal disease is directly related to bacterial accumulation on the teeth surface, around the gingival margin. *Agregatibacter actinomicetemcomitans* plays an important part in the induction and progression of periodontal disease, which cannot be overlooked. Thus a large number of studies concentrated on finding a positive effect of PDT on this bacterium. The results of these studies highlighted the effectiveness of using a photosensitizer (e.g.: Curcumin, Chlorhexidine) in preventing the growth of *A. actinomicetemcomitans*, a result which was even more effective when used simultaneously with PDT (7).

Periodontal therapy's main focus is to significantly reduce or eliminate oral bacteria and periodontal pathogens, to stop disease progression and stabilize periodontal attachment levels. These goals can alternatively be achieved by using an unconventional method of therapy such as PDT, in addition to the traditional procedures such as scaling, root planning and antibiotic administration. Photodynamic therapy has an antimicrobial effect on *P. gingivalis*, a pathogen implied in periodontal disease, that's the reason why this approach is considered promising as a new way of bacterial elimination in periodontal therapy (13). Most of the studies results were consistent and proved that the use of photodynamic therapy results in decrease of bacterial content of biofilm, and also the structural disruption of biofilm matrix can make the pathogens more sensitive to eradication. More than that, adding hydrogen peroxide to photosensitizers during PDT-plus results in a high degree of antimicrobial photodynamic activity against planktonic periodontal pathogens and on bacteria organized in biofilms (14).

In root canal treatment, one of the most important stages is the complete elimination of bacteria from the root canal system. Endodontic pathogens have proved capable of developing a strong resistance in order to survive in adverse conditions, a situation which rendered the 'sterilization' of the root canal system to be almost impossible. The more conventional chemo-mechanical approach and the antimicrobial intracanalar medicaments frequently used for disinfection of the root canals often fail to obtain the desired effect of removing bacterial biofilms completely because of various microbiological and anatomical factors. And in this context, photo activation therapy is an antimicrobial strategy which proved more effective in microbial elimination thus emerging as an alternative approach to eradicate endodontic pathogens (2,15,16). The effect of PDT on E. faecalis, which is a major part of secondary endodontic infections, using reduced graphene-oxide-curcumin as a photosensitizer may be a promising therapeutic option for treating persistent endodontic infections (17).

Recently, a number of *in vitro* and *in vivo*- studies were conducted on human extracted teeth and their results showed that the root canals treated by photo activated disinfection exhibited high percentage of reduction of bacteria (15,16,18), and can be used safely, not being harmful for the bone and periapical tissues (19). It's important to highlight the fact that all studies results concurred. *In vitro* studies conducted using PDT have concluded that this approach has excellent bactericidal potential against *E. faecalis,* a bacterium with high occurrence in cases with refractory endodontic infections (20,21). It was demonstrated PDT's effect on mixed biofilms of *E. faecalis*, *S. aureus* and *C. albicans* (22), resulting in reduction of biofilm thickness of *C.albicans* and *E.faecalis* (23). Some studies revealed that application of PDT showed antibacterial effect similar to NaOCL when these therapies were used in endodontic infection caused by *Enterococcus faecalis* (24,25). One study reported that photodynamic therapy reduced bacterial viability, but not after 10 min irradiation, not being able to significantly eliminate *E. faecalis* contamination (26), the efficacy remaining questionable (27).

Photo-Activated Disinfection, as an innovative treatment method that utilizes two non-toxic components can also be used against cariogenic bacteria. The spreading zone of demineralization in a tooth decay is preceded by a layer of partially demineralized dentin infected with bacteria. PDT has shown good results in the process of disinfecting and to re-mineralize the partially infected area (2).

Photo-Activated Disinfection can also be used in treatment of all type of carious lesions before sealing. In decays, some disinfected demineralized tissue can be left before PDT is used. Several recent studies managed to prove that photo-sensitized cariogenic bacteria can be eradicated by directly applied visible light. The technique involves applying a photosensitizer on the operative surfaces and using a source of visible illumination which cause cytotoxic bacterial reactions that result in selective destruction of the targeted pathogens (2,28). Also in deep carious lesions, where *S. mutans* concentration is usually very high, PDT managed to reduce the metabolism of the bacteria and also the biofilm (29,30), being effective on many bacterial species like *L.casei*, *Fusobacterium nucleatum*, *Atopobium rimae*, except for *S.sobrinus* (31). Comparing photo-activated disinfection with the conventional methods used before, it was shown PDT's effectiveness over the cavity disinfectants or antibacterial materials (18,32). One study indicated that PDT with 0.01% Methylene blue was not able to reduce bacteria from deep carious lesions and cannot be considered a viable clinical approach (33).

Photo-Activated Disinfection has proved a positive effect in reducing cell viability of pathogens and it can be considered an important additional method of therapy to traditional techniques in treating several diseases in dental context.

PDT is currently being used in many medical areas, in dentistry is used in the fields of endodontics, periodontology, prosthodontics and carious lesions. For the treatment of all these diseases, PDT could prove out to be a promising therapeutic approach (8).

PDT as a dental treatment technique uses various light sources such as lasers (argon, diode or neodymium doped: yttrium, aluminum and garnet), and also less expensive and more cost effective LED lamps, in addition to a wide range of photosensitizers whose different effectiveness was demonstrated in may studies (4).

Photodynamic therapy also has other advantages such as immediate effects, improved access to complex areas as furcation, pits over the root, selectivity, it reduces the possibility of bacteremia in immuno-compromised systemic patients, decreases patient discomfort, reduced dentin sensitivity after root planning, reduced pain and edema after surgery, it is time saving and cost effective. Also, it has low toxicity levels and causes minimal damage to host tissue, for example human fibroblasts (34).

The mechanism of action of PDT involves a photo activated sensitizer that can interact with the biological substrate and resulting in the production of highly reactive oxygen species, such as singlet oxygen and free radicals, which can kill bacteria by damaging essential cellular molecules.

In prosthodontics, PDA is used in many ways, from the disinfection of prepared tooth before final cementation to interventions on the bone or gingival tissue (35). In periodontology, there is also an increasing interest in alternative approaches and PDT is proactively becoming an adjunct method to the conventional therapy. There are many clinical trials that showed substantially better clinical short term healing outcomes with adjunctive PDT. In endodontics, the use of photo activated therapy may be an important step for a better disinfection of infected root canals than the traditional methods can achieve and in shortening the number of visits at the dental office. Recent studies and case reports reported a significant reduction of bacterial load in the root canals after photodynamic therapy. Bacteria play a very important role in the initiation and progression of dental cavities.

Conclusion

Photo activated therapy brings a new perspective on the methods of therapy in the dental field and it can be considered a new complementary way of treatment. Considering the information highlighted previously, PDT has potential application to fight off many oral infections, it has low local toxicity, it can speed up dental treatment, several photosensitizers are disposable for each type of laser wavelenght and the treatment will not be harmful for the patient. Thus, this is clear that PDT is a promising method to combat the bacteria causing oral diseases.

References :

1. Garcez A., Nunez S., Azambuja N., et al. Effects of Photodynamic Therapy on Gram – Positive and Gram-Negative Bacterial Biofilms by Bioluminescence Imaging and Scanning Electron Microscopic Analysis. Photomed Laser Surg 2013;11 : 519-525.
2. Ifzah, Patel Z. Photo Activated Disinfection in Dentistry. European Journal of Pharmaceutical and Medical Research 2016;3 : 249-251.
3. Aparecida C., Borges A.C., et al. Photodynamic inactivation of *Streptococcus mutan*s and *Streptococcus sanguinis* biofilms in vitro. Lasers Med Sci 2013;28 : 859-864.
4. Asnaashari M., Mojahedi S.M., Asadi Z., Azari-Marhabi S., Maleki A. A comparison of the antibacterial activity of the two methods of photodynamic therapy (using diode laser 810 nm and LED lamp 630 nm) against *Enterococcus faecalis* in extracted human anterior teeth. Photodiagnosis and Photodynamic Therapy 2016;13 : 233-237.
5. Cieplik F., Spath A., et al. Photodynamic biofilm inactivation by SAPYR-An exclusive singlet oxygen photosensitizer. Free radical biology and medicine 2013;65 : 477-487.
6. Alvarenga L., Prates R.A., et al. *Aggregatibacter actitinomicetemcomitans* biofilm can be inactivated by methylene blue-mediated photodynamic therapy. Photodiagnosis and Photodynamic Therapy 2015;12 : 131-135.
7. Najafi S., Bahador A., et al. An In Vitro Comparison of Antimicrobial Effects of Curcumin –Based Photodynamic Therapy and Chlorhexidine, on Aggregatibacter actinomicetemcomitans. Journal of Lasers in Medical Sciences 2016;7 : 21-25.
8. Nielsen H., Garcia J., Vaeth M., Schlafer S. Comparison of Riboflavin and Toluidine Blue 0 as Photosensititizers for Photoactivated Disinfection on Endodontic and Periodontal Pathogens *In Vitro*. PLOS ONE (2015).
9. Vahabi S., Fekrazad R., Ayremlou S., Taheri S., Zangeneh N. The Effect of Antimicrobial Photodynamic Therapy with Radachlorin and Toluidine Blue on Streptococcus Mutans: An in Vitro Study. Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran 2011; Vol.8, No.2.
10. Costa L.M., Matos F.S., Correia A.M.O., Carvalho N.C., Faria-e-Silva A.L., Paranhos L.R., Ribeiro M.A.G. Tooth color change caused by photosensitizers after photodynamic therapy: an in vitro study. Journal of Photochemistry & Photobiology, B: Biology 2016;16: 225–228.
11. Nardinia E.F., Almeidaa T., Yoshimurab T., Ribeirob M., Cardosoa R., Garceza A. The potential of commercially available phytotherapeutic compounds as new photosensitizers for dental antimicrobial PDT: A photochemical and photobiological in vitro study. Photodiagnosis and Photodynamic Therapy 2019;27 :248–254.
12. Carrera E.T., Dias H.B., Corbi S.C.T., Marcantonio R.A.C., Bernardi A.C.A., Bagnato V.S., Hamblin M.R., Rastelli A.N.S. The application of antimicrobial photodynamic therapy (aPDT) in dentistry: a critical review. Laser Phys . Author manuscript, 2017.
13. C. Chui1, A. Aoki1, Y. Takeuchi1, Y. Sasaki, K. Hiratsuka , Y. Abiko, Y. Izumil. Antimicrobial effect of photodynamic therapy using high-power blue light emitting diode and red-dye agent on Porphyromonas gingivalis. J Periodontal Res 2013.
14. Decker E.M., Bartha V., Ohle C. Improvement of Antibacterial Efficacy Through Synergistic Effect in Photodynamic Therapy Based on Thiazinium Chromophores Against Planktonic and Biofilm-Associated Periodontopathogens. Photomedicine and Laser Surgery Volume 35, Number 4, 2017.
15. Charis B., Nikolaos E., Hercules S., Chrissanthy P., Theodoros L. Evaluation of Antimicrobial Photodynamic Therapy Using Indocyanine Green and Near-Infrared Diode Laser Against Enterococcus faecalis in Infected Human Root

Canals. Photomedicine and Laser Surgery, 2016.
16. Rios A., He J., Glickman G., Spears R., Schneiderman E., Honeyman A. Evaluation of photodynamic therapy using a light-emitting diode lamp against enterococcus faecalis in extracted human teeth. JOE — Volume 37, Number 6, June 2011.
17. Roghayeh G., Hadi A., Nasim C., Steven P., Babak P., Babak E., Mohammad Y.A., Abbas B. Modulation of virulence in Enterococcus faecalis cells surviving antimicrobial photodynamic inactivation with reduced graphene oxide-curcumin :An ex vivo biofilm model. Photodiagnosis and Photodynamic Therapy 29 (2020) 101643
18. Arash A., Samira S., Maryam R.A.R., Shirin L. Effect of photodynamic therapy with two photosensitizers on Streptococcus mutants: In vitro study. Accepted Manuscript, 2016.
19. Sarda R.A., Shetty R.M., Tamrakar A., Shetty S.Y. Antimicrobial Efficacy of Photodynamic Therapy, Diode Laser, and Sodium Hypochlorite and Their Combinations on Endodontic Pathogens. Journal Pre-proof, 2019.
20. Hirais M.C., Al-Huwaizi H.F. Evaluation of antibacterial action of photosensitizer solution activated by diode lamp and three intracanal medicaments (in vitro study). J Bagh College Dentistry 25(2013).
21. Abdollah G., Abbas B., Pegah S., Roya A., Reza F., Sohrab Asefi. Ex vivo comparison of antibacterial efficacy of conventional chemomechanical debridement alone and in combination with lightactivated disinfection and laser irradiation against Enterococcus faecalis biofilm. Photodiagnosis and Photodynamic Therapy 29 (2020) 101648
22. Ivan K., Ana B., Zrinka B., Suzana J., Ivica A. The photo-activated and photo-thermal effect of the 445/970 nm diode laser on the mixed biofilm inside root canals of human teeth in vitro:A pilots tudy. Photodiagnosis and Photodynamic Therapy 2019;26: 277–283.
23. Eslami L.M., Vatanpour M., Aminzadeh N., Mehrvarzfar P., Taheri S. The comparison of intracanal medicaments, diode laser and photodynamic therapy on removing the biofilm of Enterococcus faecalis and Candida albicans in the root canal system (ex-vivo study). Photodiagnosis and Photodynamic Therapy 26 (2019).
24. Fabio C.A., P. Salmeron-Lozano, Y. Martınez-Beneyto. Effects of photodynamic therapy, 2 % chlorhexidine, triantibiotic mixture, propolis and ozone on root canals experimentally infected with Enterococcus faecalis: an in vitro study. Odontology, 2016.
25. Yildirim C., Karaarslan E.S., Ozsevik S., Zer Y., Sari T., Usumez A. Antimicrobial efficiency of photodynamic therapy with different irradiation durations. European Journal of Dentistry, Vol 7 / Issue 4 / Oct-Dec 2013.
26. Matheus F., Juliane M.G.T., Mario T.F., Vanderlei S.B., Camila G.E., Fabio L.C., Villela B. Photodynamic therapy in root canals contaminated with Enterococcus faecalis using curcumin as photosensitizer. Lasers Med Sci, 2014.
27. Siddiqui S.H., Awan K.H., Javed F. Bactericidal efficacy of photodynamic therapy against enterococcus faecalis in infected root canals : a systematic literature review. Photodiagnosis and Photodynamic Therapy, 2013.
28. Natalia C.A., Rebeca F.M., Vanda S. M.C., Alexandrino P.S.N, Carla R.F., Vanderlei S.B., Catherine M.H., Marleny E.M.G. Photodynamic Inactivation of Cariogenic Pathogens Using Curcumin as Photosensitizer. Photomedicine and Laser Surgery,2012.
29. Shima A., Maryam P., Nasim C., Mahdi A., Abbas B. Anti-biofilm and anti-metabolic effects of antimicrobial photodynamic therapy using chlorophyllin-phycocyanin mixture against Streptococcus mutans in experimental biofilm caries model on enamel slabs. Photodiagnosis and Photodynamic Therapy 29 (2020).
30. Garcia M.T., Pereira A.H.C., Jorge A.O.C.J., Strixino J.F., Junqueira J.C. Photodynamic Therapy mediated by chlorin-type photosensitizers against Streptococcus mutans biofilms. Accepted Manuscript, 2018.
31. Steiner-Oliveira C., Longo P.L., Aranha A.C.C., Ramalho K.M., Mayer M.P.A., Eduardo C.P. Randomized in vivo evaluation of photodynamic antimicrobial chemotherapy on deciduous carious dentin. Journal of Biomedical Optics 20(10), 108003 (October 2015).
32. Fatma A.B., Ismail O., Bilge H.C., Emine S.K., Melis C., Merve A. Effects of photodynamic therapy with indocyanine green on Streptococcus mutans biofilm. Photodiagnosis and Photodynamic Therapy 2019;26: 229–234.

33. Neves P.A.M., Lima L.A., Rodrigues F.C.N., Leitao T.J., Ribeiro C.C.C. Clinical effect of photodynamic therapy on primary carious dentin after partial caries removal. Braz. Oral Res. 2016;30(1).
34. Masuda Y., Sakagami H., Horiike M., Kadokura H., Yamasaki A., Klokkevold P.R., Takei H., Yokose S. Photodynamic Therapy with Pyoktanin Blue and Diode Laser for Elimination of Enterococcus faecalis. In vivo 2018;32: 707-712 .
35. Gounder R., Gounder S. Laser Science and its Applications in Prosthetic Rehabilitation. Journal of Lasers in Medical Sciences 2016;7 : 209-213.

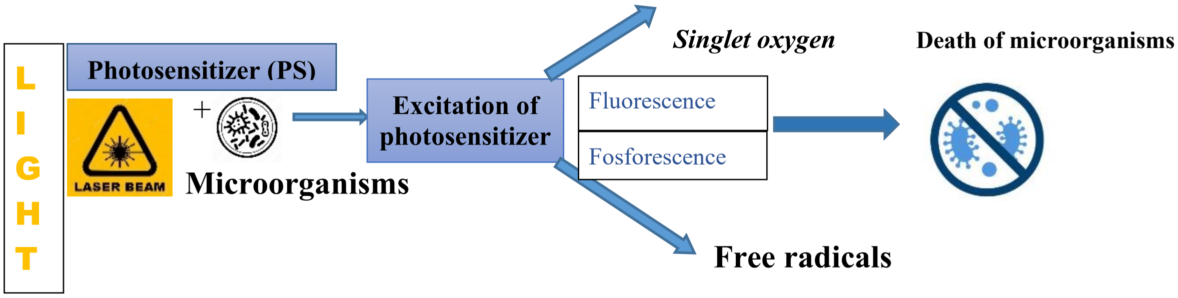

Figure 1. Photodynamic therapy mechanism : the microorganisms absorb the photosensitizer and after exposure to light at a specific wavelength it starts the excitation of PS, which generates oxygen singlet and free radicals which lead to the death of bacteria.

Table 1 Antimicrobial effect of photodynamic therapy studies.

Authors	**Study**	**Conclusion**
Garcez et al	Tested the effects of a 66o nm diode laser and methylene blue on gram-positive and gram-negative bacterial biofilms.	PDT successfully reduced *P. aeruginosa* and *E. faecalis* cells in biofilms, disrupting the structure of the biofilm.
Cieplik et al	Investigated the photodynamic biofilm inactivation properties of SAPYR, against monospecies and polyspecies biofilms.	SAPYR can interrupt biofilm structure and also has a high killing efficacy against bacteria.
Nielsen et al	Investigated the effect of PDT using riboflavin and a led lamp and comparing it to PDT using toluidine blue and a red light.	Limited microbial kills were found when using riboflavin/blue light PDT. PDT with toluidine blue/red light showed great efficacy on all investigated species.
Asnaashari et al	Investigated the effects of PDT using a diode laser (810 nm) and a diode lamp (630 nm) on *E. faecalis* biofilm in anterior extracted human teeth.	PDT with 810 nm diode laser could evidently reduce the amount of bacteria from root canals. Effect of diode lamp of 630 nm against *E faecalis* was evident.
Pereira et al	Investigated the effects of photodynamic inactivation against *S. mutans* and *S. sanguinis* using erytrosine (ER) and Rose Bengal (RB).	In vitro biofilms formed by *S. mutans* and *S. sanguinis* were sensitive to PDT using blue led associated with photosensitizers ER or RB.
Hirais et al	Evaluated the effectiveness of PDT using a diode lamp and toluidine blue against the microorganisms from the root canal systems.	Root canals treated by PDT showed high percentage of bacteria reduction after 30 second from treatment and after 7 days (96.36, 99.48) respectively.

Alvarenga et al	Evaluated the antibacterial effects of PDT against *Aggregatibacter actinomycetemcomitans* using methylene blue as a photosensitizer.	Laser irradiation (660 nm and 100 mW) showed a reduction of *A. actinomycetemcomitans* by up to 99.85% on the group exposed to PDT for 5 min of irradiation.
Shima et al	Evaluated the influence of PDT using a diode laser (635 nm) and clorophyllin – phycocyanin mixture as photosensitizer on bacteria within a preformed biofilm caries model on enamel slabs of Streptoccocus mutans.	At a maximum concentration of CHL-PC (5000 microlitres/ml) and 3 min. DL irradiation time reduced the cariogenic biofilm of S. mutans by 36.93% and reducing its metabolic activity.
Abdollah et al	Compared and tested antibacterial efficacy of chemomecanical debridement (CCMD) alone, CCMD+ light activated disinfection (LAD 810 nm 0.3 W) and CCMD + DL (810 nm 2W) and indocyanine green against Enterococcus faecalis biofilm.	All methods resulted in partial elimination of Enterococcus faecalis from the root canal biofilm but CCMD + LAD showed a higher efficacy from the three metods.
Ivan et al	Tested the antibacterial effect of 445/660/970 nm diode laser on a mixed biofilm of S. Aureus, C. Albicans and E. faecalis inside root canals of extracted human teeth.	A significant reduction in the microbial population was observed in all treatments. C.albicans appears to be more sensitive to laser irradiation than other bacteria.
Natalia et al	Investigated the effect of PDT on Streptococcus mutans and Lactobacillus acidophilus when grown simultaneously in dentine carious lesions. Different concentrations of curcumin photosensitizer where used (0.75, 1.5, 3.0, 4.0, 5.0 g/L) and activated through LED source with 450 nm wavelength at two intensities (19 and 47.5 mw/cm2)	Notable bacterial reduction was observed at both intensities of the the LED light with 5.0 g/L curcumin concentration and 5 minutes irradiation time. In order to have a succesful procedure it is required a higher concentration of curcumin as photosensitizer.
Fatma et al	Compared the antibacterial effect of PDT using diode laser with indocyanine green (ICG) on Streptococcus mutans biofilm with CHX, NaOCL, gaseous ozone, YAG laser and diode laser.	Similar and significant bacterial reduction was observed in all groups, but lower in the ICG group. Still PDT using diode laser with ICG may be suggested on cavity disinfection as an alternative to conventional methods.
Azizi et al	Compared the antimicrobial effect of PDT with Indocyanine green (IG 0.2 %) and Methylene Blue (MB 2%) on on extracted teeth contamined with *Streptococcus mutans.*	PDT with a combination of Methylene blue and Indocyanine green have the ability to completely eradicate *Streptococcus mutans* bacterial colonies.
Beltes et al	Evaluated the effect of PDT in root canals infected with *Enterococcus faecalis* using Indocyanine green as photosensitizer and a near infrared diode (NIR)	All methods had bactericidal effect to Enterococcus faecalis (60 -99.9 % reduction) from the root canals with ICG-mediated PDT activated by NIR diode (810 nm) with 60 seconds irradiation time.

Camacho et al	Evaluated the antibacterial effect of PDT (660 nm) , and methylene blue as sensitizer and also compared with 2% CHX, propolis, ozone and triantibiotic in root canal systems.	Enterococcus faecalis inside root canals can be reduced by up to 98.13% if treated with PDT for 60 seconds, because of the penetration capacity in dentinal tubules of laser therapy.
Chuic et al	Tested the effect of PDT using a high-power blue LED (425-470 nm) and red-dye agent in periodontal therapy on *Porphyromonas gingivalis*.	Blue light irradiation in combination with Rose Bengal photosensitizer demonstrated a significat antimicrobial/growth-inhibiting effect on *P. gingivalis* and shows promise as a treatment method in elimination of bacteria in periodontal disease.
Da Frota et al	Evaluated the effect of PDT in root canal therapy contaminated with *E. faecalis* using blue LED (450 nm) and curcumin as sensitizer	PDT combined with curcumin sensitizer (5 min irradiation time) reduced bacterial viability but it did not significantly eliminate contamination with *E. faecalis.*
Vahabi et al	Tested the effect of PDT using diode laser (633 nm) in combination with toluidine blue O (TBO) on the viability of *S. mutans* in vitro.	The results demonstrated that PDT with diode laser (644 nm and 3 J/cm2 energy density) in combination with toluidine blue 0 (TBO) is effective in reducing the viability of *S. mutans* on pure cultures.
Rios et al	Evaluated the effect PDT in disinfecting root canals using toluidine blue and low energy LED on extracted human teeth contaminated with *Enterococcus faecalis.*	PDT using toluidine blue and LED after 30 seconds irradiation time showed a 97.1% reduction of *E. faecalis* viability and has the potential to be used in endodontic therapy as adjuvant antimicrobial method.
Neves et al	Investigated the clinical effect of PDT in decontamination of deep dentin in teeth submitted to partial elimination of carious tissue using methylene blue dye (0.01%) irradiated by a diode laser at 660 nm.	Limited bactericidal effects were found when using PDT in deep dentin when using methylene blue 0.01% and diode laser on *Lactobacillus spp.* and *S. mutans*.
Steiner et al	Compared antimicrobial effects in primary carious dentin of PDT using diode laser (660 nm) with methylene blue and LED (630 nm) with toluidine blue in a randomized in vivo study with 6 and 12 months follow-up periods.	Both PDT methods have shown to be effective in reducing the number of bacteria and can be considered modern and minimal approaches for the treatmet of deep primary caries.
Yildirim et al	Evaluated the efficiency of PDT at different durations of irradiation (1, 2, 4 min) on *E. faecalis* inoculated teeth. It was used methylene blue activaded by a 660 nm diode laser.	Between 99.8% and 99.9% reduction of viability was obtained proving that PDT with 660 nm diode and methylene blue is effective against *E. faecalis.*