Critical review

Oral Microbiology

Photodynamic antimicrobial chemotherapy for prevention and treatment of dental caries: a critical review

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Abstract

Introduction

Photodynamic antimicrobial chemotherapy studies regarding dental caries have been present more frequently in the literature. However, photodynamic antimicrobial chemotherapy depends on the adjustment of variables such as the type of light source and, photosensitisers target microorganism; this makes it difficult to draw meaningful comparisons. The purpose of this paper was to provide a critical review related to this coadjuvant approach in the prevention and treatment of dental caries.

Materials and Methods

A database search was made via Medline/PubMed (keywords: photodynamic therapy and dental caries) and 33 articles were found.

Results

Twelve articles were included after using the filter tool, being excluded reviews and manuscripts reporting works not related to the studied area.

Conclusion

The manuscripts showed that photodynamic therapy presents optimal results against dental caries, even though better understanding of photodynamic antimicrobial chemotherapy and its components are necessary before the clinical application of this alternative modality in the dental practice.

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Introduction

The human oral cavity is heavily colonised by a complex, relatively specific and highly interrelated range of microorganisms (as many as 1000 different species have been detected) collectively known as *normal oral microflora*[1]. A peculiarity of this environment is that most of the bacteria found here are present in complex aggregates (known as biofilms)[2]. Microbial biofilms are composed of microorganisms adhered both to each other and to dental surfaces (or interfaces) and embedded in an extracellular polymeric matrix, which includes water and nutrient channels[3]. A change in a key environmental factor will trigger a shift in the balance of the resident microflora, which will promote the emergence of acidogenic/aciduric bacteria. The constant accumulation of these kind of bacteria change the equilibrium towards dental demineralisation (dental caries lesions)[4].

The constituents of diet present an important role in the development of dental caries. Sucrose is considered the most cariogenic dietary carbohydrate, because it is fermentable and serves as a substrate for the synthesis of extracellular polysaccharides (EPS) and intracellular polysaccharides in cariogenic dental plaque[4].

In addition, the presence of EPS (mainly insoluble glucan) promote bacterial adherence to the tooth surface and contribute to the structural integrity of dental biofilms. Yet, there is a clear evidence showing that sucrose exposure and insoluble EPS lead to a more cariogenic biofilm[5].

Dental caries is among the most significant human chronicle infectious diseases and results in the progressive dissolution of enamel. With the disease progression, it can lead the underlying dentine compromising the vitality of the element and its fixation in the maxillomandibular complex[6].

Prevention of dental caries can be achieved by controlling the accumulation of dental plaque by mechanical removal[7]. In cases of insufficient biofilm disorganisation, the association with antimicrobial chemical agents, such as chlorhexidine may help in the decreasing of pathogenic bacteria levels[7]. Unfortunately, this preventive approach does not reach the population as a whole, allowing dental cavity formation. Treatment of the carious lesion involves the removal of infected dentine with posterior restoration of the affected tooth with any of the variety of materials, for example mercury amalgam, resin composite and glass ionomer cements[8]. Due to emergence of antibiotic resistant strains, alteration in taste, burning sensation, increase of calculus formation and staining of the teeth and restorative materials stimulated a search for alternative treatments[9].

Recently, approaches that might offer the possibility of efficient intraoral bacterial count reduction with minimum damage to systemic health (preventive approach) and avoid secondary caries development reducing the chance of material substitution and pulp inflammation as well (curative approach) are necessary. For these circumstances, photodynamic antimicrobial chemotherapy (PACT) offers the possibility of a novel modality to reduce pathogenic bacteria, and consequently, prevent against (new) dental caries lesions[10].

PACT is based on the combined use of a photosensitive drug (usually a dye) and an appropriate wavelength of visible light. Once exposed to light, the photosensitisers are activated to a short-lived excited state that then converts to a long-lived triplet state. This state may generate free radicals or superoxide ions resulting from hydrogen or electron transfer (type I) and can produce oxygen singlets or reactive oxygen species (ROS) (type II) (Figure 1). The abundant ROS generated reacts with the surrounding molecules and exerts a bactericidal effect on microorganisms[10].

Overall, PACT has been extensively investigated for the treatment of several microorganisms, including bacterial oral pathogens, in both *in vitro* and clinical trials[11]. As a rapid, non-toxic and non-invasive antimicrobial approach, PACT emerges as a suitable process to reduce bacterial contamination, increasing the success of the treatment[12]. Furthermore, another advantage of PACT compared to antibiotics is that bacteria do not develop resistance to oxygen species, since there is no target specificity. As compared to chlorhexidine, PACT does not exert the reported side-effects[12].

Thus, based on the information highlighted above, the aim of this present work was to investigate the status of this coadjuvant approach, specifically in the prevention and treatment of dental caries, by revising critically the specific current literature.

Materials and Methods
Database search
An electronic search (executed on 17 October 2013) was performed in Medline/PubMed database using the following keywords (after consulting and adequacy to the controlled vocabulary descriptors MeSH): photodynamic therapy and dental caries. No limits regarding year of publication or type of study was made, in exception to published language (American/British English) and investigations that offer abstract and full text as well.

Results
The search resulted in 33 studies in which just 12 were included due to matching with the objective of the present investigation. These studies involved *in vitro*, *in situ* and *in vivo* experiments with a diversity of PACT protocols that were published between 2004 and 2012. Figure 2 describes a flux gram used to filter articles resulting in the number cited above. In addition, the main characteristics of these works are described in Table 1.

Discussion
The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

It has been known since the beginning of the last century that microorganisms can be killed by the combination of dyes and light. In 1900, Raab[13] reported the lethal effect of acridine and visible light on *Paramecium caudatum* and the
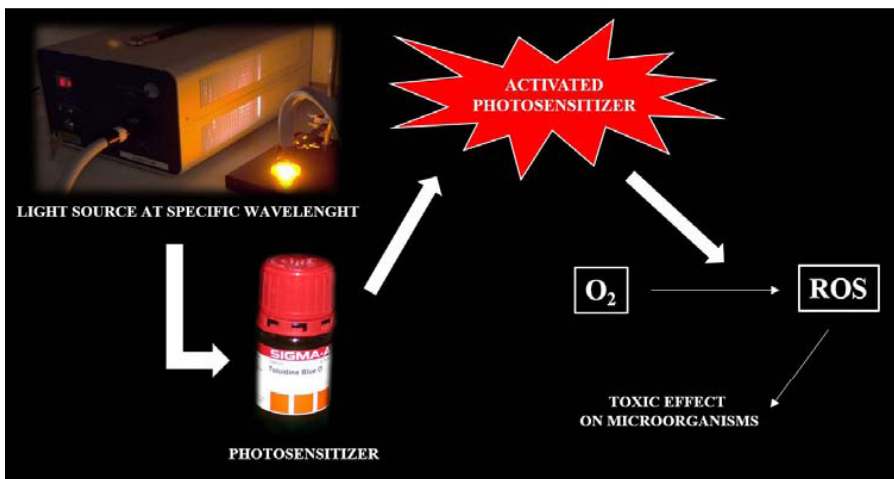
Figure 1: Schematic representation of PACT way of action. Source of light of specific wavelength is absorbed by a proper photosensitizer, which allows a transition form of low short-energy of oxygen to the excited long-excited singlet state. ROS and singlet oxygen formed are able to damage nucleic acid and plasmatic membrane with consequent microorganism death (photodamage or photosensitization effect).
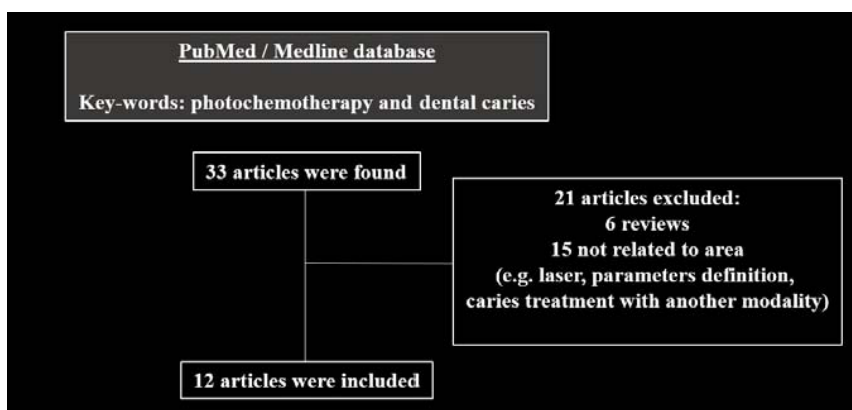
Figure 2: Flux gram showing the result of articles search.

Critical review

Table 1 Studies related to PACT to prevent and treat dental caries and some parameters				
Study	**Type of study and MO***	**Photosensitizer**	**Light source**	**Dose of energy**
Wilson (2004)[22]	*In vitro—Streptococcus* spp.	Toluidine blue (50 ug/mL) and phthalocyanine	He–Ne laser and GaAlAs laser diode	No available data
Müller et al. (2007)[18]	*In vitro—S. sobrinus* and *S. oralis*	Methylene blue	Soft laser (Helbo Theralite Laser)	75 mW
Lima et al. (2009)[19]	*In situ—S. mutans* and *L. acidophilus*	Toluidine blue (100 ug/mL)	Red LED	47 and 94 J/cm^2
Costa et al. (2010)[26]	*In vitro—S. mutans*	Erythrosine (24 uM) and rose bengal (24 uM)	LED	95 J/cm^2 and 526 mW/cm^2
Vahabi et al. (2011)[23]	*In vitro—S. mutans*	Toluidine blue (0.1%) and radachlorin (0.1%)	Diode laser	3 and 12 J/cm^2
Longo et al. (2012)[27]	*In vitro* and *In vivo—* cariogenic bacteria	Phthalocyanine	Red laser	*In vitro* (6.85, 20.57 and 61.71 J/cm^2); *In vivo* (180 J/cm^2)
Guglielmi et al. (2011)[20]	*In vivo—S. mutans* and *Lactobacillus* spp.	Methylene blue (0.01%)	InGaAlP laser	320 J/cm^2
Baptista et al. (2012)[24]	Animal study—*S. mutans*	Methylene blue (100 uM)	Red light-emitting diode	480 mW/cm^2
Mang et al. (2012)[28]	*In vitro—S. mutans*	Photofrin (125 ug/mL)	KTP:YAG laser	100 mW/cm^2
Ishiyama et al. (2012)[29]	*In vitro—S. mutans*	Erythrosine, rose bengal and phloxine (25 and 100 mM)	Nd:YAG laser	80 mW/cm^2
Teixeira et al. (2012)[25]	*In vitro* and *In situ—S. mutans*	Toluidine blue (100 ug/mL)	Red LED	55 J/cm^2
Araújo et al. (2012)[30]	*In vitro—S. mutans* and *L. acidophilus*	Curcumin (0.75 and 1.5 g/L)	Blue LED	5.7 J/cm^2

essential involvement of light and oxygen in the process was shortly thereafter demonstrated by von Tappeiner, who inserted the term 'photodynamic'[14]. However, the potential of PACT against microbial diseases was not exploited for several decades, especially for two basic reasons: (i) some well-known pathogens (specially gram-negative and protozoa) are poorly affected by most traditional photosensitising agents (e.g. xanthene, acridine dyes and porphyrins) and; (ii) the discovery of antibiotics raised the belief that microbiologically based diseases would have been gradually reduced to a level that no longer had a serious impact on human health. On the other hand, the rapid emergence of resistance to even those antibiotics which initially appeared to be highly effective disappointed such expectations[15].

Decades of epidemiological, biochemical and animal studies reported that streptococci generally comprise the majority of dental plaque microorganisms and implicated *Streptococcus mutans* as the principal agent of dental caries[16]. In the included articles in this review, *S. mutans* was the most studied bacteria, since it is one of the microorganisms involved with the first stages of dental caries[17]. As the lesion progresses to deeper

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dentin, anaerobic species start to thrive and a transition takes place from predominantly facultative gram-positive bacteria to strictly anaerobic gram-positive rods and cocci, and gram-negative rods. Thus, other specimens were also evaluated by the authors like *Lactobacillus acidophilus*, *Streptococcus sobrinus* and *Streptococcus oralis*[18–20].

The effectiveness of PACT is based on some aspects: photosensitiser capability of interacting with the bacterial membrane, photosensitiser ability of penetration and action inside the cell, and ROS formation around the bacterial cell by illumination of the PS. In this review, all the selected papers evaluated and achieved optimal results against gram-positive bacteria (Table 1). In particular, gram-positive bacteria present at the outer wall (15–80 nm thick) containing up to 100 peptidoglican layers, which are intimately associated with lipotheicoic and negative charged teichuronic acids. This wall displays a relatively high degree of porosity, since various macromolecules, such as glycopeptides and polysaccharides were found to readily diffuse to the inner plasma membrane[21]. This fact allied to the absence of protective external membrane can explain the good PACT outcomes.

Seven of the 12 articles used photosensitisers of the phenotiazinium group (toluidine blue and methylene blue)[18–20,22–25]. This group presents key characteristics that can explain their widespread use in PACT: capability of inactivating both gram-positive and gram-negative bacteria, their positively charged molecule, which may bind to the polyphosphates of the outer membrane and rapidly attracted by negatively charged mitochondria and inner organelles, high efficiency in production of ROS species and low costs[25].

In the same way, anionic photosensitisers were used as well (erythrosine, radachlorin, phthalocyanines, rose bengal and photofrin) in five

investigations[22,23,26–29]. In these studies, low concentrations were able to photoinactivate planktonic suspensions, biofilm structures and dental plaque scraping of *S. mutans* with different light sources and exposure times. Costa et al.[26] achieved a log bacterial reduction of 5–6 when using rose bengal and erythrosine, whereas Ishiyama et al.[29] reduced in 1–3 of *S. mutans* bacterial suspension. Since these compounds are used as plaque disclosing agents and are activated with lights easily found at dental offices, it seems to be more appropriate for PACT application. Just one article used a natural photosensitiser (curcumin) presented an optimal bacterial reduction after proper light illumination exposure[30]. The authors cite easy manipulation, high rate of ROS formation and low costs as advantages of using these kind of photosensitisers[30].

For efficiency of PACT, the dye must be activated by proper wavelengths from light sources. Thus, the basic requirement for lights is that they match the activation absorption spectrum of the photosensitiser and provide adequate dose of energy that are able to transit to a higher-energy triplet state[27].

The literature presents three main classes of clinical PACT light sources: lasers, presents three main classes (LED) and halogen lamps. Laser has some advantages, such as monochromaticity and high efficiency (>90%) and high potency as well; however, they do have a high cost and requires a separate unit for each photosensitiser due to the different absorption wavelengths. On the other hand, the main advantages of LED over lasers are their low cost, portability, easy configuration arrays into different irradiation geometries and it demonstrated the same antimicrobial effects on *S. mutans* biofilm viability as stated by a recent publication[31]. Filtered halogen lamps have the advantage that they can be spectrally filtered to match any photosensitiser; however, they cannot be efficiently

coupled into optical fibre bundles or liquid light guides.

The manuscripts included in this review (Table 1) contain publications using lasers and LED devices to activate different photosensitisers. Most of the papers employed red lights to activate phenotizinium dyes (toluidine blue and methylene blue)[18–20,22–25], since the maximum absorbance of these components occur at 600–660 nm. Other blue/green photosensitisers were porphyrin (photofrin)[28] and phthalocyanine derivatives[27], which have the maximum absorbance for red light at 630 nm and 600—700 nm, respectively. These photosensitisers were reported to be successfully activated by compatible lights with their maximum absorbance. Other photosensitisers reported in the articles were red coloured, such as rose bengal[26,29] and erythrosin[26,29], which absorb lights at 561 and 530 nm, respectively. For rose bengal, the employed light sources emitted wavelengths between 400 and 500 nm. The advantages employing these agents is that both photosensitisers (plaque disclosing agents) and light sources such as halogen lamps and LED at blue wavelength are present in the dental routine and can be used in PACT without requiring acquisition of new equipment[26,29].

It is worth to highlight that, as another new modality of treatment, toxicity studies and more detailed *in situ* and animal models need to be used before clinical application; it can explain, in part, the fact that there are only two *in vivo* investigations[20,27] among the selected articles.

In summary, PACT will probably not replace classic therapy for dental caries. However, the photodynamic approach may improve, accelerate and lower the cost of dental treatment, with several advantages. It may be used in the future as a coadjuvant therapy for dental caries treatment, sterilising the dental surface during treatment. It may also have benefits on endodontic and periodontal treatments.

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Conclusion

This critical review showed promising results for the use of photodynamic chemotherapy against cariogenic bacteria. However, it is known that the oral cavity environment is different from the laboratorial culture or *in vitro* environment, which limits the therapeutical application for PACT. Although the studies referred in this review are extremely important for the increased knowledge on the PACT potential, further *in situ* and *in vivo* studies, including toxicity investigations, should be performed before PACT is established as an antimicrobial option for dental preventive and restorative routine.

Abbreviations list

EPS, extracellular polysaccharides; LED, light-emitting diode; PACT, photodynamic antimicrobial chemotherapy; ROS, reactive oxygen species.

References

1. Schuster GS. Oral flora and pathogenic organisms. Infect Dis Clin North Am. 1999 Dec;13(4):757–74.
2. Wilson M. Bacterial biofilms and human disease. Sci Prog. 2001;84(Pt 3):235–54.
3. Paes Leme AF, Koo H, Bellato CM, Bedi G, Cury JA. The role of sucrose in cariogenic dental biofilm formation–new insight. J Dent Res. 2006 Oct;85(10):878–87.
4. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res. 1994 Jul;8(2):263–71.
5. Marsh PD. Sugar, fluoride, pH and microbial homeostasis in dental plaque. Proc Finn Dent Soc. 1991;87(4):515–25.
6. Radcliffe CE, Akram NC, Hurrell F, Drucker DB. Effects of nitrite and nitrate on the growth and acidogenicity of Streptococcus mutans. J Dent. 2002 Sep–Nov; 30(7–8):325–31.
7. Paraskevas S, Versteeg PA, Timmerman MF, Van der Velden U, Van der Weijden GA. The effect of a dentifrice and mouth rinse combination containing amine fluoride/ stannous fluoride on plaque and gingivitis: a 6-month field study. J Clin Periodontol. 2005 Jul;32(7):757–64.
8. Summitt JB. Conservative cavity preparations. Dent Clin North Am. 2002 Apr; 46(2):171–84.
9. Arweiler NB, Auschill TM, Reich E, Netuschil L. Substantivity of toothpaste slurries and their effect on reestablishment of the dental biofilm. J Clin Periodontol. 2002 Jul;29(7):615–21.
10. Dougherty TJ. An update on photodynamic therapy applications. J Clin Laser Med Surg. 2002 Feb;20(1):3–7.
11. Wilson M, Dobson J, Harvey W. Sensitization of oral bacteria to killing by low-power laser radiation. Curr Microbiol. 1992 Aug;25(2):77–81.
12. Bevilacqua IM, Nicolau RA, Khouri S, Bruqnera A Jr, Teodoro GR, Zângaro RA, Pacheco MT. The impact of photodynamic therapy on the viability of Streptococcus mutans in a planktonic culture. Photomed Laser Surg. 2007 Dec;25(6):513–8.
13. Raab O. Uber die wirkung fluoriziender stoffe auf infusorien. Zeit Biol. 1900;39:524–46.
14. Von Tappeiner H. Zur kenntis der lichtwirkenden (fluoreszierenden) stoffe. Dtsch Med Wochen. 1904;1:579–80.
15. Tünger O, Dinç G, Ozbakkaloglu B, Atman UC, Algün U. Evaluation of rational antibiotic use.Int J Antimicrob Agents. 2000 Jul;15(2):131–5.
16. Lemos JA, Burne RA. A model of efficiency: stress tolerance by Streptococcus mutans. Microbiology. 2008 Nov;154(Pt 11):3247–55.
17. Mikkelsen L, Jensen SB, Jakobsen J. Microbial studies on plaque from carious and caries-free proximal tooth surfaces in a population with high caries experience. Caries Res. 1981;15(5):428–35.
18. Müller P, Guggenheim B, Schmidlin PR. Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro. Eur J Oral Sci. 2007 Feb;115(1):77–80.
19. Lima JP, Sampaio de Melo MA, Borges FM, Teixeira AH, Steiner-Oliveira C, Nobre Dos Santos M, et al. Evaluation of the antimicrobial effect of photodynamic antimicrobial therapy in an in situ model of dentine caries. Eur J Oral Sci. 2009 Oct;117(5):568–74.
20. Guglielmi Cde A, Simionato MR, Ramalho KM, Imparato JC, Pinheiro SL, Luz MA. Clinical use of photodynamic antimicrobial chemotherapy for the treatment of deep carious lesions. J Biomed Opt. 2011 Aug;16(8):088003.
21. Friedrich CL, Moyles D, Beveridge TJ, Hancock RE. Antibacterial action of structurally diverse cationic peptides on grampositive bacteria. Antimicrob Agents Chemother. 2000 Aug;44(8):2086–92.
22. Wilson M. Lethal photosensitisation of oral bacteria and its potential application in the photodynamic therapy of oral infections. Photochem Photobiol Sci. 2004 May;3(5):412–8.
23. 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. J Dent. 2011;8(2):48–54.
24. Baptista A, Kato IT, Prates RA, Suzuki LC, Raele MP, Freitas AZ, Ribeiro MS. Antimicrobial photodynamic therapy as a strategy to arrest enamel demineralization: a short-term study on incipient caries in a rat model. Photochem Photobiol. 2012 May–Jun;88(3):584–9.
25. Teixeira AH, Pereira ES, Rodrigues LK, Saxena D, Duarte S, Zanin IC. Effect of photodynamic antimicrobial chemotherapy on in vitro and in situ biofilms. Caries Res. 2012;46(6):549–54.
26. Costa AC, Chibebe Junior J, Pereira CA, Machado AK, Beltrame Junior M, Junqueira JC, Jorge AO. Susceptibility Of planktonic cultures of Streptococcus mutans to photodynamic therapy with a light-emitting diode. Braz Oral Res. 2010 Oct–Dec;24(4):413–8.
27. Longo JP, Leal SC, Simioni AR, de Fátima Menezes Almeida-Santos M, Tedesco AC, Azevedo RB. Photodynamic therapy disinfection of carious tissue mediated by aluminum-chloride-phthalocyanine entrapped in cationic liposomes: an in vitro and clinical study. Lasers Med Sci. 2012 May;27(3):575–84.
28. Mang TS, Tayal DP, Baier R. Photodynamic therapy as an alternative treatment for disinfection of bacteria in oral biofilms. Lasers Surg Med. 2012 Sep;44(7): 588–96.
29. Ishiyama K, Nakamura K, Ikai H, Kanno T, Kohno M, Sasaki K, Niwano Y. Bactericidal action of photogenerated singlet oxygen from photosensitizers used in plaque disclosing agents. PLoS One. 2012;7(5):e37871.
30. Araújo NC, Fontana CR, Bagnato VS, Gerbi ME. Photodynamic effects of curcumin against cariogenic pathogens. Photomed Laser Surg. 2012 Jul;30(7):393–9.
31. Zanin IC, Gonçalves RB, Junior AB, Hope CK, Pratten J. Susceptibility of Streptococcus mutans biofilms to photodynamic therapy: an in vitro study. J Antimicrob Chemother. 2005 Aug;56(2): 324–30.