Photodynamic therapy in the control of endodontic infections

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ABSTRACT

Introduction: Effective decontamination of the root canal system is key to the success of endodontic treatment. Photodynamic Therapy (PDT) may be an important tool in the reduction of root canal pathogens. **Objectives:** This study aimed to review the literature on the use of this therapy in Endodontics, and which clinical protocol has been used. **Methods:** A literature search was carried out using the following databases: PubMed and Scielo, and the following key-words: *endodontics; photodynamic therapy; photodynamic therapy endodontics; light-activated disinfection root canal; light activated disinfection endodontics; photo-activated disinfection endodontics; photo-activated disinfection*. **Results:** We selected 18 articles which fit the inclusion criteria: original and relevant articles, clinical and laboratory research, in permanent human teeth, as well as articles reporting the use of photodynamic therapy as a single or adjunct treatment to root canal disinfection, in addition to its efficacy in bacterial reduction. **Conclusions:** PDT has been shown to be an effective method in microbial reduction of root canals; however, it should be used as an additional method to conventional treatment.

Keywords: Photochemotherapy. Endodontics. Dental pulp cavity

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Introduction

The main reason for failures in endodontic treatment is the persistence of infection.[1] Success of this therapy depends on elimination of pathogenic microorganisms from the root canal system after chemical-mechanical cleaning.[2]

New technologies and material have been developed in an attempt to increase effectiveness of disinfection in root canals, and photodynamic therapy (PDT) has been proposed in the literature as a promising treatment for elimination of microorganisms.[3]

PDT is the use of a light source with affinity for malignant cells, fungi or bacteria, and a photosensitizing substance in the presence of oxygen. The molecules of the photosensitizer are activated by light, starting a process of energy transfer to oxygen molecules, producing singlet oxygen, superoxides, hydroxyl radicals and hydrogen peroxide, thus causing irreversible damage to the molecules, plasma membrane and genetic material of the microbial cell, therefore leading to cell death of microorganisms.[4]

Several in vitro and in vivo studies demonstrate excellent disinfection potential of root canal systems using PDT,[9-24,26] which indicates the possibility of inserting it into clinical practice.

Thus, the present study aimed to review the literature on the use of photodynamic therapy in Endodontics, showing its effectiveness in the reduction of microorganisms, and which parameters have been used in clinical practice.

Methods

A literature survey was carried out with consultation of scientific articles, using the following databases: PubMed and Scielo. A total of 42 papers published from 2006 to 2015 was analyzed. Out of these, 18 articles were selected. Inclusion criteria were: original and relevant papers; clinical and laboratory research; studies carried out with human permanent teeth; studies reporting the use of photodynamic therapy as single or adjunct treatment for canal disinfection.

Searches were performed using the following keywords: *endodontics; photodynamic therapy; photodynamic therapy endodontics; light-activated disinfection root canal; light activated disinfection endodontics; photo-activated disinfection endodontics; photo-activated disinfection*, making combinations using Boolean operators AND or OR.

Results

Eighteen studies (16 in vitro and two in vivo) were analyzed. Samples (n) used in in vitro studies ranged from 10 to 160 human teeth. In in vivo studies, 20 teeth of patients with pulp necrosis and periapical lesion[18] and 32 teeth of patients with irreversible pulpitis[20] were used (Table 1). Fifteen studies used single-rooted human teeth, one of them also used multi-rooted teeth and three did not provide this information (Table 1). Fifteen articles reported chemical-mechanical instrumentation, whether with rotary or manual instruments, prior to bacterial inoculation. Thirteen of them used irrigation with NaOCl (0.5% to 6%) + 17% EDTA, one made use of 10% citric acid + saline solution, and one used running water as irrigant (Table 2). The main parameters of photodynamic therapy in studies found for this review are detailed in Table 3.

Table 1. Types of study and number of teeth in the sample (n).

Authors and year	Type of study	n (sample)
Xhevdet et al.[9] (2014)	*In vitro*	156 single-rooted human teeth
Garcez et al.[10] (2006)	*In vitro*	30 single-rooted human teeth
Yao et al.[11] (2012)	*In vitro*	60 human teeth
Ng et al.[12] (2011)	*In vitro*	52 single- and multi-rooted human teeth
Rios et al.[13] (2011)	*In vitro*	Single-rooted human teeth
Asnaashari et al.[14] (2016)	*In vitro*	56 single-rooted human teeth
Tennert et al.[15] (2014)	*In vitro*	160 single-rooted human teeth
Soukos et al.[16] (2006)	*In vitro*	60 single-rooted human teeth
Garcez et al.[17] (2007)	*In vitro*	10 single-rooted human teeth
Garcez et al.[18] (2008)	*In vivo*	20 single-rooted human teeth with pulp necrosis and periapical lesion
Fonseca et al.[19] (2008)	*In vitro*	46 single-rooted human teeth
Bonsor et al.[20] (2006)	*In vivo*	32 human teeth of patients with irreversible pulpitis
Nunes et al.[21] (2011)	*In vitro*	60 single-rooted human teeth
Bago et al.[22] (2013)	*In vitro*	120 single-rooted human teeth
Vaziri et al.[23] (2012)	*In vitro*	90 single-rooted human teeth
Foschi et al.[24] (2007)	*In vitro*	64 single-rooted human teeth
Souza et al.[25] (2010)	*In vitro*	70 human teeth
Yildirim et al.[26] (2013)	*In vitro*	65 single-rooted human teeth

Table 2. Pre-instrumentation as a protocol before canal contamination.

Authors et al	Chemical-mechanical preparation
Xhevdet et al.[9] (2014)	Protaper + 2.5% NaOCl + 17% EDTA
Garcez et al.[10] (2006)	Manual + 0.5% NaOCl + 17% EDTA
Yao et al.[11] (2012)	Protaper + 5.25% NaOCl + 17% EDTA
Ng et al.[12] (2011)	No chemical-mechanical preparation
Rios et al.[13] (2011)	Rotary + 6% NaOCl + 17% EDTA
Asnaashari et al.[14] (2016)	Rotary + 2.25% NaOCl + 17% EDTA
Tennert et al.[15] (2014)	Protaper + 3% NaOCl
Soukos et al.[16] (2006)	Protaper + 6% NaOCl + 17% EDTA
Garcez et al.[17] (2007)	Manual + 2.5% NaOCl + 17% EDTA
Garcez et al.[18] (2008)	No previous instrumentation
Fonseca et al.[19] (2008)	Manual + 0.5% NaOCl + 17% EDTA
Bonsor et al.[20] (2006)	No chemical-mechanical preparation
Nunes et al.[21] (2011)	Manual + 1% NaOCl + 17% EDTA
Bago et al.[22] (2013)	Rotary + 2.5% NaOCl + 15% EDTA
Vaziri et al.[23] (2012)	Manual + physiological saline + 10% citric acid
Foschi et al.[24] (2007)	Rotary + 6% NaOCl + 17% EDTA
Souza et al.[25] (2010)	Manual + running water
Yildirim et al.[26] (2013)	Manual + 1% NaOCl + 17% EDTA

Table 3. Main characteristics and parameters of PDT of selected studies.

Authors et al	Methodology	Photosensitizer concentration	Laser (wavelength - nM/ power/irradiation time
Xhevdet et al.[9] (2014)	*E. faecalis* and *C. albicans* / *Group 1:* TFD 1 minute / Group 2: TFD 3 minute / Group 3: TFD 5 minutes / Group 4: 2.5% NaOCl + PUI Group 5: 2.5% NaOCl (1.2 mL for 5 seconds and 500 microL for 10 seconds) / Group 6: control	10 mg/mL Phenothiazine chloride	600 nm Diode laser/100 mW/ cm^2/1, 3 and 5 minutes
Garcez et al.[10] (2006)	*E. faecalis* / Group 1: 0.5% NaOCl (1mL for 30 minutes) / Group 2: TFD 3 minutes / Group 3: control	Azulene (AZ)/ 0.01%	685 nm Diode laser/50mW/cm^2/ 5 minutes
Yao et al.[11] (2012)	*E. faecalis* / Group 1: TFD / Group 2: 10 mL 5.25% NaOCl for 5 min / Group 3: control (saline solution)	12.7 g/mL Toluidine chloride	635 nm Diode laser/100 mW/ cm^2/ 150s
Ng et al.[12] (2011)	Group 1: NaOCl 6% (10 mL) / Group 2: TFD + NaOCl	50 µg/mL Methylene blue	665 nm Diode Laser/100 mW/ cm^2 / 5 minutes
Rios et al.[13] (2011)	*E. faecalis* / *E. faecalis* / Group 1: 6% NaOCl 30s 1mL) / Group 2: Toluidine blue 30s / Group 3: Diode Laser 30s / Group 4: Toluidine blue + Diode Laser 30s / Group 5: NaOCl + Toluidine blue + Diode Laser	0.25 mL Toluidine blue	628 nm Diode Laser/ - / 30s
Asnaashari et al.[14] (2016)	*E. faecalis* / Group 1: PDT (diode laser) / Group 2: PDT (LED) / Group 3 and 4: control	0.1 mg/mL Toluidine blue	810 nm Diode Laser/ 0.2CW/ 4x 8s LED 630 nm/ 200 mw/cm2/ 30s
Tennert et al.[15] (2014)	*E. faecalis* / Group 1: TFD 120s / Group 2: 3% NaOCl (10 mL) 3 min / Group 3: 3% NaOCl (10mL) + TFD	13-15 mg/mL Toluidine blue	LED 635 nm/ 100 mW/cm^2 / 120 s
Soukos et al.[16] (2006)	*E. faecalis* / Group 1: Photosensitizer / Group 2: Laser / Group 3: PDT / Group 4: control	25 µg/mL Methylene blue	665nm Diode Laser - 222j/cm2/ 5 minutes
Garcez et al.[17] (2007)	Gram-negative bacteria, *Proteus mirabilis* and *Pseudomonas aeruginosa* / Group 1: TFD / Group 2: 2.5% NaOCl 10 mL / Group 3: TFD + 2.5% NaOCl	Association of polyethyleneimine and Chlorine (PEI and ce6)	600nm Diode Laser/ 40 mw
Garcez et al.[18] (2008)	1.º: 10 mL 2.5% NaOCl (conventional endodontic treatment) / 2.º: + PDT	Association of polyethyleneimine and Chlorine (PEI and ce6)	600nm Diode Laser/ 40mW
Fonseca et al.[19] (2008)	*E. faecalis* / Group 1: control / Group 2: PDT	0.0125% Toluidine blue	660 nm Diode Laser / 50 mW/ 320s
Bonsor et al.[20] (2006)	1.º: 2.25% NaOCl (conventional treatment) + 20% citric acid. / 2.º: PDT	12.7 mg/mL Toluidine chloride	100mW Diode Laser / 120s
Nunes et al.[21] (2011)	*E. faecalis* / Group 1: control / Group 2: 1% NaOCl (10 mL for 15 minutes) / Group 3: PDT with optical fiber (OF) 90s / Group 4: PDT with OF 180s / Group 5: PDT without OF 90s / Group 6: PDT without OF 180s	0.01% Methylene blue	660 nm Diode Laser/ 90mW/ 90 and 180s (irradiation times)
Bago et al.[22] (2013)	*E. faecalis* / Group 1: control / Group 2: Diode Laser / Group 3: PDT / Group 4: PDT + 3D Endoprobe / Group 5: 2.5% NaOCl (5mL for 60s) / Group 6: 2.5% NaOCl + Endoactivator	155 ug ml-1 Toluidine blue/ for 1 minute and 10 mg ml-1 phenothiazine chloride.	Group 2: 975 nm Diode Laser / 2W/ 3x 20s Group 3 and 4: 660nm Diode Laser/100mW/ 60s
Vaziri et al.[23] (2012)	*E. feacalis* / Group 1: 2.5% NaOCl (5 minutes) / Group 2: Diode laser + 2.5% NaOCl / Group 3: PDT / Group 4: 2.5% NaOCl + PDT Group 5: 2% chlorhexidine / Group 6: control	15 mg/mL Toluidine blue	625nm Diode Laser / 200mW/ cm2/ 1 minute
Foschi et al.[24] (2007)	*E. faecalis* / Group 1: Photosensitizer / Group 2: diode laser / Group 3: PDT	6.25 µg/mL Methylene blue	665 nm Diode Laser/ 100 mW/ cm2/ 5 min
Souza et al.[25] (2010)	*E. faecalis* / Phase A: chemical-mechanical instrumentation (bacterial count) / Phase B: / Group 1: PDT + 2.5% NaOCl (Methylene blue) / Group 2: PDT + 2.5% NaOCl (Toluidine blue) / Group 3: PDT + NaCl (Methylene blue) / Group 4: PDT + NaCl (Toluidine blue) / (bacterial count)	15 µg/mL Methylene blue / 15 µg/mL Toluidine blue	660nm Diode Laser/ 40 mW/ 4 minutes
Yildirim et al.[26] (2013)	*E. faecalis* / Group 1: control / Group 2: 5% NaOCl (10 mL for 15 min) / Group 3: TFD 1 min / Group 4: TFD 2 min / Group 5: TFD 4 min	70 µL Methylene blue	660 nm Diode Laser/ 1, 2 and 4 minutes

Optical fiber	Results	Conclusions
Yes	Irrigation with NaOCl (70.7%) significantly differed only for Group 4 and control group, with similar results to irradiation for 5 minutes (71.5%) and 3 minutes (69.4%).	PDT was shown to be an adequate method for canal disinfection, with results similar to irrigation with NaOCl.
Yes	TFD reduced by 99.2% and irrigation with NaOCl, 93.25%.	PDT was effective in microbial reduction in root canals, being more effective than irrigation with NaOCl.
Yes	After laser irradiation or irrigation, the amount of bacteria inside the root canal decreased in the three groups.	PDT significantly reduced microorganisms, but was no more effective than irrigation with NaOCl.
Yes	Group 2 achieved better results than Group 1. 86.5% of root canals that received PDT did not present bacteria, whereas in Group 1, only 49%.	PDT significantly reduced root canal bacteria when used as adjunct to NaOCl irrigation.
Yes	The survival rate of bacteria in NaOCl/ toluidine blue/light (0.1%) was significantly lower ($p < 0.005$) than sodium hypochlorite (0.66%) and toluidine blue/light groups (2.9%).	PDT was shown to be effective in microbial reduction when used as adjunctive therapy to irrigation with NaOCl.
Yes	LED PDT was more efficient in microbial reduction than diode laser PDT.	PDT was efficient in microbial reduction of infected canals.
Yes (Light Guide Endo tip)	Root canals with primary infection = PDT alone decreased by 92.7% / NaOCl = 99.9% and NaOCl + PDT = 99.9%. Secondary infection, all three groups reached 99.9% reduction.	PDT proved effective as an adjunctive method to irrigation with NaOCl. When used alone, it was less efficient than NaOCl.
Yes	TFD reduced E. faecalis by 97% and completely eliminated other microorganisms (P. micros, P. gengivalis and F. nucleatum).	PDT significantly reduced the number of microorganisms in infected root canals.
Yes	NaOCl reduced bacteria by 90% and PDT, 95%. PDT + NaOCl reduced > 98%.	PDT was more efficient than irrigation with NaOCl. Better results were observed when it was used as an adjunctive method to conventional treatment.
Yes	Association of PDT significantly reduced the amount of bacteria from 1.08 log to 1.83 log.	PDT added to conventional endodontic treatment proved to be an effective microbial reduction method.
Yes	The test group achieved a reduction of 99.9%, while the control group had a 2.6% increase in CFU.	PDT significantly reduced bacteria from infected canals.
Yes	20% root canals presented positive culture after conventional treatment. After PDT, only one canal presented a positive culture.	PDT is an alternative as adjunctive therapy after conventional endodontic treatment, significantly reducing bacteria from infected root canals.
Yes (2 groups) No (2 groups)	Groups 2, 3, 4, 5 and 6 significantly reduced bacterial percentage in relation to Group 1. Group 2 reached the largest reduction (99.9%) and Groups 4 OF/IT180 (99.65%) and 5 NOF/IT180 (99.64%) had no significant difference between them.	PDT is effective in microbial reduction of infected canals; however, irrigation with NaOCl has been shown to be more effective.
Yes	PDT was significantly more effective than diode laser irradiation and irrigation only with NaOCl in *E. faecalis* reduction in root canals.	TFD significantly reduced the count of *E. faecalis* in infected root canals, being more effective than irrigation with NaOCl alone.
No	The combination of PDT and 2.5% NaOCl resulted in significant bacterial reduction (100%), compared to the other groups, and no viable bacteria were observed after treatment.	PDT and irrigation with NaOCl, alone, were efficient, but the combination of PDT + irrigation with NaOCl was shown to be more effective in bacterial reduction in infected root canals.
Yes	PDT reached bacterial reduction by 77.5%. Methylene blue and LED alone reduced bacterial viability to 19.5% and 40.5%, respectively.	PDT significantly reduced bacterial count in infected canals.
Yes	Phase A instrumentation significantly reduced bacteria compared to PDT. PDT did not improve disinfection after irrigation.	PDT did not have an additional effect to the chemical-mechanical instrumentation in the disinfection of the root canals. NaOCl proved to be more effective.
Yes	PDT resulted in significant reduction from 98.8% to 99.9%, comparable to irrigation with NaOCl, which reduced it by 99.9%.	PDT is as effective as conventional irrigation with 5% NaOCl in disinfection of contaminated root canals.

Discussion

Complexity of root canal system anatomy is responsible for many difficulties during endodontic treatment;[27] and for this reason, cleaning, disinfection, modeling and filling can be affected.[28]

The mode of action and efficacy of antimicrobial agents and disinfectants have been investigated, as well as the effects and results of current endodontic techniques.[6] Within the new approaches, in order to optimize and improve bacterial elimination, PDT has been extensively studied, and its protocol is based on interaction between three components: a photosensitizer, a light source and oxygen. The mechanism of action occurs when a non-toxic dye sensitive to light, followed by irradiation of visible light with a suitable wavelength, absorbs photons from the light source and its electrons enter an excited state, also known as the triplet state. Upon returning to its fundamental state, in the presence of a substrate such as oxygen, it transfers energy and/or electrons to this substrate, forming free radicals with high cytotoxicity, such as singlet oxygen and superoxides.[7,8] These highly reactive species can cause serious damage to microorganisms by irreversible oxidation of cellular components, resulting in cell death.[5]

Photodynamic therapy reveals efficacy in microbial reduction of root canals,[9-24,26] even when used without associating it or comparing it with any other treatment.[14,16,19,24] Reduction of microorganisms can reach 99.9% when associated with irrigation with NaOCl.[21] *E. faecalis* is the most studied microorganism in the researches.

PDT is also efficient, especially as adjunct to chemical-mechanical instrumentation (with NaOCl).[12,13,18-20,23] However, it may be more efficient than chemical-mechanical instrumentation in elimination of bacteria from root canals.[10,17,22] Results of PDT and chemical-mechanical cleaning may be similar,[9,26] or even more, PDT may be less effective than irrigation with NaOCl.[11,15,21]

When comparing the efficacy of PDT with irrigation with 1% NaOCl, irrigation with NaOCl is more efficient (99.9%), but with no significant difference, since PDT reaches a reduction of 99.65%.[21] The two methods compared may also result in larger microbial reduction with NaOCl (99.9%) irrigation than PDT alone (92.7%), with statistically significant difference.

Although most studies report the antimicrobial efficacy of PDT,[9-24,26] it may not be efficient and has no additional effect on chemical-mechanical instrumentation,[25] which may occur due to low concentration of oxygen available in the canals, especially in irregularities and dentinal tubules.[25]

The elements involved in PDT should be taken into account, since their variables can affect the result, such as: the concentration of photosensitizer, laser wavelength, power output, the use of optical fiber, and the time of irradiation.[29]

Most photosensitizers are activated by light with wavelengths between 630 and 700 nm, corresponding to depth penetration of 0.5 cm (630 nm) to 1.5 cm (700 nm). The major photosensitizers found in the literature are derivatives of hematoporphyrin (620-650 nm), phenothiazine, such as toluidine blue and methylene blue (620-700 nm), cyanine (600-805 nm), phytotherapics (550-700 nm), and hytalocyanine (660-700 nm).[30,31,32] In Endodontics, toluidine blue and methylene blue stand out, corroborating our results.

These two photosensitizers do not present significant difference in terms of efficacy.[25] Azulene,[10] toluidine chloride,[11,20] phenothiazine chloride[9] and the combination of polyethyleneimine and chlorine (PEI and ce6)[17,18] can also be used. Phenothiazine chloride plus toluidine blue may yield satisfactory results,[22] as well as the use of a combination of polyethyleneimine and chlorine (PEI and ce6).[17,18]

In relation to the light source used, there is a wide variety that can be used in PDT in Endodontics. Low-power lasers, such as helium-neon (He-Ne) and diodes, are the most commonly used radiation sources for microbial reduction in the oral cavity because they allow rapid repair of periapical tissues and reduction of post-instrumentation discomfort.[33] When used at low power, it exerts an antimicrobial effect due to the association between light and exogenous photosensitizers, initiating a cascade of events leading to cell death.[34]

Its light may or may not be directed by means of an optical fiber to provide the adequate amount,[34] enhancing the effectiveness of therapy due to the ability of the fiber-optic beam to evenly distribute light by 360° throughout the system of root canals with a minimum of loss, in addition to compatibility with root canals dimensions.[35] Thus, the action of light

is able to extend to areas of difficult access, easily reaching the apical third, even in molars with curved roots, and even to biofilm external to the root apex.[8] The studies in this review using optical fiber are listed in Table 3; and among all studies, only one did not use optical fiber.[23]

Diode lasers are most commonly used because they are more compact, easier to handle, more versatile and well absorbed by biological tissues.[8] Recently, other light sources, such as LED, have been successfully applied as alternative energy sources in PDT due to their low cost, lower energy consumption and ease of manipulation.[5] Moreover, they have lower thermal productivity and cause minimal tissue injury.[36] Although not mentioned, PDT having LED lamp (810nm) as irradiation source is as effective as diode laser (630nm) in microbial reduction.[14]

On the efficiency of therapy without presence of photosensitizer or without activation, used alone,[13,16,17,24] there is minimal microbial reduction when compared to conventional treatment, PDT (Photosensitizer + light) and the association of the two (PDT + NaOCl). The survival rate of bacteria in the NaOCl + TFD group is 0.1%, significantly lower ($p < 0.005$) than sodium hypochlorite (0.66%) and PDT alone (2.9%).[13]

The studies analyzed in the present study used wavelengths ranging from 600 nm to 810 nm. Exposure to irradiation has great variability, but the most frequently used are as follows: 2, 3, 5 and 15 minutes.[21] Power must also be taken into account and usually ranges from 50 to 150 mW/cm^2, the irradiation time for sources of low intensity being necessarily longer.[37] In this study, the power used ranged from 40 to 100mW/cm^2, and irradiation time ranged from 30 seconds to 5 minutes.

Influence of irradiation time on the efficacy of photodynamic therapy has been investigated.[9,21,26] Root canals exposed to laser for 1, 3 and 5 minutes were analyzed.[9] When contaminated with C. albicans, there was no significant difference, since 1 minute of laser was enough to eliminate approximately 82% of bacteria. In canals infected with *E. faecalis*, there were significant differences between the 5-minute and 1-minute exposure times, with no significant difference from 3-minute exposure time. This indicates long periods of irradiation for better disinfection of root canals.

However, increasing irradiation time may not significantly influence bacterial reduction. Even with an increase in time from one to two minutes, the antimicrobial effect was slightly higher, without statistically significant differences.[26]

PDT as adjunct in cases of endodontic retreatment of periapical lesion has already been used with 150 μg methylene blue photosensitizer, irradiated with 40 mW diode laser coupled to optical fiber, with no information on irradiation time. This resulted in repair of periapical lesion, indicating effective decontamination and absence of toxic effects that could alter the repair process.[38]

The methodologies used are inconsistent among studies, as well as the parameters of PDT and sodium hypochlorite in relation to concentration and time of irrigation. Therefore, the efficacy of therapy may differ according to these variables, influencing the end result. This diversity of research parameters, coupled with the limited number of selected articles that were relevant to the present study, make comparisons laborious.

Conclusion

Photodynamic therapy proved to be efficient in disinfection of root canals. However, it is recommended to use it as an additional method to conventional treatment. Results should be interpreted and analyzed with caution, since a standardized protocol for this therapy has not yet been established due to diversity of parameters studied.

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