The Effect of Photodynamic Therapy in Root Canal Disinfection: A Systematic Review

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Abstract

Introduction: Effective root canal disinfection is a fundamental component of successful root canal treatment. Photodynamic therapy (PDT) has been proposed as a new adjunctive method for additional disinfection of the root canal system with the possibility of improved treatment outcomes. The aim of this systematic review was to investigate the effect of PDT on bacterial load reduction during root canal disinfection. **Methods:** Two reviewers independently conducted a comprehensive literature search using a combination of medical subject heading terms and key words to identify studies relevant to the Population Intervention Control Outcome question. The selection of articles for inclusion was performed in 2 phases based on predetermined eligibility criteria according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Inter-reviewer agreement for each phase was recorded. The effect of PDT on bacterial load reduction during root canal disinfection was evaluated as the primary outcome variable during data extraction. **Results:** The literature search provided 57 titles and abstracts. Three articles met the inclusion criteria and were selected for this systematic review. The reasons for study exclusion in each phase were recorded. Because of the heterogeneity in clinical indications and PDT protocols among the included studies, a meta-analysis could not be performed. All included studies showed a positive effect of PDT in the reduction of microbial load in root canal treatment ranging from 91.3%–100%. **Conclusions:** Limited clinical information is currently available on the use of PDT in root canal disinfection. If supported by future clinical research, PDT may have efficacy for additional root canal disinfection, especially in the presence of multi–drug-resistant bacteria. *(J Endod 2014;40:891–898)*

Key Words

Antibacterial, bacteria reduction, light-activated disinfection, photodynamic therapy, reactive oxygen species, root canal disinfection

Effective root canal disinfection is undoubtedly a fundamental component of successful root canal treatment. Contemporary techniques include the mechanical debridement and shaping of the root canal system with emphasis on various nickel-titanium (NiTi) rotary systems, intracanal irrigation with antimicrobial/tissue dissolving agents, and interappointment dressings. However, several studies have reported that rotary and hand instruments are equally effective in bacteria reduction, and despite the improved efficiency of NiTi systems, there is no difference in antimicrobial reduction (1, 2). Regarding chemotherapeutic agents, sodium hypochlorite (NaOCl) has been considered as the "gold standard" because of its antibacterial and tissue dissolution properties (3, 4). Nevertheless, numerous studies have verified that complete elimination of bacteria from the root canal system cannot be consistently achieved with any of the currently used techniques and combinations (1, 5–9).

In search of new methods to provide additional disinfection for the root canal system and presumably improve treatment outcome, novel techniques including various laser wavelengths (10); hydraulic (eg, Endo-Vac; SybronEndo Corporation, Orange, CA) (11), sonic (12), and ultrasonic (13, 14) irrigation; and gaseous ozone (15) and photodynamic therapy (PDT) (16) have been proposed in the literature. PDT is an antimicrobial strategy defined as "light induced inactivation of cells, microorganisms and molecules" (17). In principle, it uses a nontoxic photosensitizer that is selectively absorbed in a target tissue and a low-intensity light source. Upon photo-induced activation of the photosensitizer, in the presence of oxygen, a series of reactions produce free radicals and singlet oxygen molecules leading to bacterial eradication. Singlet oxygen diffuses to a distance of approximately 100 nm with a half-life of <0.04 microseconds (18). The photodynamic effect or the extent of tissue/cell damage depends on the type, dose, incubation time, and localization of the photosensitizer; the availability of oxygen; the wavelength of light (nm); the light power density measured in mW/cm^2; and the light energy fluency. Of all the PDT dosimetry parameters, fluency appears to cause some confusion. Some authors use the cross-sectional area of the light source, whereas others use a light effect on a determined area. In either case, fluency represents the rate of deposited energy in a specified area and is expressed in J/cm^2. Because of its high antibacterial potential, PDT has been suggested as an adjunct to conventional endodontic disinfection protocols.

Currently, the use of PDT in endodontic therapy has been tested in terms of bacterial load reduction *in vivo* (16, 19, 20) as well as *in vitro* (21, 22) and *ex vivo* (23) and has shown promising results. A recent systematic review of PDT against *Enterococcus faecalis* provides a direct comparison of these studies (24). Despite recent research efforts to study the effect of PDT on the disinfection of the root canal system, no effort has been made to evaluate the efficacy of this approach by means of a system-

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atic review of the literature. Thus, the aim of this systematic review was to investigate the effect of PDT on bacteria load reduction during root canal disinfection.

Materials and Methods

Population Intervention Control Outcome Question

To address the aim of this systematic review, the following question was constructed based on the Population Intervention Control Outcome (PICO) principle: "For patients undergoing root canal treatment, does the use of PDT compared with conventional chemomechanical techniques alone further reduce the bacterial load?"

Search Strategy

A search was conducted for articles related to the PICO question, which were published from January 2000 to June 2013. The literature search included an electronic search of the PubMed database of the US National Library of Medicine and the Cochrane Central Register of Controlled Trials (CENTRAL) using the following combination of medical subject heading terms and key words: "photodynamic" OR "light activated disinfection" OR "photodynamic therapy" OR "photo-activated disinfection" AND "endodontics" OR "photodynamic therapy endodontics" OR "light activated disinfection root canal" OR "photodynamic therapy endodontic" OR "light activated disinfection endodontics" OR "photo-activated disinfection endodontics." Reference lists from identified articles or literature reviews were also searched to identify other potentially relevant articles.

Study Selection and Quality Assessment

The titles and abstracts of all articles identified from the electronic search were screened independently and in duplicate by 2 reviewers. The review process was performed to eliminate articles that clearly failed to meet the search criteria. Any disagreement between the authors was resolved via discussion.

Full-text copies of all remaining articles were obtained and further examined independently by each reviewer to determine whether or not they were eligible for this study based on specific inclusion and exclusion criteria.

Published studies were selected for inclusion based on the following criteria:

1. Human studies
2. Randomized controlled trials, prospective clinical cohort, or crossover studies
3. Use of photodynamic therapy as adjunctive treatment for the disinfection of root canal systems
4. Inclusion of permanent teeth with fully formed apices undergoing endodontic treatment
5. Report of outcomes of reduction in bacterial load
6. English language

Studies were excluded if they were animal studies or *in vitro* investigations, did not quantify the antimicrobial effect of PDT, did not specify the pulp and periradicular status, or did not follow a consistent endodontic procedure for all participants.

The investigators then scrutinized the remaining list of articles to reach a consensus that the inclusion and exclusion criteria were followed and that key studies were not missed. In case of a disagreement that was not resolved with discussion, the opinion of a senior reviewer was sought to determine definitive inclusion or exclusion of the article. Inter-reviewer agreement was assessed using Cohen kappa statistics.

Quality assessment of randomized clinical trials and observational studies was performed using the CONSORT (CONsolidated Standards Of Reporting Trials) statement criteria (25) and the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) statement criteria (26), respectively. The risk of bias for each of the included studies was reported as low, moderate, or high (27).

Data Extraction

Two reviewers independently mined data regarding the year of publication, location of data, source of funding, number of participants in each group, number of interventions, and outcomes for each study and entered them in an electronic sheet. Because of the limited follow-up time in the published studies, the effect of PDT on the reduction of the bacterial load during root canal disinfection was considered to be an acceptable surrogate outcome measure and was evaluated as the primary outcome variable.

Results

A total of 57 titles and abstracts were identified after an electronic search in both electronic databases using the specific combination of terms and key words. No additional studies were identified as relevant after a search of the reference lists. After the first phase of selection, 53 articles were excluded based on the predefined exclusion criteria (inter-reviewer agreement: $\kappa = 0.938$). Reasons for exclusion were studies identified as irrelevant to the specific PICO question ($n = 16$), *in vitro* studies ($n = 29$), an animal study ($n = 1$), and review articles ($n = 7$).

Full-text articles were retrieved for the remaining 4 articles and underwent independent review by each of the reviewers. After scrutiny, 1 article was excluded because it was a duplicate report of findings from another study (16, 28) (inter-reviewer agreement: $\kappa = 1$). The remaining 3 studies (16, 19, 20) fulfilled the inclusion criteria and were included in this systematic review.

Figure 1 presents a flowchart of the systematic review process according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The basic characteristics of the included studies are presented in Table 1. One study was a crossover clinical study (16), and 2 were uncontrolled clinical studies (19, 20). All studies were conducted in private practice settings, and treatment was performed by a single practitioner in each study. In 1 study, tolonium chloride was added as a photosensitizer for 60 seconds and then activated using a diode laser (ie, wavelength: 633 ± 2 nm, power: 100 mW, time: 120 seconds). The laser emitter was inserted in the root canal within 4 mm from the working length before photoactivation (16). In the studies by Garcez et al (19, 20), the photosensitizing agent used was a polyethylenimine and chlorine (e6) conjugate (ie, 60 μmol added for 120 seconds) photoactivated with a diode laser (ie, wavelength:660 nm, power: 40 mV, time: 240 seconds). The laser fiber was placed in the apical portion of the root canal until resistance was felt and then moved from the apical to the coronal direction with manual spiral movements (19, 20). None of the included studies reported any adverse events associated with the use of PDT. The small number of studies and the heterogeneity noted in the PDT protocol and inclusion criteria among the included studies did not allow us to conduct a meta-analysis.

Primary Outcomes

The primary outcome in each study was the effect of PDT on the microbial load (or number of microbial species) in the root canal system as measured using microbiological sampling methods and analyses (Table 2).

Effect of PDT on Initial Root Canal Treatment

Bonsor et al (16) compared the antimicrobial efficacy of PDT to 2.5% NaOCl in the initial root canal treatment and concluded that

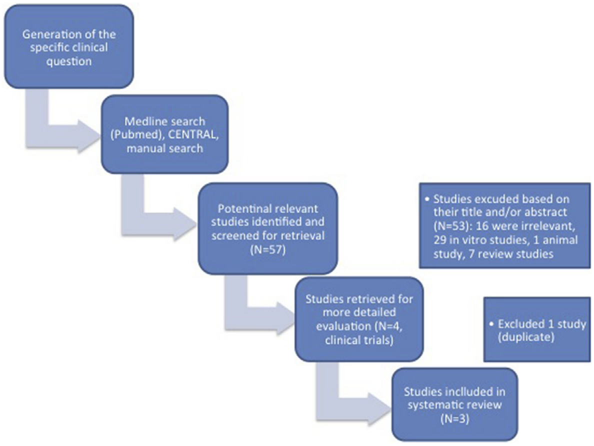

Figure 1. A flow diagram representing the systematic review process. Modified from the PRISMA Statement guidelines (55)

both treatment modalities resulted in highly significant bacterial load reduction compared with baseline measurements. In this study, samples were obtained from 44 infected root canals with a 0.02 taper NiTi hand file 1 size larger than the master apical file (16). PDT application yielded 91.3% reduction of bacteria load, whereas 2.5% NaOCl led to 80.9% reduction. However, the level of additional reduction was not significant when treatments were alternated between groups (ie, crossover design). The authors concluded that these results indicate that PDT could be as effective as NaOCl in root canal disinfection (16).

Garcez et al (20) investigated the effect of PDT application after conventional chemomechanical debridement in the initial root canal treatment of necrotic teeth (N = 22). Even though this study lacked a control group, samples obtained at each clinical session after access preparation (sample 1) and after conventional chemomechanical debridement (sample 2) served as quasi-controls for the samples obtained after the application of PDT (sample 3). The collection of bacterial samples was performed with 3 sterile paper points previously left in the canal system for 1 minute each. All 3 paper points were combined for microbiological analysis (20). Results showed that PDT significantly further decreased the bacterial count from 91% to 98.1% ($P < .01$) in comparison with conventional chemomechanical debridement. In the same study, $Ca(OH)_2$ was applied for 1 week as an intracanal medicament, and a second chemomechanical debridement followed by PDT was performed at the second visit. The second reduction achieved by PDT was significantly greater than the first ($P < .01$), and the overall reduction achieved by the 2 visits was more than 99.9%. This reduction was found to be significantly greater than the one achieved at the first visit alone ($P < .01$) (20).

Effect of PDT on Root Canal Retreatment

Garcez et al (19) investigated the effect of PDT in the retreatment of teeth with necrotic pulp infected with microflora resistant to a previous antibiotic therapy/root canal treatment (N = 30 infected root canals). As in the previous study performed by the same group of investigators, bacterial samples were collected with 3 sterile paper points previously left in the canal system for 1 minute each (19).

Based on patients' reports, a systemic antibiotic was prescribed by the previous dentist who performed the initial root canal treatment. Information on the type and dosage of the systemic antibiotic was not available. To verify the presence of bacteria resistant to antibiotics, the authors performed antibiogram analysis on the first microbiological samples collected from the root canal systems (ie, after access). The antibiogram confirmed that all patients included in this study had at least 1 microorganism resistant to antibiotic medication. The application of PDT after conventional chemomechanical preparation rendered the root canal system free of microorganisms, indicating the potential use of PDT against multi–drug-resistant bacteria (19).

Discussion

Contemporary chemomechanical debridement techniques do not consistently eliminate bacteria during root canal treatment. The significance of complete or near complete bacterial load reduction has been underlined by several *in vivo* studies showing that the remaining bacterial load may negatively affect the treatment outcome (29, 30). The presence of persistent cultivable bacteria at the time of obturation has been shown to reduce the treatment success rate from 94% to 68% (29). Similarly, Molander et al (30) found that the success rate in teeth with negative cultures before obturation was significantly higher than the success rate in teeth with positive cultures, which was reported to be as low as 49%. Thus, there is merit in identifying more effective disinfection methods to further improve root canal treatment outcome. Because the goal of total bacterial elimination in the root canal system still remains elusive, the quest for identification of the ideal disinfection protocol continues.

PDT is a recent advance in the field of disinfection protocols. It has shown potent antimicrobial properties, and its oral applications have been extensively tested. PDT's group of indications encompasses therapy for tumors, periodontitis, oral lesions, and premalignant diseases (31). Regarding the clinical protocol, a photosensitizing agent is applied on a target tissue that is subsequently irradiated with light of an appropriate wavelength in the presence of oxygen to produce free radicals, singlet oxygen, and other reactive oxygen species (17). When the photosensitizer is activated, it undergoes transition from a low-energy level to a higher-energy level called the "triplet state." As a result, it transfers its energy to a biomolecule or to molecular oxygen, resulting in the generation of these cytotoxic species. The bactericidal action of these cytotoxic species is attributed to 2 main pathways: damaging of the cellular plasma membrane and/or damaging of the cell DNA. Both outcomes result in cell death (17, 32–34). One of PDT's advantages with high clinical relevance is the absence of thermal side effects in the periradicular tissues (35). The lethal action of PDT is based on photochemical events and not thermal effects, as opposed to many laser therapy techniques (36, 37). The absence of a thermal effect of PDT makes it potent in eradicating microorganisms such as bacteria (35), fungi (38), and viruses (39) without causing overheating of the adjacent tissues (35).

Even though the application of PDT has significant advantages, potential adverse events have been reported previously. Tooth discoloration may be an adverse effect that follows the use of PDT in root canal treatment when methylene blue (MB) is used as the photosensitizer (40). However, 2.5% NaOCl with or without the use of Endo-PTC cream (a cream consisting of 10% urea peroxide, 15%, Tween 80 [detergent], and 75% carbowax) (Fórmula e Ação, São Paulo, SP, Brazil) has been shown to be effective in preventing tooth staining related to the application of MB (40). None of the studies included in this review reported tooth discoloration associated with the use of PDT. Another area of concern is the potential cytotoxicity of PDT. *In vitro* and *ex vivo* studies have been performed that aimed to investigate the safety profile of PDT for potential *in vivo* applications (41, 42). In a proof-of-principle study, MB was activated with red light at 20 and 40 mW/cm^2 and was shown to have modest effects on osteoblasts at 24 hours. The authors concluded that under a therapeutic window PDT is safe toward mammalian cells (42). Another study by George and Kishen (41)

TABLE 1. Summary of the Main Characteristics of Studies Included after the Second Phase of Selection

Study	Country	Funding source	Study design	Random allocation	Population characteristics	n (sample size)	Microbiological sampling method	Photosensitizer	Laser, wavelength (nm), power (mW), emission time
Bonsor et al, 2006 (15)	Private office setting, United Kingdom	Financial support from the device manufacturer reported	Crossover clinical study (2 experimental groups with first intervention at T2 and crossover at T3).	Yes	64 randomly selected healthy patients (64 teeth), age 16–70 years, diagnosed with irreversible pulpitis or periradicular periodontitis (lesion) similarly distributed in 2 groups (*n* = 32). Group 1: 73% molars. Group 2: 76% molars, with remaining teeth being single rooted.	44 infected root canals (23/21)	Group 1: 3 microbiological samples. T1: After access preparation and debridement with a#15 NiTi file. T2: After Profile instrumentation 2-mm short from WL with citric acid 20%, sterile saline, and PDT. T3: After Profile instrumentation at WL with NaOCl 2.25% and citric acid 20%. Group 2: T1: As in Group 1. T2: After Profile instrumentation 2-mm short from WL with NaOCl 2.25%, citric acid 20%. T3: After Profile instrumentation at WL with citric acid 20%, saline, and PDT. Samples were taken with endodontic hand file.	TC for 60 seconds	Diode laser; 633 ± 2 nm; 10 mW for 120 seconds
Garcez et al, 2008 (22)	Private office setting, Brazil	No external funding reported. NIH grant support reported for 1 coauthor	Uncontrolled clinical study (1 experimental group and no control group sampled at 2 different appointments. Quasi-control between T2 and T3 in each appointment).	NA	20 randomly selected healthy patients, age 21–35 years, diagnosed with pulp necrosis and periapical periodontitis. Anterior teeth with single relatively straight root canals.	20 infected root canals	Microbiological samples. T1: After access preparation and debridement with a#10 K file with 1 mL saline. T2: After chemomechanical debridement to size 40.02 with 10 mL NaOCl 2.5%, 10 mL H_2O_2 3%, 5 mL EDTA 17 %, 5 mL saline. T3: After PDT and final rinse with 10 mL saline. Placed $Ca(OH)_2$ for 1 week and repeated sampling procedure as in first appointment (T1: Baseline. T2: After 2nd chemomechanical instrumentation, T3: After 2nd PDT). Samples were taken with 3 paper points.	PEI/ce6 conjugate in PBS solution (60 μmol/L) for 120 seconds	Diode laser; 660 nm; 40 mW for 240 seconds

(*Continued*)

TABLE 1. (*Continued*)

Study	Country	Funding source	Study design	Random allocation	Population characteristics	n (sample size)	Microbiological sampling method	Photosensitizer	Laser, wavelength (nm), power (mW), emission time
Garcez et al, 2010 (21)	Private office setting, Brazil	No external funding reported	Uncontrolled clinical study (1 experimental group and no control group. Quasi-control between T2 and T3).	NA	21 randomly selected patients, age 17–52 years, diagnosed as previously treated with endodontic treatment and antibiotic therapy and concurrent diagnosis of periapical periodontitis. Anterior single-rooted teeth	30 infected root canals	Microbiological samples. T1: After access preparation and removal of previous filling with a#15 H file with 1 mL saline. T2: After chemomechanical debridement to average size 45.02 with 10 mL NaOCl 2.5%, 10 mL H_2O_2 3%, 5 mL EDTA 17%, 5 mL saline. T3: After PDT and final rinse with 10 mL saline. Samples were taken with 3 paper points.	PEI/ce6 conjugate in PBS solution (60 μmol/L) for 120 seconds	Diode laser; 660 nm; 40 mW for 240 seconds

ce6, chlorine e6; NA, not applicable; PBS, phosphate buffered saline; PDT, photodynamic therapy; PEI, polyethylenimine; TC, tolonium chloride; WL, working length.

showed that when MB was irradiated for 20 minutes with a 30-mW laser, it resulted in 97% reduction of *E. faecalis*, whereas it resulted in the death of only 30% of fibroblast cells. Results from the same study showed that PDT cytotoxicity was significantly less compared with NaOCl when used for root canal disinfection (41). In the literature, residual systemic photosensitization has also been reported as a potentially adverse event associated with the use of intravenous photosensitizers (43); however, there seems to be no implication with oral applications of PDT.

The role of PDT in endodontic therapy has been tested using different combinations of photosensitizers and light sources and has shown divergent results (16, 19–21, 23, 44–50). Its use against *E. faecalis in vitro* has revealed a promising bactericidal potential (44–48). However, several limitations have been associated with PDT and its antimicrobial efficacy. The species of bacteria in the root canal system and their growth mode were found to influence their susceptibility to PDT in a dose-dependent manner (51). Furthermore, dentin, dentin matrix, pulp tissue, bacterial lipopolysaccharides, and bovine serum albumin were found to significantly decrease PDT antimicrobial efficacy (52). An effort to enhance the photodynamic effect by encapsulating and delivering MB in polymeric nanoparticles appears promising (47). Other strategies include the use of a photosensitizer solvent (53), efflux pump inhibitors (51), or photoactivated functionalized chitosan nanoparticles for disinfection and stabilization of the dentin matrix (54).

Preclinical data suggest its use after conventional chemomechanical debridement for further reduction of bacteria load (23). Yet, there are conflicting data on its supplemental effect to disinfection after conventional chemomechanical debridement (50). Thus, the following clinical question emerges: "Does the use of PDT in root canal therapy compared with conventional chemomechanical techniques alone further reduce the bacterial load?"

The present systematic review revealed only 3 clinical studies that fulfilled our inclusion criteria and were relevant to the PICO question (16, 19, 20). A limitation of the present systematic review is that none of the included studies correlated their results to clinical outcomes. In clinical research, it can be difficult to investigate the effect of PDT on the success rate of root canal treatment with appropriate longitudinal follow-up data. Therefore, in this review, we used the effect of PDT on the reduction of the bacterial load during root canal disinfection as the primary outcome. All included studies concluded that PDT application enhances disinfection during root canal therapy. Garcez et al (19, 20) used PDT in combination with conventional chemomechanical debridement. Their protocol included instrumenting with K-files to size 40–45 and irrigating with 2.5% NaOCl, 3% H_2O_2, and 17% EDTA before PDT application (19, 20). They presented promising results of adjunctive PDT application. When they evaluated their protocol in teeth with necrotic pulps undergoing initial root canal treatment, they found that significantly greater reduction in the bacterial count occurred after the additional application of PDT. If they allowed for weekly treatment with $Ca(OH)_2$ as an intracanal medicament and performed a second session of chemomechanical debridement followed by PDT, a near-complete bacterial elimination (99.9%) was noted. Nevertheless, it should be noted that their sampling technique using paper points lacks the ability to test the efficacy of PDT on biofilms. The use of PDT as the main disinfection protocol has been also evaluated. Bonsor et al (16) found that the use of PDT in lieu of NaOCl was equally effective as 2.25% NaOCl combined with 20% citric acid irrigation after crown-down instrumentation.

Regarding tooth selection, the following reasonable clinical questions arise: (1) Will PDT be effective in all types of root canal anatomy? and (2) Will light delivery be hindered by the complex

TABLE 2. Summary of the Results of Studies Included after the Second Phase of Selection

Study	Interventions	Primary outcomes	Results of intragroup comparison	Results of intergroup comparison	Adverse effects	Risk of bias
Bonsor et al, 2006 (15)	Group 1: ProTaper crown-down/citric acid 20%/saline → PDT → Profile/ NaOCl 2.25%/citric acid 20% → $Ca(OH)_2$ → 2nd visit: obturation Group 2: GT Rotaries crown-down → Profile/NaOCl 2.25%/citric acid 20%/saline → PDT → $Ca(OH)_2$ → 2nd visit: obturation	Reduction in microbial load of facultative anaerobes (6 gradients, 0–5)	Statistically significant reduction in microbial load in comparison to baseline in both groups	Not significant reduction of microbial load after crossover	None reported	High
Garcez et al, 2008 (22)	1st visit: Gates Glidden, K-files crown-down/NaOCl 2.5%/H_2O_2 3%/ EDTA 17%/saline → PDT → 1-week $Ca(OH)_2$ 2nd visit: repeated chemomechanical debridement and PDT → obturation	Reduction in microbial load of facultative anaerobes and microaerophilic (CFU determination)	Statistically significant reduction in microbial load. 1. Between conventional chemomechanical debridement and total disinfection with adjunctive PDT both at 1st and 2nd visit. 2. Between adjunctive disinfection with PDT at 1st versus 2nd visit. 3. Between total disinfection with adjunctive PDT at 1st versus 2nd visit.	NA	None reported	Moderate
Garcez et al, 2010 (21)	1st visit: Retreatment with H-files → K-files crown-down/ NaOCl 2.5%/H_2O_2 3%/EDTA 17%/PBS/ saline → PDT → 1-week $Ca(OH)_2$ 2nd visit: Repeated chemomechanical debridement and PDT → obturation	Microbiological identification and determination of number of species per root canal	T1: 100% of samples were found to have at least 1 resistant bacterial species. T2: 33% of samples had 100% bacterial elimination. T3: 100% of samples had 100% bacterial elimination. (Only descriptive statistics were reported in this study.)	NA	None reported	Moderate

CFU, colony-forming unit; NA, not applicable; PBS, phosphate-buffered saline; PDT, photodynamic therapy.

anatomy or curvature of specific teeth groups? In 2 of the included studies, tooth selection was limited to anterior teeth with single straight canals (19, 20). Yet, 1 study included both posterior and anterior teeth with posterior teeth representing the majority of the included teeth (73%–76%). Results showed that PDT can be effective in molar root canal systems as well (16). Although the majority of *in vitro* (47, 48, 50) and *ex vivo* (23) studies use an intracanal fiber in successful PDT bacterial load reduction, Nunes et al (46) also showed *in vitro* that PDT can be effective with or without the use of an intracanal fiber, meaning that light delivery may not drastically affect its antimicrobial action.

Another limitation is the heterogeneity among presently available studies that do not allow for quantitative synthesis of the results. Each of the included studies used a different study design, with 2 studies including teeth requiring initial root canal treatment (16, 20) and 1 study including previously endodontically treated teeth (19). In addition, there were 2 different combinations of light sources and photosensitizers mentioned. Two studies used polyethylenimine chlorine (e6) with a 660-nm wavelength diode laser at 40-mV power for 240 seconds (19, 20). The remaining study used tolonium chloride activated using a 633-nM wavelength diode laser at 100-mV power for 120 seconds (16). Because the application of PDT for additional reduction of the microbial load of root canal systems seems promising, future work should be performed to strengthen the currently available level of evidence for its use. It would be beneficial to identify the ideal combination of photosensitizer and light wavelength via preclinical studies and conduct future randomized controlled trials to test the effect of PDT on root canal disinfection in a large array of indications.

It is also important that future clinical studies explicitly report adverse events associated with PDT so that an estimation of the benefit-to-risk ratio from the use of PDT is feasible. Although there was no adverse effect mentioned in the included studies of the current review, not all of the included studies mentioned that a record of adverse events was maintained during the clinical application of PDT.

Conclusion

Limited clinical information is currently available on the use of PDT in root canal disinfection. All available studies showed a positive effect of PDT in the reduction of microbial load in root canal treatment. We cautiously conclude that a potential additive benefit from the use of PDT in root canal disinfection may exist, especially in the presence of multi–drug-resistant bacteria. A direction for future work would be to optimize and standardize PDT dosimetry with the use of an appropriate photosensitizing agent that yields the most potent antimicrobial results while maintaining a high safety profile. There is also a need for future randomized, controlled, clinical trials that will report clinical treatment outcomes.

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The authors deny any conflicts of interest related to this study.

References

1. Siqueira JF Jr, Lima KC, Magalhaes FA, et al. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. J Endod 1999;25:332–5.
2. Dalton BC, Orstavik D, Phillips C, et al. Bacterial reduction with nickel-titanium rotary instruments. J Endod 1998;24:763–7.
3. Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endod 1978;4:60–4.
4. Siqueira JF Jr, Rocas IN, Favieri A, et al. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. J Endod 2000;26:331–4.
5. Card SJ, Sigurdsson A, Orstavik D, et al. The effectiveness of increased apical enlargement in reducing intracanal bacteria. J Endod 2002;28:779–83.
6. Mickel AK, Chogle S, Liddle J, et al. The role of apical size determination and enlargement in the reduction of intracanal bacteria. J Endod 2007;33:21–3.
7. Rodig T, Hulsmann M, Muhge M, et al. Quality of preparation of oval distal root canals in mandibular molars using nickel-titanium instruments. Int Endod J 2002;35:919–28.
8. Shuping GB, Orstavik D, Sigurdsson A, et al. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000;26:751–5.
9. Paiva SS, Siqueira JF Jr, Rocas IN, et al. Clinical antimicrobial efficacy of NiTi rotary instrumentation with NaOCl irrigation, final rinse with chlorhexidine and interappointment medication: a molecular study. Int Endod J 2013;46:225–33.
10. Sahar-Helft S, Stabholtz A, Moshonov J, et al. Effect of Er:YAG laser-activated irrigation solution on *Enterococcus faecalis* biofilm in an *ex-vivo* root canal model. Photomed Laser Surg 2013;31:334–41.
11. Heilborn C, Reynolds K, Johnson JD, et al. Cleaning efficacy of an apical negative-pressure irrigation system at different exposure times. Quintessence Int 2010;41:759–67.
12. Huffaker SK, Safavi K, Spangberg LS, et al. Influence of a passive sonic irrigation system on the elimination of bacteria from root canal systems: a clinical study. J Endod 2010;36:1315–8.
13. Huque J, Kota K, Yamaga M, et al. Bacterial eradication from root dentine by ultrasonic irrigation with sodium hypochlorite. Int Endod J 1998;31:242–50.
14. Paiva SS, Siqueira JF Jr, Rocas IN, et al. Molecular microbiological evaluation of passive ultrasonic activation as a supplementary disinfecting step: a clinical study. J Endod 2013;39:190–4.
15. Huth KC, Quirling M, Maier S, et al. Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. Int Endod J 2009;42:3–13.
16. Bonsor SJ, Nichol R, Reid TM, et al. An alternative regimen for root canal disinfection. Br Dent J 2006;201:101–5. discussion 98; quiz 20.
17. Gursoy H, Ozcakir-Tomruk C, Tanalp J, et al. Photodynamic therapy in dentistry: a literature review. Clin Oral Investig 2013;17:1113–25.
18. Moan J, Berg K. The photodegradation of porphyrins in cells can be used to estimate the lifetime of singlet oxygen. Photochem Photobiol 1991;53:549–53.
19. Garcez AS, Nunez SC, Hamblim MR, et al. Photodynamic therapy associated with conventional endodontic treatment in patients with antibiotic-resistant microflora: a preliminary report. J Endod 2010;36:1463–6.
20. Garcez AS, Nunez SC, Hamblin MR, et al. Antimicrobial effects of photodynamic therapy on patients with necrotic pulps and periapical lesion. J Endod 2008;34:138–42.
21. Fimple JL, Fontana CR, Foschi F, et al. Photodynamic treatment of endodontic polymicrobial infection *in vitro*. J Endod 2008;34:728–34.
22. Foschi F, Fontana CR, Ruggiero K, et al. Photodynamic inactivation of *Enterococcus faecalis* in dental root canals *in vitro*. Lasers Surg Med 2007;39:782–7.
23. Ng R, Singh F, Papamanou DA, et al. Endodontic photodynamic therapy *ex vivo*. J Endod 2011;37:217–22.
24. Siddiqui SH, Awan KH, Javed F. Bactericidal efficacy of photodynamic therapy against *Enterococcus faecalis* in infected root canals: a systematic literature review. Photodiagnosis Photodyn Ther 2013;10:632–43.
25. Juni P, Altman DG, Egger M. Systematic reviews in health care: assessing the quality of controlled clinical trials. BMJ 2001;323:42–6.
26. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med 2007;4:e296.
27. Kotsakis GA, Konstantinidis I, Karoussis IK, et al. A systematic review and meta-analysis of the effect of various laser wavelengths in the treatment of peri-implantitis. J Periodontol 2014 Jan 20 [Epub ahead of print].
28. Bonsor SJ, Nichol R, Reid TM, et al. Microbiological evaluation of photo-activated disinfection in endodontics (an *in vivo* study). Br Dent J 2006;200:337–41. discussion 29.
29. Sjogren U, Figdor D, Persson S, et al. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30:297–306.
30. Molander A, Warfvinge J, Reit C, et al. Clinical and radiographic evaluation of one- and two-visit endodontic treatment of asymptomatic necrotic teeth with apical periodontitis: a randomized clinical trial. J Endod 2007;33:1145–8.
31. Sharwani A, Jerjes W, Salih V, et al. Fluorescence spectroscopy combined with 5-aminolevulinic acid-induced protoporphyrin IX fluorescence in detecting oral premalignancy. J Photochem Photobiol B 2006;83:27–33.
32. Lee MT, Bird PS, Walsh LJ. Photo-activated disinfection of the root canal: a new role for lasers in endodontics. Aust Endod J 2004;30:93–8.
33. Sharman WM, Allen CM, van Lier JE. Photodynamic therapeutics: basic principles and clinical applications. Drug Discov Today 1999;4:507–17.
34. Takasaki AA, Aoki A, Mizutani K, et al. Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases. Periodontol 2000 2009;51:109–40.
35. Soukos NS, Chen PS, Morris JT, et al. Photodynamic therapy for endodontic disinfection. J Endod 2006;32:979–84.
36. Amyra T, Walsh LT, Walsh LJ. An assessment of techniques for dehydrating root canals using infrared laser radiation. Aust Endod J 2000;26:78–80.
37. Walsh LJ. The current status of low level laser therapy in dentistry. Part 1. Soft tissue applications. Aust Dent J 1997;42:247–54.
38. Bliss JM, Bigelow CE, Foster TH, et al. Susceptibility of Candida species to photodynamic effects of photofrin. Antimicrob Agents Chemother 2004;48:2000–6.
39. Ceballos-Salobrena A, Gaitan-Cepeda LA, Ceballos-Garcia L, et al. Oral lesions in HIV/AIDS patients undergoing highly active antiretroviral treatment including protease inhibitors: a new face of oral AIDS? AIDS Patient Care STDS 2000;14:627–35.
40. Carvalho Edos S, Mello I, Albergaria SJ, et al. Effect of chemical substances in removing methylene blue after photodynamic therapy in root canal treatment. Photomed Laser Surg 2011;29:559–63.
41. George S, Kishen A. Advanced noninvasive light-activated disinfection: assessment of cytotoxicity on fibroblast versus antimicrobial activity against *Enterococcus faecalis*. J Endod 2007;33:599–602.
42. Xu Y, Young MJ, Battaglino RA, et al. Endodontic antimicrobial photodynamic therapy: safety assessment in mammalian cell cultures. J Endod 2009;35:1567–72.
43. Konopka K, Goslinski T. Photodynamic therapy in dentistry. J Dent Res 2007;86:694–707.
44. Kishen A, Upadya M, Tegos GP, et al. Efflux pump inhibitor potentiates antimicrobial photodynamic inactivation of *Enterococcus faecalis* biofilm. Photochem Photobiol 2010;86:1343–9.
45. Nagayoshi M, Nishihara T, Nakashima K, et al. Bactericidal effects of diode laser irradiation on *Enterococcus faecalis* using periapical lesion defect model. ISRN Dent 2011;2011:870364.
46. Nunes MR, Mello I, Franco GC, et al. Effectiveness of photodynamic therapy against *Enterococcus faecalis*, with and without the use of an intracanal optical fiber: an *in vitro* study. Photomed Laser Surg 2011;29:803–8.
47. Pagonis TC, Chen J, Fontana CR, et al. Nanoparticle-based endodontic antimicrobial photodynamic therapy. J Endod 2010;36:322–8.

48. Rios A, He J, Glickman GN, et al. Evaluation of photodynamic therapy using a light-emitting diode lamp against *Enterococcus faecalis* in extracted human teeth. J Endod 2011;37:856–9.
49. Silva LA, Novaes AB Jr, de Oliveira RR, et al. Antimicrobial photodynamic therapy for the treatment of teeth with apical periodontitis: a histopathological evaluation. J Endod 2012;38:360–6.
50. Souza LC, Brito PR, de Oliveira JC, et al. Photodynamic therapy with two different photosensitizers as a supplement to instrumentation/irrigation procedures in promoting intracanal reduction of *Enterococcus faecalis*. J Endod 2010;36:292–6.
51. Upadya MH, Kishen A. Influence of bacterial growth modes on the susceptibility to light-activated disinfection. Int Endod J 2010;43:978–87.
52. Shrestha A, Kishen A. The effect of tissue inhibitors on the antibacterial activity of chitosan nanoparticles and photodynamic therapy. J Endod 2012; 38:1275–8.
53. George S, Kishen A. Influence of photosensitizer solvent on the mechanisms of photoactivated killing of *Enterococcus faecalis*. Photochem Photobiol 2008;84: 734–40.
54. Shrestha A, Hamblin MR, Kishen A. Photoactivated rose bengal functionalized chitosan nanoparticles produce antibacterial/biofilm activity and stabilize dentin-collagen. Nanomedicine 2013 Nov 4 [Epub ahead of print].
55. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.