

Original

Efficacy of antimicrobial photodynamic therapy for root canals infected with *Enterococcus faecalis*

Ricardo A. S. Arneiro¹), Ryan D. Nakano¹), Livia A. A. Antunes²), Gustavo B. Ferreira³),
Karla B. F. C. Fontes²), and Leonardo S. Antunes²)

¹School of Dentistry, Fluminense Federal University, Nova Friburgo, RJ, Brazil

²Department of Specific Formation, School of Dentistry, Fluminense Federal University,
Nova Friburgo, RJ, Brazil

³School of Dentistry, Fluminense Federal University, Niterói, RJ, Brazil

(Received July 18, 2014; Accepted October 14, 2014)

Abstract: Effective decontamination of root canal systems is a constant concern in clinical practice. In this article, we compare the performance of photodynamic therapy (PDT) and sodium hypochlorite (NaOCl) in reducing the amount of *Enterococcus faecalis* in root canals. Relevant studies were identified by searching electronic databases, including Web of Science, PubMed, BVS (Medline, Scielo, Lilacs and BBO), Scopus, and Cochrane, and by manually searching the references of identified studies. The terms used in the literature search were “photodynamic therapy” and “*Enterococcus faecalis*”. We selected 13 experimental studies that exclusively assessed the performance of PDT in reducing *E. faecalis* in root canals of human teeth. In an evaluation of methodological quality, 12 articles were classified as moderate-quality reports and 1 as a high-quality report. No article needed to be excluded because of low-quality methodology. The results showed that PDT had a better antimicrobial effect when used as an adjuvant endodontic treatment to NaOCl. However, this finding should be carefully interpreted, as there are few relevant studies and the methods of the selected studies varied.

(J Oral Sci 56, 277-285, 2014)

Keywords: antimicrobial photodynamic therapy; endodontic treatment; *Enterococcus faecalis*; lasers; systematic review.

Introduction

Effective elimination of microorganisms in infected root canal systems is an important concern in endodontics (1,2). Chemical-mechanical technique is the most important step in controlling root canal infection. Antimicrobial irrigants, especially sodium hypochlorite (NaOCl), are very effective in reducing bacteria populations, as they have a proteolytic effect. However, NaOCl has cytotoxic and neurotoxic effects when extruded into periapical tissues (3). Although NaOCl acts directly on target bacteria, several factors—such as the anatomical complexities of root canals, deep invasion of microorganisms into dentinal tubules, and formation of biofilm on the surface of the root apex—make it difficult to completely eliminate microorganisms from root canals and periapical lesions (4,5).

Enterococcus faecalis is a bacterium commonly associated with chronic periodontitis and failed root canal treatment (6,7). *E. faecalis* is a Gram-positive, non-sporing, facultative anaerobe that is sometimes highly resistant to medicaments and antimicrobial irrigants during endodontic treatment (8). Protocols for enhancing disinfection can use alternative methods, such as substances for chemical-mechanical preparation, or other approaches for complementing the effects of conventional disinfection. Dental applications of

Correspondence to Dr. Leonardo Santos Antunes, Department of Specific Formation, School of Dentistry, Fluminense Federal University, Rua Doutor Silvio Henrique Braune, 22 Centro, Nova Friburgo, Rio de Janeiro 28625-650, Brazil
Fax: +55-22-25287168 E-mail: leonardoantunes@id.uff.br

doi.org/10.2334/josnusd.56.277

DN/JST.JSTAGE/josnusd/56.277

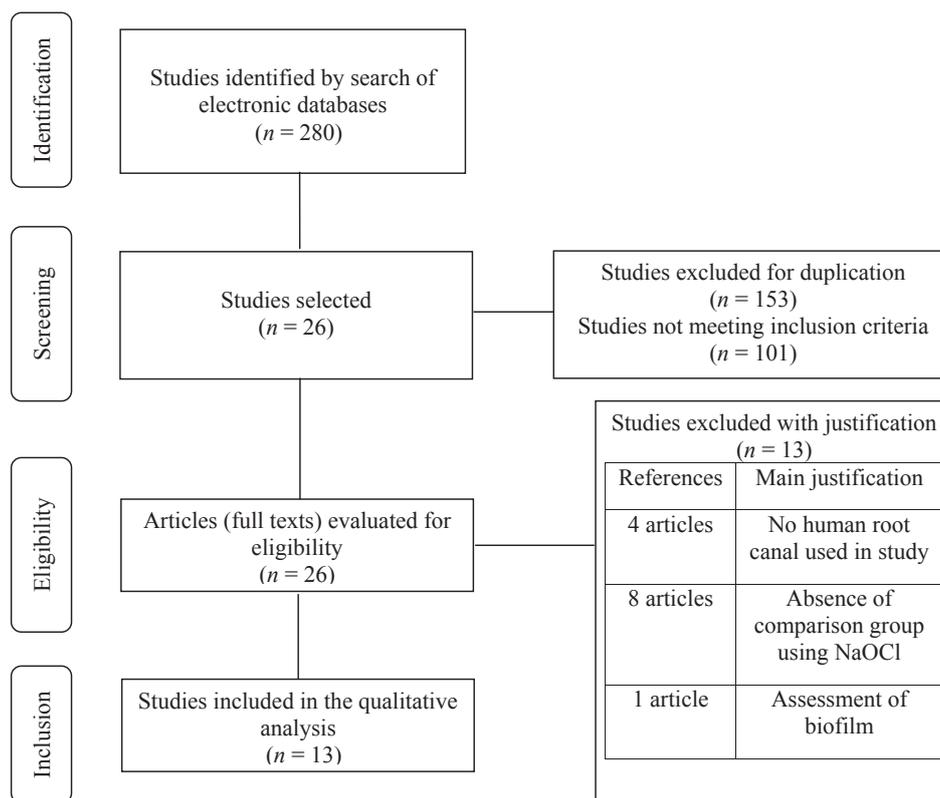


Fig. 1 Flowchart of process used to search and select research studies, using items recommended for systematic reviews (PRISMA).

photodynamic therapy (PDT) are increasing rapidly, and several studies have shown that oral bacteria are susceptible to PDT (9,10). PDT has recently been used to eradicate microorganisms from root canal systems, which suggests that it might be useful as adjunctive therapy to current endodontic disinfection techniques.

Photodynamic inactivation of microorganisms by topical application of photosensitizer and light restricts the action of highly reactive oxygen species and neutralizes systemic effects on non-pathogenic bacterial flora (11). In addition, unlike antibiotics, which have unique microbial targets, reactive oxygen generated from a photodynamic reaction has multifunctional characteristics and can damage several cellular structures, thus decreasing possible development of PDT-resistant bacteria (12).

A number of experimental studies have investigated the effect of PDT on reducing *E. faecalis* inside root canals. We critically examined the available literature to identify, and assess the methodological quality of, all relevant studies, which were then systematically reviewed to compare the effects of PDT and NaOCl in reducing *E. faecalis* inside root canals.

Methods

Research question

Is photodynamic therapy more effective than NaOCl in reducing the amount of *E. faecalis* inside root canals?

Strategy for identifying and selecting studies

A comprehensive search of the literature was performed by two independent researchers. Only articles published before July 2014 were considered for review. The selection process is shown in Fig. 1. The following electronic databases were used to identify potentially relevant studies: Web of Science, PubMed, BVS (Medline, Lilacs, and BBO), Scopus, and Cochrane. The key words used in the bibliographic search were “photodynamic therapy” and “*Enterococcus faecalis*”. To refine the search results, the terms were combined.

The key words were chosen from the structured and trilingual vocabulary DeCS (Descriptors of Health Science), which was developed based on the Medical Subject Headings (MeSH) of the National Library of Medicine in the United States, to allow terms used in Portuguese, Spanish, or English. This method provided a unique and consistent way to retrieve information regardless of the language used. The above-mentioned

Table 1 Model for qualitative evaluation

Item	Quality assessment	Yes	No
Study Design			
1	Methodology presenting reproducibility	1	0
2	Presence of control group	1	0
3	Group pairing if more than one group	1	0
4	Statement that only one investigator performed the experiments or that investigators were calibrated	1	0
5	Calculation or justification of sample size	1	0
Description of statistical methods			
6	Use of appropriate statistical tests to assess main results	1	0
Results			
7	Results judiciously interpreted by considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	1	0
8	Discussion of study limitations that accounts for potential sources of bias or inaccuracies	1	0
Total			

key words yielded the best search results during the pre-test phase.

After an initial electronic search, abstracts and titles were evaluated. In cases in which the abstract and title were unclear, the complete article was read to minimize the possibility of excluding important studies. In addition, a manual search of the references of the identified studies was performed.

Criteria for study selection

Studies satisfying all the following criteria were included:

- i) Experimental studies,
- ii) Studies of root canals of human teeth,
- iii) Studies of the effects of photodynamic therapy on *E. faecalis* numbers only,
- iv) Studies comparing photodynamic therapy and NaOCl,
- v) Studies with no language restriction.

Literature reviews, dissertations, theses, qualitative studies, case reports, textbooks, textbook chapters, and annals of congresses were excluded.

Critical evaluation of the studies

Full copies of potentially relevant studies were independently obtained and analyzed by two of the authors (R.S.A. and R.D.N.). The selected studies were then compared, and any disparity was discussed so that a consensus could be achieved in combination with a third investigator (L.S.A.). After assessing the articles, studies that did not clearly meet the inclusion criteria were excluded.

Criteria for quality assessment

The articles selected were assessed by considering the criteria described in Table 1 and then classified into three categories, as follows:

- Category A: high-quality methodology, i.e., an article satisfying 7 or 8 of the proposed criteria
- Category B: moderate-quality methodology, i.e., an article satisfying 4 to 6 of the proposed criteria
- Category C: low-quality methodology, i.e., an article satisfying 3 or fewer of the proposed criteria.

Studies in category C were not included in the present analysis.

Data extraction

Data were independently retrieved by two investigators (R.A.S.A. and R.D.N.), who fully read the articles and considered the methodology (number of teeth/canals and groups analyzed), parameters for NaOCl (concentration and duration) and PDT (laser wavelength, fiber diameter, photosensitizer, irradiation duration, active laser media, energy dose, and potency), results, and conclusions.

Results

Search and selection of articles

First, abstracts from 280 articles were identified in electronic databases, as follows: 66 articles from VHS, 59 from PubMed, 2 from Cochrane, 69 from Scopus, and 94 from Web of Science. After disregarding duplicate studies and analyzing the titles and abstracts, 26 articles were selected.

In the final search and selection process, the 26 identified articles were analyzed in detail, on the basis of our inclusion and exclusion criteria, and 13 studies

Table 2 Qualitative analysis of selected studies

Evaluation criteria	Studies												
	Meire et al. 2009	Poggio et al. 2011	Souza et al. 2010	Rios et al. 2011	Nunes et al. 2011	Ng et al. 2011	Yao et al. 2012	Vaziri et al. 2012	Cheng et al. 2012	Bago et al. 2013	Miranda et al. 2013	Yildirim et al. 2013	Xhevdet et al. 2014
Methodology presenting reproducibility	1	1	1	0	1	1	1	1	1	1	1	1	1
Presence of control group	1	1	0	1	1	1	1	1	1	1	1	1	1
Group pairing if more than one group	1	1	1	1	1	1	1	1	1	1	1	1	1
Statement that only one investigator performed the experiments or that investigators were calibrated	0	1	0	0	0	0	0	0	0	0	0	0	0
Calculation or justification of sample size	0	0	0	0	0	0	0	0	0	0	0	0	0
Use of appropriate statistical tests to assess main results	1	1	1	1	1	1	1	1	1	1	1	1	1
Results judiciously interpreted by considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	1	1	1	1	1	1	1	1	1	1	1	1	1
Discussion of study limitations that accounts for potential sources of bias or inaccuracies	1	1	1	1	1	1	1	1	1	1	1	1	1
Total	6	7	5	5	6	6	6	6	6	6	6	6	6
Category	B	A	B	B	B	B	B	B	B	B	B	B	B

Table 3 Characteristics of studies that met inclusion criteria

Author/year	Methodology		NaOCl parameters		Results	Conclusion
	Number of teeth/canals	Groups assessed	Concentration	Duration (min)		
Meire et al. 2009	60/60	1- Nd:YAG laser 2- KTP laser 3- PDT 4- NaOCl 5- Positive/negative control	2.5%	15	High-power lasers (1 and 2) did not reduce EF in root canals. PDT treatment considerably reduced EF vs. control. Reductions were greater in NaOCl treatment group.	Laser systems and PDT were less efficacious than NaOCl in reducing EF, either in aqueous solution or in an infected-tooth model.
Poggio et al. 2011	100/100	1- PDT 2- PDT + NaOCl 3- TB 4- Prolonged PDT 5- 5% NaOCl (positive control) 6- Negative control	5%	3	Groups 2 and 5 had greater bacterial reduction. Group 4 had a greater reduction than group 1. Group 3 had the lowest percentage of bacterial reduction in all strains tested.	The in vitro study confirmed that the antimicrobial efficacy of 5% NaOCl is greater than that of PDT. NaOCl can be used as an auxiliary procedure for endodontic treatment. A longer irradiation time may be necessary to improve effectiveness.
Souza et al. 2010	70/70	1- PDT with MB + 2.5% NaOCl 2- PDT with TB + 2.5% NaOCl 3- PDT with MB + 0.85% NaCl 4- PDT with TB + 0.85% NaCl	2.5% or 0.85%	-	Comparisons between groups 1 and 2 and groups 3 and 4 revealed no significant differences. However, when groups 1 and 3 and groups 2 and 4 were compared, a significant bacterial reduction in the root canals was observed.	PDT with either MB or TB did not result in significant improvement as auxiliary treatment for instrumentation/irrigation in intracanal disinfection.
Rios et al. 2011	Unknown	1- 6% NaOCl 2- TB 3- PDT with no TB 4- PDT + TB 5- PDT + TB + NaOCl Positive and negative controls	6%	0.5	The survival rate of EF with PDT + TB + 6% NaOCl (0.1%) was significantly lower ($P < 0.005$) than that with NaOCl (0.66%) and PDT (2.9%)	PDT with TB might be useful as auxiliary therapy for conventional endodontic treatment in reducing the amount of bacteria.
Nunes et al. 2011	60/60	1- Control group 2- NaOCl 3- PDT with OF + 90-s ET 4- PDT with no OF + 180-s ET 5- PDT with no OF + 90-s ET 6- PDT with no OF + 180-s ET	1%	15	A greater reduction in EF was obtained with 1% NaOCl. PDT significantly reduced the amount of EF in the following groups (in descending order of EF reduction): OF + 180-s ET, no OF + 180-s ET, OF + 90-s ET, and no OF + 90-s ET. No statistically significant differences were observed among the groups.	PDT was effective against EF, regardless of the use of intracanal optical fiber.

Table 3, continuation

Author/year	Methodology		NaOCl parameters		Results	Conclusion
	Number of teeth/canals	Groups assessed	Concentration	Duration (min)		
Ng et al. 2011	52/101	1- NaOCl 2- PDT + NaOCl	6%	3	86.5% of samples had no bacterial colony units after PDT application; 49% of samples had no bacterial colony units after use of NaOCl.	PDT used as an auxiliary procedure for MCP significantly enhanced elimination of EF in root canals.
Yao et al. 2012	60/60	1- PDT 2- NaOCl 3- 0.9% saline solution	5.25%	5	No bacteria were detected after NaOCl irrigation, but after 72 h, bacteria were found in 11 samples. Bacteria were detected in all other groups. PDT was significantly more efficacious than saline solution in reducing the amount of bacterial cells in root canals ($P < 0.05$).	PDT had a bactericidal effect that increased linearly with the irradiation energy dose.
Vaziri et al. 2012	90/90	1- NaOCl 2- Diode laser + NaOCl 3- PDT 4- NaOCl + PDT 5- Irrigation with chlorhexidine 6- Control group	2.5%	5	Combination of PDT and NaOCl achieved maximum reduction of bacteria. No bacteria were observed after treatment with PDT + 2.5% NaOCl.	The combination of PDT and NaOCl was efficacious in eliminating EF in dentinal tubules.
Cheng et al. 2012	220/220	1- Nd: YAG 2- Er: YAG + NaOCl+ NaCl+ DW 3- Er: YAG + NaCl + DW 4- Er, Cr: YSGG 5- PDT Controls Positive: 5.25% NaOCl Negative: 0.9% NaCl	5.25%	1	Bacterial reductions in the experimental groups and positive control group were significantly greater than in the negative control ($P < 0.001$). However, only in group 2 (Er:YAG/NaOCl/NaCl/DW) did the bacterial reduction reach 100% on the surface of the root canal walls or 100/200 mm of the dentinal tubules.	All the protocols tested, especially that for group 2, had an efficacious bactericidal effect on experimentally infected root canals. Thus, the group 2 protocol seems ideal for disinfection of root canals during endodontic treatment.
Bago et al. 2013	120/120	1- NaOCl 2- NaOCl (EndoActivator) 3- Diode laser 4- PDT 5- PDT + Endoprobe 3D 6- Positive control	2.5%	1	There was a significant reduction in the amount of EF after treatments ($P < 0.01$). The reduction in CFUs was significantly greater in groups 2, 4, and 5 than in groups 1 and 3 ($P < 0.05$).	PDT and EndoActivator were more successful than a diode laser and irrigation with NaOCl, performed separately, in reducing root canal infection.
Miranda et al. 2013	125/125	1- CMP + NaOCl 2- CMP + NaOCl EndoVac 3- CMP + NaOCl + PDT 4- CMP + NaOCl EndoVac + PDT	5.25%	1	A significant reduction ($P < 0.05$) in mean EF count was observed in all groups. No differences were detected among groups.	Adjuvant use of EndoVac and PDT, in combination or not, was as efficacious as CMP+NaOCl in association with CaOH2 in reducing EF count.
Yildirim et al. 2013	60/60	Control group 1- NaOCl 2- PDT 1 min 3- PDT 2 min 4- PDT 4 min	5%	15	The results of bacterial load reduction in the PDT groups (99.8% to 99.9%) and in the 5% NaOCl irrigation group (99.9%) were comparable, indicating that PDT was as efficacious as conventional NaOCl irrigation.	PDT was as efficacious as conventional irrigation with 5% NaOCl in reducing EF. Irradiation for 1 min was sufficient to achieve the antimicrobial effect of PDT.
Xhevdet et al. 2014	156/156	1- PDT 1 min 2- PDT 3 min 3- PDT 5 min 4- NaOCl 5- 10-s PUI + NaOCl 6- Positive control group	2.5%	5 s	NaOCl irrigation significantly differed only with PUI and positive control. Five-minute laser irradiation did not significantly differ in disinfection effectiveness as compared with 3-min laser irradiation or NaOCl irrigation.	PDT was an adequate disinfectant agent for EF-infected root canals. However, it did not totally eradicate microorganisms.

Abbreviations: PDT, photodynamic therapy; NaOCl, sodium hypochlorite; NaCl, saline solution; DW, distilled water; CFU, colony forming units; CMP, chemical-mechanical preparation; TB, toluidine blue; MB, methylene blue; EF, *Enterococcus faecalis*; OF, optical fiber; ET, exposure time; PUI, passive ultrasonic irrigation.

were excluded because they did not satisfy the inclusion criteria for this article, namely, eight had no comparison group with NaOCl (10,13-19), four used no human teeth (20-23), and one used a biofilm created with four types of different bacteria (24). Thus, 13 articles were eligible for quality assessment: one high-quality article and 12 moderate-quality articles. No article was excluded for

low-quality methodology (Table 2).

Sample selection and preparation

Data extraction from selected articles is described in Tables 3 and 4. In the studies assessed, 60 to 220 root canals were used; most studies used 60 to 125 teeth (25-34), and only two studies used more than 125 teeth,

Table 4 PDT parameters in the selected studies

Author/year	Laser wavelength (nm)	Fiber diameter (μM)	Photosensitizer (concentration in $\mu\text{g/mL}$)	Duration of irradiation (min)	Active laser media	Energy dose	Power
Meire et al. 2009	1- 1,064	1- 200	TB	1- 5 \times 5 s, with 20 s intervals	1- YAG laser	1- 37.5 J	1- 1.5 W
	2- 532	2- 200	12.7 mg mL ⁻¹	2- 5 \times 5 s, with 20 s intervals	2- KTP laser	2- 25 J	2- 1 W
	3- 635	3- 400		3- Uniformly irradiated for 150 s	3- diode laser	3- 15 J	3- 100 mW
Poggio et al. 2011	628	500	TB Between 10 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$	1- 0.5 2- 0.5 3- 0 4- 1.5	-	-	-
Souza et al. 2010	660	300	1- MB 2- TB Both 15 $\mu\text{g/mL}$	4	Diode laser	-	40 mW
Rios et al. 2011	628	-	TB 0.25 mL	0.5	Diode laser	-	-
Nunes et al. 2011	660	216	MB 100 $\mu\text{g/mL}$	1 min and 30 s (IT 90) or 3 min (IT 180)	Diode laser	IT 90 -8 J IT 180 -16.2 J	90 mW
Ng et al. 2011	665	250	MB 50 $\mu\text{g/mL}$	Light applied for 2.5 min, followed by a break of 2.5 min and a second light exposure for 2.5 min.	Diode laser	30 J/cm ²	1 W
Yao et al. 2012	635	400	TB 12.7 $\mu\text{g/mL}$	5- 55 s	Diode laser	0.5-5.5 J	50 e 100 mW
Vaziri et al. 2012	625	-	TB 15 $\mu\text{g/mL}$	1	Diode laser	-	200 mW/cm ²
Cheng et al. 2012	Nd: YAG: 1064 Er: YAG: 2940 Er, Cr: YSGG: 2780 Diode laser: 660	Nd: YAG: 200 Er: YAG: 300 Er, Cr: YSGG: 415 diode laser: 2 mm	MB 10 $\mu\text{g/mL}$	Nd: YAG: laser activated for 4 s; procedure repeated 4 times with a 15-s interval Er: YAG: activated for 20 s with a 15-s interval Er, Cr: YSGG: withdrew 1 mm/s with laser activated; procedure repeated 4 times with a 15-s interval PDT: Activated for 60 s	Nd: YAG; Er: YAG; Er, Cr: YSGG; diode laser	-	Nd: YAG: 1.5 W Er: YAG: 0.3 W Er, Cr: YSGG: 1 W diode laser: 0.2 W
Bago et al. 2013	3- 975	3- 320	4- TB (155 $\mu\text{g/mL}^{-1}$)	3- 3 \times 20 s	Diode laser	-	3- 2 W
	4- 660	4- 320	5- phenothiazine chloride (10 mg/mL ⁻¹)	4- 1			4- 100 mW
	5- 660			5- 1			5- 100 mW
Miranda et al. 2013	660	300	MB 25 $\mu\text{g/mL}^{-1}$	5	Diode laser	-	40 mW
Yildirim et al. 2013	660	-	MB	Three exposure times (1, 2, and 4 min)	Diode laser	-	-
Xhevdet et al. 2014	660	-	Phenothiazine chloride 10 mg/mL	Application of laser for 1, 3 and 5 min	Diode laser	-	100 mW/cm ²

Abbreviations: TB, toluidine blue; MB, methylene blue.

namely, Xhevdet et al. (35) used 156 root canals and Cheng et al. (36) used 220 root canals. Of the 13 articles selected, 12 used single-rooted teeth with only one radiographically demonstrated canal (25,26,28-37), and one also used multi-rooted teeth (27).

During sample preparation, root canals were sterilized and prepared before bacterial inoculation in all but three studies (26,27,33). With regard to instrumentation procedures, four studies used manual chemical-mechanical preparation with conventional files (25,28,31,34), whereas eight used rotary instrumentation with nickel-titanium files (26,27,29,30,32,33,35,37). After all samples had been sterilized and properly instrumented, bacterial inoculation was performed before the experiments.

PDT parameters

The most frequently used photosensitizers in the studies were toluidine blue (TB) and methylene blue (MB). Meire et al. (25), Poggio et al. (29), Yao et al. (30), Vaziri et al. (31), and Rios et al. (37) used TB only in their studies, whereas Ng et al. (27), Nunes et al. (28), Miranda et al. (33), Yildirim et al. (34), and Cheng et al. (36) used MB only. Xhevdet et al. (35) used phenothiazine chloride (PC) only. Bago et al. (32) used TB in one group and PC in another group. Souza et al. (26) used TB and MB as photosensitizers in different groups. Two of the studies included a group to test the use of photosensitizers without photoactivation (29,37), whereas two other studies included a group to test the use of lasers without photosensitizer (32,37).

Each article assessed in the present study had included a parameter for performing PDT, as summarized in Table 4. The use of intracanal fiber was not universal, since Vaziri et al. (31) did not describe its use.

Studies that investigated the exclusive use of photosensitizers (i.e., with no photoactivation), such as Poggio et al. (29) and Rios et al. (37), or lasers (without application of photosensitizers), such as Bago et al. (32) and Rios et al. (37), found minimal or no bacterial reduction.

With regard to the effect of PDT in reducing *E. faecalis*, some studies found it less effective than NaOCl (25,29-31,37), others found them similarly effective (34,35), and others found that PDT had better results when associated with NaOCl (27,29,31,33,37).

Discussion

PDT is regarded as an effective method for eliminating oral bacteria; however, few clinical studies have assessed its action in root canals. In addition, parameters for its clinical use in endodontics have not been standardized.

We assessed the methodological quality of *in vitro* studies comparing the efficacy of PDT and NaOCl in reducing *E. faecalis* in root canals. *In vitro* studies were selected in an attempt to identify parameters for PDT application, since experimental studies establish the conditions for further clinical studies. *In vitro* study protocols with a qualified and tested high-quality methodology can be reproduced with greater safety and efficiency in clinical applications.

There were few studies on the question of interest. Because systematic reviews use inclusion and exclusion criteria to select studies, which are then categorized according to extremely rigorous methodological criteria, the number of articles tends to decrease, mainly when new criteria are imposed by the authors, as in the present study.

We noted several methodological differences in the selected *in vitro* studies. Most studies (25,28-32,34-37) reported instrumentation of the root canals before bacterial inoculation. This impedes the mechanical action of the instruments and limits the reduction in *E. faecalis*.

Other methodological differences were the concentration and duration of action of NaOCl in the root canal. NaOCl concentration varied from 1% to 6%, and duration of action varied from 15 s to 15 min. The efficacy of antimicrobial action may vary depending on these variables; thus, it is important to note that the results of the studies may have been influenced by these factors.

The use of a photosensitizer to achieve an antimicrobial effect during PDT application is widespread, and MB and TB are the most common options. Our results show

that the choice of photosensitizer was quantitatively balanced. Bago et al. (32) opted to use phenothiazine chloride as a photosensitizer in addition to TB. Souza et al. (26) used both photosensitizers in different groups to compare their efficacy, but no significant difference was found. In their comparative study of MB and TB, Usacheva et al. (38) concluded that both were efficient in reducing the amount of bacteria, although MB was more efficient than TB. The use of a laser without a photosensitizer, as well as photosensitizers with no photoactivation, resulted in a minimal reduction in the amount of bacteria in root canals (29,37).

All but one study (31) used optical fibers with lasers for photoactivation. Nunes et al. (28) compared groups with and without optical fibers, and their results suggest that the use of an intracanal fiber is not a crucial factor in eliminating bacteria in root canals. The authors explained that the photosensitizer used (i.e., MB) could be photoactivated without direct contact with light, thus requiring no optical fiber, as photoactivation occurred at a distance.

Lasers can have different powers and active media. Meire et al. (25) used low- and high-power lasers and found that the latter had no antimicrobial effects. The use of high-power lasers can alter the tooth surface and produce undesirable effects, such as thermal injuries to periodontal tissues. Therefore, they must be used with caution.

Different light sources can be used in endodontic PDT, such as argon lasers, helium-neon lasers, metal-vapor lasers, and diode lasers. When used at low power they exert an antimicrobial effect because of the association between light and exogenous photosensitizers, thereby starting a cascade of events that leads to cell death (39). Low-power lasers are used in endodontics because they allow for rapid repair of periapical tissues and reduction of post-instrumentation discomfort (40). All the selected studies used diode lasers, possibly because they are the most commonly available on the market.

The PDT wavelength ranges from 600 to 1,200 nm in the electromagnetic spectrum, and all the studies assessed used a wavelength within this spectrum. It should be emphasized that the wavelength must be directly correlated with the wavelength to be absorbed by the photosensitizer, to produce a photochemical cascade. Because laser power differed by study, we were unable to compare these parameters.

Regarding the efficacy of PDT as an antimicrobial agent, five articles in this review reported that the synergistic effect between NaOCl and PDT yielded better results. In addition, combined therapy was more efficient than conventional treatment in eliminating *E. faecalis* in

root canals (27,29,31,33,37).

It is important to emphasize that the authors of the selected studies adopted different parameters for PDT (see Table 4) and NaOCl (concentration and duration of action). This lack of methodological standardization made it impossible to compare the results in a meta-analysis. This diversity in study parameters, in conjunction with the limited number of selected articles that were relevant to the study question, makes comparisons difficult and less reliable.

To establish a safe, effective protocol that can be reproduced in clinical settings, additional *in vitro* studies will be needed in order to standardize the PDT parameters.

The present findings should be interpreted with caution as the studies used different methods and parameters, despite their relatively high methodological quality. The articles selected in this article reported that PDT was effective in reducing *E. faecalis* inside root canals. Therefore, the use of PDT as adjunctive therapy to current endodontic disinfection techniques is recommended.

References

- Siqueira JF Jr (2001) Aetiology of root canal treatment failure: why well-treated teeth can fail. *Int Endod J* 34, 1-10.
- Nair PN, Henry S, Cano V, Vera J (2005) Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 99, 231-252.
- Gatot A, Arbelle J, Leiberman A, Yanai-Inbar I (1991) Effects of sodium hypochlorite on soft tissues after its inadvertent injection beyond the root apex. *J Endod* 17, 573-574.
- Vertucci FJ (1984) Root canal anatomy of the human permanent teeth. *Oral Surg Oral Med Oral Pathol* 58, 589-599.
- Noguchi N, Noiri Y, Narimatsu M, Ebisu S (2005) Identification and localization of extraradicular biofilm-forming bacteria associated with refractory endodontic pathogens. *Appl Environ Microbiol* 71, 8738-8743.
- Sundqvist G, Figdor D, Persson S, Sjögren U (1998) Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 85, 86-93.
- Wong R (2004) Conventional endodontic failure and retreatment. *Dent Clin North Am* 48, 265-289.
- Rôças IN, Siqueira JF Jr, Santos KR (2004) Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J Endod* 30, 315-320.
- Bonsor SJ, Nichol R, Reid TM, Pearson GJ (2006) Microbiological evaluation of photo-activated disinfection in endodontics (an *in vivo* study). *Br Dent J* 25, 337-341.
- Soukos NS, Chen PS, Morris JT, Ruggiero K, Abernethy AD, Som S et al. (2006) Photodynamic therapy for endodontic disinfection. *J Endod* 32, 979-984.
- Engelhardt V, Krammer B, Plaetzer K (2010) Antibacterial photodynamic therapy using water-soluble formulations of hypericin or mTHPC is effective in inactivation of *Staphylococcus aureus*. *Photochem Photobiol Sci* 9, 365-369.
- Konopka K, Goslinski T (2007) Photodynamic therapy in dentistry. *J Dent Res* 86, 694-707.
- Foschi F, Fontana CR, Ruggiero K, Riahi R, Vera A, Doukas AG et al. (2007) Photodynamic inactivation of *Enterococcus faecalis* in dental root canals *in vitro*. *Lasers Surg Med* 39, 782-787.
- Bergmans L, Moisiadis P, Huybrechts B, Van Meerbeek B, Quirynen M, Lambrechts P (2008) Effect of photo-activated disinfection on endodontic pathogens *ex vivo*. *Int Endod J* 41, 227-239.
- Fonseca MB, Júnior PO, Pallota RC, Filho HF, Denardin OV, Rapoport A et al. (2008) Photodynamic therapy for root canals infected with *Enterococcus faecalis*. *Photomed Laser Surg* 26, 209-213.
- Pagonis TC, Chen J, Fontana CR, Devalapally H, Ruggiero K, Song X et al. (2010) Nanoparticle-based endodontic antimicrobial photodynamic therapy. *J Endod* 36, 322-328.
- Schlafer S, Vaeth M, Hørsted-Bindslev P, Frandsen EV (2010) Endodontic photoactivated disinfection using a conventional light source: an *in vitro* and *ex vivo* study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 109, 634-641.
- Silva FC, Freitas LRP, Lourenço APA, Braga Junior ACR, Jorge AOC, Oliveira LD et al. (2010) Analysis of the effectiveness of the instrumentation associated to antimicrobial photodynamic therapy and root canal dressing in the elimination of *Enterococcus faecalis* in root canals. *Braz Dent Sci* 13, 31-38.
- Garcez AS, Fregnani ER, Rodriguez HM, Nunez SC, Sabino CP, Suzuki H et al. (2013) The use of optical fiber in endodontic photodynamic therapy. Is it really relevant? *Lasers Med Sci* 28, 79-85.
- Nagayoshi M, Nishihara T, Nakashima K, Iwaki S, Chen KK, Terashita M et al. (2011) Bactericidal effects of diode laser irradiation on *Enterococcus faecalis* using periapical lesion defect model. *ISRN Dent*, 870364.
- Meire MA, Coenye T, Nelis HJ, De Moor RJ (2012) Evaluation of Nd:YAG and Er:YAG irradiation, antibacterial photodynamic therapy and sodium hypochlorite treatment on *Enterococcus faecalis* biofilms. *Int Endod J* 45, 482-491.
- Hecker S, Hiller KA, Galler KM, Erb S, Mader T, Schmalz G (2013) Establishment of an optimized *ex vivo* system for artificial root canal infection evaluated by use of sodium hypochlorite and the photodynamic therapy. *Int Endod J* 46, 449-457.
- Zand V, Milani AS, Amini M, Barhaghi MH, Lotfi M, Rikhtegaran S et al. (2014) Antimicrobial efficacy of photodynamic therapy and sodium hypochlorite on monoculture biofilms of *Enterococcus faecalis* at different stages of development. *Photomed Laser Surg* 32, 245-251.
- Muhammad OH, Chevalier M, Rocca JP, Brulat-Bouchard N, Medioni E (2014) Photodynamic therapy versus ultrasonic

- irrigation: interaction with endodontic microbial biofilm, an ex vivo study. *Photodiagnosis Photodyn Ther* 11, 171-181.
25. Meire MA, De Prijck K, Coenye T, Nelis HJ, De Moor RJ (2009) Effectiveness of different laser systems to kill *Enterococcus faecalis* in aqueous suspension and in an infected tooth model. *Int Endod J* 42, 351-359.
 26. Souza LC, Brito PR, de Oliveira JC, Alves FR, Moreira EJ, Sampaio-Filho HR et al. (2010) Photodynamic therapy with two different photosensitizers as a supplement to instrumentation/irrigation procedures in promoting intracanal reduction of *Enterococcus faecalis*. *J Endod* 36, 292-296.
 27. Ng R, Singh F, Papamanou DA, Song X, Patel C, Holewa C et al. (2011) Endodontic photodynamic therapy ex vivo. *J Endod* 37, 217-222.
 28. Nunes MR, Mello I, Franco GC, de Medeiros JM, Dos Santos SS, Habitante SM et al. (2011) Effectiveness of photodynamic therapy against *Enterococcus faecalis*, with and without the use of an intracanal optical fiber: an in vitro study. *Photomed Laser Surg* 29, 803-808.
 29. Poggio C, Arciola CR, Dagna A, Florindi F, Chiesa M, Saino E et al. (2011) Photoactivated disinfection (PAD) in endodontics: an in vitro microbiological evaluation. *Int J Artif Organs* 34, 889-897.
 30. Yao N, Zhang C, Chu C (2012) Effectiveness of photoactivated disinfection (PAD) to kill *enterococcus faecalis* in planktonic solution and in an infected tooth model. *Photomed Laser Surg* 30, 699-704.
 31. Vaziri S, Kangarlou A, Shahbazi R, Nazari Nasab A, Naseri M (2012) Comparison of the bactericidal efficacy of photodynamic therapy, 2.5% sodium hypochlorite, and 2% chlorhexidine against *Enterococcus faecalis* in root canals; an in vitro study. *Dent Res J (Isfahan)* 9, 613-618.
 32. Bago I, Plečko V, Gabrić Pandurić D, Schaperl Z, Baraba A, Anić I (2013) Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. *Int Endod J* 46, 339-347.
 33. Miranda RG, Santos EB, Souto RM, Gusman H, Colombo AP (2013) Ex vivo antimicrobial efficacy of the EndoVac system plus photodynamic therapy associated with calcium hydroxide against intracanal *Enterococcus faecalis*. *Int Endod J* 46, 499-505.
 34. Yildirim C, Karaarslan ES, Ozsevik S, Zer Y, Sari T, Usumez A (2013) Antimicrobial efficiency of photodynamic therapy with different irradiation durations. *Eur J Dent* 7, 469-473.
 35. Xhevdet A, Stubljarić D, Kriznar I, Jukic T, Skvarc M, Veranic P et al. (2014) The disinfecting efficacy of root canals with laser photodynamic therapy. *J Lasers Med Sci* 5, 19-26.
 36. Cheng X, Guan S, Lu H, Zhao C, Chen X, Li N et al. (2012) Evaluation of the bactericidal effect of Nd:YAG, Er:YAG, Er,Cr:YSGG laser radiation, and antimicrobial photodynamic therapy (aPDT) in experimentally infected root canals. *Lasers Surg Med* 44, 824-831.
 37. Rios A, He J, Glickman GN, Spears R, Schneiderman ED, Honeyman AL (2011) Evaluation of photodynamic therapy using a light-emitting diode lamp against *Enterococcus faecalis* in extracted human teeth. *J Endod* 37, 856-859.
 38. Usacheva MN, Teichert MC, Biel MA (2001) Comparison of the methylene blue and toluidine blue photobactericidal efficacy against gram-positive and gram-negative microorganisms. *Lasers Surg Med* 29, 165-173.
 39. Kawamoto K, Senda N, Shimada K, Ito K, Hirano Y, Murai S (2000) Antibacterial effects of yellow He-Ne laser irradiation with crystal violet solution on *Porphyromonas gingivalis*: an evaluation using experimental rat model involving subcutaneous abscess. *Lasers Med Sci* 15, 257-262.
 40. Lage-Marques JL, Gouw-Soares S, Bello-Silva MS, Ribeiro AC (2010) Laser insertion in endodontics practice. In: *Fundamentals of dentistry: lasers in dentistry*, Eduardo CP ed, Guanabara Koogan, Rio de Janeiro, 127.