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Can Antimicrobial Photodynamic Therapy (aPDT) Enhance the Endodontic Treatment?

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Abstract

In order to achieve a long-lasting effect, one of the main goals in root canal treatment is to eliminate the endodontic bacteria. Conventional chemomechanical debridement is considered as the basic treatment in root canal therapy, but adjunctive techniques such as antimicrobial photodynamic therapy (aPDT) can also be helpful. The aim of this study was to evaluate reports in the scientific literature that used different photosensitizers (PSs) for bacterial reduction. The literature search was conducted using databases including PubMed, Scopus, and Google Scholar with the keywords "photodynamic therapy," "antimicrobial photodynamic therapy," or "photoactivated disinfection" and "endodontic," "*Enterococcus faecalis*," or "root canal treatment," from 2000 to 2015. By evaluating different studies, it was concluded that aPDT should be applied in combination with conventional mechanical debridement and irrigants. However, it is also important to note that the success rate is critically dependent on the type of the PS, output power of the laser used, irradiation time, pre-irradiation time, and type of tips used.

Keywords: Antimicrobial, Endodontic, Photodynamic therapy.

Introduction

Bacteria are considered the main etiology of pulp and periapical lesions due to the production of toxins that irritate the pulpal and periradicular tissue.[1,2] The lack of suitable accessibility of the immune system to the root canal space can lead to an incomplete elimination of endodontic infection.[3] Therefore, root canal therapy is needed to eliminate infection and return the periradicular tissue to full health.[4,5] One of the primary aims in endodontic treatment is to reduce the number of microorganisms by root canal disinfection. This goal is achieved by chemomechanical preparation of the root canal, application of irrigants and antimicrobial agents, and sealing of the root space.[6,7] Although chemomechanical debridement is a basic part of root canal treatment, several studies show that this technique has some restrictions due to deep penetration of the bacteria into anatomical structures such as accessory canals, apical branches, isthmus, and dentinal tubules, particularly in the apical one-third part of the root canal.[8,9] The number of bacteria is significantly reduced in the root canal by the simultaneous application of mechanical debridement and antibacterial medicaments.[10] The long-term success of this treatment is dependent of the anatomy of the root canal system and bacterial resistance.[11] Generally, the main cause of failure is the presence of microorganisms; the main microorganism in root canal treatment failure is *Enterococcus faecalis*, which is the most resistant form of bacteria that is reported in primary and secondary endodontic infection.[8,12]

Conventional antibacterial irrigants showed some cytotoxicity, which should be taken into account when considering periradicular tissue.[13]

To reduce these unfavorable side effects, laser-assisted endodontic disinfection has gained special attention.[14] Although different wavelengths can be used for this purpose, the near-infrared wavelengths between 810 and 1064 nm are the most suitable due to their greater penetration depth compared to mid- and far-infrared lasers.[15] However, lasers use the method of photothermal effects to reduce bacterial counts, and this can be a concern.[16]

Antimicrobial photodynamic therapy (aPDT) is based on the application of a photosensitizer (PS), a light source, and oxygen for bacterial damage.[17] After the application of a PS to the site of infection, the light source, which coincides with the peak absorption of the PS, is illuminated to produce singlet oxygen and free radicals, which results in bacterial cell damage.

This technique is minimally invasive, non-resistant, and repeatable.[18]

The aim of this review study was to evaluate the effectiveness of photodynamic therapy for bacterial reduction compared to conventional methods.

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Methods

Search Strategy

The search was done in electronic databases, including PubMed, Scopus, and Google Scholar, using the keywords "photodynamic therapy," "antimicrobial photodynamic therapy," or "photoactivated disinfection" and "endodontic," "*Enterococcus faecalis,*" or "root canal treatment" from 2000 to 2015.

After an evaluation of the articles with related topics to aPDT, 43 articles were selected. Then, a table of data extraction was prepared, and the papers reviewed. The in vitro, in vivo, and clinical trials studies were included. Finally, the studies were categorized according to the type of PS used.

Results

Forty-three studies were found related to keywords used for this review. Among these studies on different PSs, 18 were on the topic of toluidine blue (TBO) (Table 1), 21 were on the topic of methylene blue (MB) (Table 2), 3 were on the topic of curcumin (Table 3), and 1 was on the topic of indocyanine green (ICG) (Table 4).

Discussion

Different techniques have been developed to enhance root canal disinfection. Among these techniques, aPDT has gained special attention as it can improve the success rate in just one treatment. It can also be applied as a supplement to chemomechanical treatment.[60]

To achieve the best results, there are some factors that should be taken into consideration, such as the concentration of the PS, laser wavelength, output power, irradiation time, incubation time, and the type of tips used (e.g., flat or diffuser).[17]

Certain studies, such as the one by Bago et al, showed that the use of TBO was more effective in reducing bacterial count; however, most studies suggested that the application of aPDT, accompanied by sodium hypochlorite (NaOCl), led to maximum reduction in the number of bacteria.[25]

Conversely, there are some studies indicating that aPDT was not an effective disinfectant. Gergova et al concluded that NaOCl, followed by chlorhexidine irrigant, were the most effective at disinfecting biofilms in root canals.[19] In addition, Hecker et al compared NaOCl with aPDT, and found that aPDT was a less effective disinfectant.[24]

In other studies, MB was used as a PS for aPDT to remove endodontic bacteria.

Some studies indicated that MB has the potential to eliminate most pathogens involved in endodontic infection, except *E. faecalis*. It was also found that the success rate in curing endodontic infections is mainly dependent on an appropriate choice of parameters. Yildirim et al, in evaluation of the effects of different light exposure durations on *E. faecalis* reduction, concluded that irradiation for 1 minute is adequate to achieve the antimicrobial effect.[42] Moreover, they found that by increasing the irradiation dose the antimicrobial effect was slightly greater, although this difference was not significantly important. In addition, Nunes et al reported comparable insignificant antimicrobial efficiency for different irradiation time in groups using MB and a diode laser for aPDT.[48]

Only one study related to the application of aPDT using ICG as a PS was found. In 2011, Nagayoshi et al used ICG accompanied by a diode laser with different irradiation times, and came to the conclusion that the application of ICG with a diode laser had the same antimicrobial effect as 2.5% NaOCl.[59] However, the fibroblasts can be damaged by 2.5% NaOCl, so aPDT is useful as a treatment option which does not cause damage to surrounding tissues. Recently, the application of curcumin as a PS has gained special attention. Curcumin has a peak absorption at around 450 nm, which is similar to LED devices.[61] It is thus essential to connect a fiber optic to the LED in order to provide a more effective photodynamic therapy. In addition, the fiber optic should be moved inside the root canal. After assessing the different studies, which used curcumin as a PS, it was concluded that the application of a PS accompanied by LED irradiation enhanced the results.[57,58]

Overall, the literature survey suggested that the best approach is to use TBO and MB with concentrations under 100 μg/mL and an incubation time of 1 to 5 minutes, followed by laser irradiation for a period of time that is mainly dependent on the output power of the device which defines the dose. It should also be kept in mind that aPDT should be performed following conventional instrumentation and irrigation.

When using ICG as the PS, the commercially available concentrations of 1 mg/mL can be applied inside the root canal. After 5 minutes, which is considered pre-irradiation time, ICG can be activated using a diode laser at a wavelength of 808 nm with a fiber of 200 μm for 10 seconds to control the thermal effects. This wavelength has a greater penetration depth compared to other wavelengths used in aPDT, which is favorable in the elimination of bacteria in endodontic treatment. On the other hand, the reaction between ICG and the 808 nm diode laser is more a thermal than a chemical process, which enhances bacterial death.

Finally, for curcumin, it has been suggested that it should be dissolved in a 0.5% dimethyl sulfoxide solution, as this has the potential to provide more free radicals after irradiation. The pre-irradiation time for the curcumin solution can be only 5 minutes, and then it is activated by illuminating with an LED for 5 minutes; this rapid method can thus save time.

Conclusion

The technique of aPDT can be applied alongside conventional chemomechanical techniques to improve the reduction in the number of endodontic bacteria, or the behavior of the bacteria by altering the virulence factors, which can reduce their ability to form biofilms.

Conflict of Interest

The author has no conflict of interest to declare.

Table 1. Studies Using TBO as PS

Author/Year	Type	Bacteria	Groups	PS	Wavelength	Results
Gergova et al[19] 2015	In vitro	*Enterococcus faecalis* and other gram-positive cocci	1) Control group; 2) Laser group; 3) PDT; 4) Different chemical substances; 5) Different solution	TBO 0.1 mg/mL	660 nm P: 100 mW T: 5 min	The PDT with FotoSan, hydrogen peroxide, and all tested types of iontophoresis all showed strong disinfection potential with very small, insignificant differences.
Muhammad et al[20] 2014	In vitro	*Enterococcus faecalis*, *Streptococcus salivarius*, *Porphyromonas gingivalis* and *Prevotella intermedia* bacteria	1) Aseptim Plus® photo-activated (LED) disinfection system; 2) Group by a 650 nm diode laser and toluidine blue as photosensitizer; 3) Control group, by ultrasonic irrigation (PUI) using EDTA 17% and NaOCl 2.6% solutions	TBO 15 µg/mL	650 nm P: 60 mW T: 120 s	There was no statistically significant difference between results obtained from groups treated by Aseptim Plus® and diode laser.
Tennert et al[21] 2014	In vitro	Clinical isolate of *E. faecalis*	1) PDT group; 2) NaOCl;) NaOCl-PDT group	TBO 13-15 mg/mL	635 nm P:100 mW T: 120 s	Antimicrobial treatment of root canals caused a significant reduction of bacterial load in all groups.
Pinheiro et al[22] 2014	In vitro	*Enterococcus faecalis*	1) Manual instrumentation; 2) Rotary instrumentation; (Toluidine blue O/laser, fuchsin/halogen light and fuchsin/LED)	TBO 0.005%	660 nm P: 100 mW T: 60 s	It may be concluded that both rotary and manual instrumentation reduced *E. faecalis*. PDT can be used as an adjuvant to conventional endodontic treatment.
Schiffner et al[23] 2014	In vitro	*E. faecalis* with mixed aerobic or anaerobic microbial populations	1) Untreated; 2) NaCl alone; 3) PAD (PS plus irradiation) for 60 s; 4) The PS alone; 5) Irradiation for 60 s alone.	TBO not mentioned	632-644 nm P: 200 mW T: 60 s	The bactericidal activity of PAD appears to be enhanced by serum proteins in vitro, but is limited to bacteria present within the root canal.
Hecker et al[24] 2013	In vitro	*E. faecalis*	1) Disinfection with NaOCl (0.5%, 1.0% or 3.0% for 30 or 60 or 600 s); 2) Disinfection with NaOCl (as above) followed by application of neutralizing solution; 3) Disinfection with the PACT 200 system.	TBO not mentioned	635 nm P: 200 mW T: 4 or 6 min	The antibacterial PDT system did not achieve sufficient disinfection.
Bago et al[25] 2013	In vitro	*E. faecalis*	1) Diode laser irradiation (2 W; 3, 9, and 20 s); 2) PAD (100 mW, 60 s); 3) PAD with 3D Endoprobe (100 mW, 60 s); 4) 30-gauge syringe irrigation with NaOCl (60 s); 5) sonic agitation of NaOCl with the EndoActivator system (60 s); 6) 30-gauge syringe irrigation with NaCl (60 s).	TBO 155 µg/mL	660 nm P:100 mW T: 60 s	The PAD and EndoActivator system were more successful in reducing the root canal infection than the diode laser and NaOCl syringe irrigation alone.
Yao et al[26] 2012	In vitro	*Enterococcus faecalis*	Energy dose ranging from 0.5 to 5.5 J. 50 or 100 mW, irradiation time 5 to 55 s. part II: PAD therapy; 5.25% NaOCl irrigation and saline	TBO 12.7 µg/mL	635 nm P: 100 mW E: 0.5 to 5.5 J	PAD could decrease *E. faecalis* in root canals effectively, but was no more effective than 5.25% NaOCl.
Vaziri et al[27] 2012	In vitro	*E. faecalis*	1) Sodium hypochlorite (NaOCl) irrigation; 2) Diode laser plus 2.5% NaOCl; 3) PDT; 4) 2.5% NaOCl plus PDT; 5) Chlorhexidine irrigation; 6) Control groups	TBO 15 µg/mL	625 nm 200 mw/cm^2 60 s	Combination of PDT and 2.5% NaOCl achieved maximum reduction in recovered viable bacteria, no viable bacteria was observed after treatment of PDT + 2.5% NaOCl.

Table 1. Continued

Meire et al[28] 2012	In vitro	*E. faecalis*	1) aPDT (Denfotex Helbo system); 2) Er:YAG laser irradiation (2940 nm, 50 mJ or 100 mJ, 15 Hz, 40 s); 3) Er:YAG laser irradiation (2940 nm, 100 mJ, 15 Hz, 40 s); 4) Nd:YAG laser irradiation (1064 nm, 2 W, 15 Hz, 40 s); 5) immersion in 2.5% (w/v) NaOCl for 1 min; 6) immersion in 2.5% (w/v) NaOCl for 5 min; 7) immersion in 2.5% (w/v) NaOCl for 10 min; immersion in 2.5% (w/v) NaOCl for 30 min in control group; 8) NaOCl 0.25% + Er:YAG	TBO 12.7 mg /mL	635 nm P: 100 mw T: 10 s	The use of both commercial aPDT systems resulted in a weak reduction in the number of *E. faecalis* cells.
Poggio et al[29] 2011	In vitro	*Enterococcus faecalis*, *Streptococcus mutans* and *Streptococcus sanguis* strains	1) Teeth treated with PAD (FotoSan system); 2) teeth treated with PAD and with 5% NaOCl solution; 3) teeth irrigated with TBO; 4) teeth treated with PAD for longer time (FotoSan system);5) teeth irrigated with 5% NaOCl solution (positive control).	TBO 100 µg/mL	628 nm P:1 w T: 30 or 90 s	PAD applied for a longer time (in respect to manufacturer's instructions) or PAD associated to 5% NaOCl showed the significantly higher antibacterial effects.
Rios et al[30] 2011	In vitro	*E. faecalis*	Five experimental groups and three control groups: 1) NaOCl; 2) toluidine blue O (TBO); (3) light: canals were filled with sterile saline; (4) TBO/light; (5) NaOCl/TBO/light	TBO low viscosity	635 nm 30 s	PDT using TBO and a LED lamp has the potential to be used as an adjunctive antimicrobial procedure in conventional endodontic therapy.
Schlafer et al[31] 2010	In vitro	*Escherichia coli*, *Candida albicans*, *Enterococcus faecalis*, *Fusobacterium nucleatum*, and *Streptococcus intermedius*	1) *T–L–*, No TBO, no light (negative control treatment); 2) *T+L–*, TBO, no light; 3) *T–L+*, no TBO irradiation; 4) *T+L+*, TBO + irradiation	TBO 100 µg/mL	628 nm 30 s	PAD yielded significant reductions in the viable counts of all organisms in planktonic suspension.
Souza et al[32] 2010	In vitro	*E. faecalis*	1) MB/NaOCl (PDT with MB and NaOCl as the irrigant), 2) TB/NaOCl (PDT with TBO and NaOCl as the irrigant), 3) MB/NaCl (PDT with MB and NaCl as the irrigant), 4) TB/NaCl (PDT with TBO and NaCl as the irrigant)	MB 15 µg/mL	660 nm 40 mW T: 4 min	These in vitro results suggest that PDT with either MB or TBO may not exert a significant supplemental effect to instrumentation/irrigation procedures.
Meire et al[33] 2009	In vitro	*E. faecalis*	1) Nd:YAG laser 2) KTP laser 3) PAD treatment 4) NaOCl solution 5) positive control	TBO 12.7 mg /mL	635 nm P:100 mW T: 150 s Energy: 15 J	In aqueous suspension, PAD and NaOCl resulted in a significant reduction in the number of *E. faecalis.*
Bergmans et al[34] 2008	In vitro	*Streptococcus anginosus*, *E. faecalis* or *Fusobacterium nucleatum*	1) PAD 2) Laser 3) Dye 4) Positive control	TBO 12.7 mg/mL	635 nm P:100 mW T: 150 s	Treatment of root canals with PAD (15 J) caused a significant reduction of the bacterial load, resulting in a 93.8% kill of *S. anginosus* ($P < 0.0001$), a 88.4% kill of *E. faecalis.*
Fonseca et al[35] 2008	In vitro	*E. faecalis*	1) Control group and 2) test group	TBO 0.0125%	660 nm P: 50 mW	The mean decrease in CFU/mL was 99.9% in the test group.
Bonsor et al[36] 2006	In vivo	*Fusobacterium nucleatum*, *Prevotella intermedia*, *Streptococcus intermedius* and *Peptostreptococcus micros*	A microbiological sample of the canal was taken on accessing the canal, after conventional endodontic therapy, and finally	TBO	635 nm 60 s at 100 mW	The PAD system offers a means of destroying bacteria remaining after using conventional irrigants in endodontic therapy.

Table 2. Studies Using MB as PS

Author/Year	Type	Bacteria	Groups	PS	Wavelength/ parameter	Results
de Oliveira et al[37] 2015	In vitro	*E. faecalis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*	1) 1% NaOCl, 2) 5.25% NaOCl, 3) saline+PDT, 4) 1% NaOCl+PDT, 5) 5.25% NaOCl+PDT, 6) Positive control, 7) Negative control	MB 15 µg/mL	660 nm P:100 mW T:90 s	The result shows that PDT can be useful to improve the root canal disinfection.
Wang et al[38] 2014	In vitro	*E. faecalis*	1) ultrasonic irrigation with 2.5% NaOCl, 2) methylene blue (MB)-mediated PDT, 3) ultrasonic irrigation and PDT	MB 100 µM	670 nm P:50 mw/100 mW	The combination of ultrasonic irrigation and PDT produced significant antibacterial efficacy against *E. faecalis* during root canal disinfection.
Silva et al[39] 2014	In vitro	*E. faecalis*	1) MG 30 s, 2) MB 30 s, 3) MG 60 s, 4) MB 60 s, 5) MG 120 s, 6) MB 120 s, 7) NaOCl 120 s, 8) Normal saline 120 s	MB	660 nm P: 40 mW, 120 J/cm^2 T: 30, 60, or 120	PDT using MB and MG have antibacterial effect against *E. faecalis*, showing potential to be used as an adjunctive antimicrobial procedure in endodontic therapy.
Bumb et al[40] 2014	In vitro	*E. faecalis*	1) Control group, 2) PDT group	MB 25 mg/mL	910 nm P: 1 W, T:3*20 s	It was found that percentage of CFU/mL reduction in PDT group was 96.70%. The result of the study suggested the potential of PDT to be used as an adjunctive antimicrobial procedure after standard endodontic chemo-mechanical debridement.
Xhevdet et al[41] 2014	In vitro	*E. faecalis, Candida albicans*	1) PDT 1 min, 2) PDT 3 min, 3) PDT 5 min, 4) NaOCl + PBS + FBS, 5) PUI with NaOCl control	Phenothiazine chloride 10 mg/mL	660 nm 100 mw/cm^2 1, 3 and 5 min	Longer times of PDT were recommended. Irrigation with 2.5% NaOCl showed similar results to 5 min irradiation.
Yildirim et al[42] 2013	In vitro	*E. faecalis*	1) A 5% sodium hypochlorite (NaOCl), 2) PDT 1 min, 3) PDT 2 min, 4) PDT 4 min	MB 10 mg/mL	660-nm 100 mW/cm^2 T: 1, 2, and 4 min	The lowest reduction in the microorganism load was observed in the 1-min irradiation group. There were no significant differences among the groups.
Miranda et al[43] 2013	In vitro	*E. faecalis*	1) Control group, 2) Endovac group, 3) PDT group, 4) Endovac + PDT group	MB 25 µg/mL	660 nm 40 mW 5 min	A significant reduction of *E. faecalis* mean counts was observed in all groups from baseline to both post-therapy samplings; no differences among the groups were detected.
Meire et al[28] 2012	In vitro	*E. faecalis*	1) aPDT (Denfotex Helbo system), 2) Er:YAG laser irradiation (2940 nm, 50 mJ or 100 mJ, 15 Hz, 40 s), 4) Er:YAG laser irradiation (2940 nm, 100 mJ, 15 Hz, 40 s), 5) Nd:YAG laser irradiation (1064 nm, 2 W, 15 Hz, 40 s), 6) immersion in 2.5% (w/v) NaOCl for 1 min, 7) immersion in 2.5% (w/v) NaOCl for 5 min, 8) immersion in 2.5% (w/v) NaOCl for 10 min, 9) immersion in 2.5% (w/v) NaOCl for 30 min in control group, 10) NaOCl 0.25% + Er:YAG	MB 10 mg /mL	660 nm P:75 mW T: 150 s	The use of both commercial aPDT systems resulted in a weak reduction in the number of *E. faecalis* cells.

Table 2. Continued

Shrestha and Kishen[44] 2012	In vitro	*E. faecalis*	CSnps and PDT using photosensitizers, rose bengal (RB), and MB	MB 0.1 and 0.3 mg/mL + CSRnp 10 µmol/L	660-nm Energy density: 5- and 10- J/cm^2 T: 1.66 and 3.33 min	The antibacterial activity of PDT using MB and RB was inhibited in a decreasing order by dentin matrix, BSA, pulp, dentin, and LPSs. The effect of tissue inhibitors was higher in the case of PDT with RB.
Cheng et al[45] 2012	In vitro	*E. faecalis*	1) Nd:YAG, Er:YAG + NaOCl + normal saline + distilled water 2) Er:YAG + normal saline + distilled water, 3) Er,Cr:YSGG, 4) aPDT, and 5) two control groups	MB 0.01 mg/mL	660 nm P: 0.2 W T: 60 s	After treatment, the bacterial reductions in the experimental groups and the positive control group were significantly greater than that of the negative control group ($P < 0.001$). However, only Er:YAG/NaOCl/NS/DW group showed no bacterial growth (the bacterial reduction reached up to 100%) on the surface of root canal walls or at 100/200 µm inside the dentinal tubules.
Ng et al[46] 2011	In vitro	Mix of 39 species in endodontic infections	1) Chemomechanical debridement (CMD group), 2) CMD + PDT	MB 50 µg/mL	665 nm P: 1 W P: 100 mW/cm^2 T: 2*2.5 min 30 J/cm^2	PDT significantly reduces residual bacteria within the root canal system.
Upadya et al[47] 2011	In vitro	*E. faecalis*	1) aqueous Ca(OH)2 in concentrations of 25%, 50%, and 100%; 2) Chitosan nanoparticles in concentrations of 10 and 20 mg/mL (3, 12, and 24 hours); 3) MB mediated LAD	MB 10, 20 mg/mL	660 nm 2-40 J/cm^2	This study highlighted the role of biofilm matrix in providing resistance to antimicrobials.
Nunes et al[48] 2011	In vitro	*E. faecalis*	1) control group (untreated), 2) conventionally-treated group (1% NaOCl irrigation), 3) PDT with optical fiber with 90 s, 4) PDT with optical fiber with 180 s, 5) PDT without optical fiber with 90 s, 6) PDT without optical fiber with 180 s	MB 100 µg/mL	660nm P: 90 mW T: 90 s, 180 s	The greatest reduction of *E. faecalis* (99.99%) was achieved with irrigation with 1% NaOCl. PDT also significantly reduced *E. faecalis* with no significant statistical difference among the groups.
Garcez et al[49] 2010	In vivo	*Enterococcus* sp, *Prevotella* sp, *Actinomyces* sp, *Peptostreptococcus* sp, *Streptococcus* sp, *Fusobacterium* sp, *Porphyromonas* sp, *Enterobacter* sp, and *Propionibacterium* sp.	(1) after accessing the root canal, (2) after endodontic therapy, (3) after PDT	chlorin(e6) 60 µmol/L	660 nm 40 mW T: 4 min E: 9.6 J	PDT is an efficient treatment to kill multi-drug resistant microorganisms.
Pagonis et al[50] 2010	In vitro	*E. faecalis*	1) No light/no MB nanoparticles (control), (2) treated only with MB-loaded nanoparticles, (3) treated with MB-loaded nanoparticles and light	MB 6.25 mg/mL	665 nm T: 10 min	The synergism of light and MB-loaded nanoparticles led to approximately 2 and 1 log reduction of colony-forming units (CFU/mL) in planktonic phase and root canals, respectively.

Table 2. Continued

Souza et al[32] 2010	In vitro	*E. faecalis*	Four experimental groups: 1) MB/NaOCl (PDT with MB and NaOCl as the irrigant), 2) TB/NaOCl (PDT with TBO and NaOCl as the irrigant), 3) MB/NaCl (PDT with MB and NaCl as the irrigant), 4) TB/NaCl (PDT with TB and NaCl as the irrigant).	MB 15 µg/mL	660 nm 40 mW T: 4 min	These in vitro results suggest that PDT with either MB or TBO may not exert a significant supplemental effect to instrumentation/irrigation procedures.
Fimple et al[51] 2008	In vitro	*Actinomyces israelii*, *Fusobacterium nucleatum* subspecies, *Porphyromonas gingivalis*, and *Prevotella intermedia*	1) No light/No MB (control group); 2) MB only, 3) Light only, 4) Light and MB	MB 25 µg/mL	665 nm P: 1 W T: 2* 2.5 min 30 J/cm^2	PDT can be an effective adjunct to standard endodontic antimicrobial treatment when the PDT parameters are optimized.
George and Kishen[52] 2008	In vitro	*E. faecalis*	1) Control group, 2) Root canal–treatment (RCT), 3) Conventional LAD group (MB in water), 4) PF4 group: using MB in emulsion, 5) RCT+PF4 group	MB 50 µmol/L	664 nm 31.84 J/cm^2	The modified photosensitizer formulation will have potential advantages in endodontic disinfection.
George and Kishen[53] 2007	In vitro	*E. faecalis*	1) MB activated by visible light, 2) sodium hypochlorite (NaOCl)	MB 10 µmol/L	664 nm T: 20 min P: 30 mW E: 36 J	*E. faecalis* cells were killed at a faster rate than fibroblasts. An irradiation dose producing 97.7% bacterial killing showed only 30% fibroblast dysfunction.
Foschi et al[54] 2007	In vitro	*E. faecalis*	1) No light/no MB (control group); 2) MB only (MB group); 3) light only (light group); 4) light and MB (PDT group).	MB 6.25 mg/mL	665 nm P: 1 W T: 10 min	PDT achieved 77.5% reduction of *E. faecalis* viability. MB alone and light alone reduced bacterial viability by 19.5% and 40.5%, respectively.
Soukos et al[55] 2006	In vitro	Endodontic pathogens in planktonic phase as well as on *E. faecalis*	1) Light+/PS+, 2) Light+/ PS-, 3) Light-/PS+, 4) Light-/PS-	MB 25 µg/mL	665 nm P: 1 W T: 5 min 30 J/cm^2	PDT may be developed as an adjunctive procedure to kill residual bacteria in the root canal system after standard endodontic treatment.

Table 3. Studies Using Curcumin as PS

Author/Year	Type	Samples	Bacteria	Groups	PS	Laser Source	Results
da Frota[56] 2015	invitro	planktonic	*E. faecalis*	1a) CUR, pre-irradiation for 5 + 5 min of irradiation, 1b) CUR, pre-irradiation for 5 + 10 min of irradiation, 2a) CUR, pre-irradiation for 5 + 5 min without irradiation, 2b) CUR pre-irradiation for 5 + 10 min of irradiation, 3a) physiological solution and irradiation for 5 min, 3b) physiological solution and irradiation for 10 min	Curcumin 20 µM	450 nm 100 mW/cm^2 T: 5 min	Curcumin as photosensitizer was effective by 5 min LED irradiation and curcumin alone was not effective in eliminating *E. faecalis*.
Neelakantan et al[57] 2015	In vitro	Biofilms	*E. faecalis*	1) sterile saline, 2) 3% sodium hypochlorite, 3) 3% sodium hypochlorite + ultrasonic files, 4) 3% sodium hypochlorite + blue light, 5) curcumin (2.5 mg/mL), 6) curcumin (2.5 mg/mL) +ultrasonic files, 7) curcumin (2.5 mg/mL) + blue light	Curcumin 2.5 mg/mL	380–515 nm 1200 mw/cm^2 T: 4 min	Light activation produced significantly higher antibacterial efficacy than ultrasonic agitation, with light activated curcumin producing the maximum elimination of biofilm bacteria within the root canal lumen and dentinal tubules.
Pileggi et al[58] 2013	In vitro	Planktonic suspensions or biofilms.	*E. faecalis*	1) Eosin-Y, 2) Rose bengal, 3) Curcumin	Curcumin 1 µM 5 µM 10 µM	(380–500 nm T: 240 s 450 mW/cm^2	Blue light irradiation alone did not alter *E. faecalis* viability. For planktonic cultures, blue light activated eosin-Y (5 µM), rose bengal (1 µM), or curcumin (5 µM) significantly reduced *E. faecalis* compared to non-irradiated group. For biofilm cultures, concentrations of light-activated eosin-Y, rose bengal, and curcumin of 100, 10, and 10 µM respectively suppressed *E. faecalis* viability.

Table 4. Studies Using Curcumin as PS

Author/Year	Type	Number of Samples	Bacteria	Groups	PS	Wavelength	Results
Nagayoshi et al[59] 2011	In vitro	8	*E. faecalis*	1) Irradiated for 30, 60, or 120 seconds with or without PS, 2) Saline, 3) NaOCl	Indocyanine green 12.5 mg/mL	805 nm P: 5 W T: 30, 60, 120 s	The viability of *E. faecalis* was significantly reduced by the combination of a photosensitizer and laser irradiation.

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