Light-activated disinfection in endodontics: A comprehensive review

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Abstract

Light-activated disinfection (LAD) has emerged as a novel approach toward antimicrobial disinfection within the root canal. This approach is based on the concept that porphyrins and photosensitizers (PSs) can be activated by light to produce cytotoxic elements that induce the desired therapeutic effect. Unlike antibiotics, LAD can act on multiple targets within a bacterial cell, including membrane lipids, genomic DNA and various proteins, including enzymes, thus reducing the ability of the organism to acquire resistance.

The aim of this review was to develop an understanding of the potential use of LAD in endodontics and to suggest strategies to maximize the antibacterial effects of LAD.

The electronic searches of the PubMed/MEDLINE, Web of Science, Scopus, and Cochrane databases were complemented by a manual hand search. A total of 303 studies were evaluated for essential parameters, which included the origin, types/variations, methodology, and application of LAD in in vitro and in vivo studies.

It can be concluded that LAD is effective against the vast majority of bacterial pathogens, including antibiotic-resistant Gram-negative and Gram-positive bacteria, along with several yeasts, viruses and protozoan species. The literature tends to suggest that LAD can be used either as a substitute or an adjunct to the conventional antimicrobial treatment regimens that are implemented to battle polymicrobial biofilms.

Keywords: biofilms, disinfection, light-activated disinfection, photosensitizing agents, root canal therapy

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Introduction

There are over 700 microbial species that can be present in the oral cavity, and an individual can have 100–200 species at any given time.[1] Usually, primary root canal infections are polymicrobial in nature and are dominated by anaerobic bacterial species.[2] The organisms frequently isolated in such cases include Gram-negative anaerobic rods, Gram-positive anaerobic cocci, gram-positive anaerobic and facultative rods, *Lactobacillus*, and *Streptococcus spp*.[2] Most anaerobes are easy to eliminate during root canal treatment, but facultative bacteria may survive the disinfection procedures.[2] *Enterococcus faecalis* is the microorganism that has been isolated in most cases of failed root canal treatment, and has therefore been mentioned in the literature as one of the chief causative agents.[3] Along with *E. faecalis*, *Staphylococcus*, *Enterococcus*, *Enterobacter*, *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Sphingomonas*, *Candida*, and *Actinomyces spp.* have also been isolated from root-filled teeth with post-treatment disease.[4–9] Antibacterial agents are widely used in the treatment of bacterial infections, but the emergence of bacterial pathogens resistant to the commonly used chemotherapeutics has led to a search for alternative drugs and/or therapies to overcome the development of resistant species.

Sodium hypochlorite (NaOCl) is the gold standard for endodontic disinfectants,[10] as it has the ability to dissolve tissue and provide broad-spectrum antimicrobial effects,[11] making it the solution of choice for the treatment of pulp necrosis and infection.[12] However, this disinfectant has several undesirable drawbacks, such as the risk of tissue damage, allergic potential, and unpleasant smell and taste. Although other irrigants, such as chlorhexidine, are more compatible than NaOCl, they lack the tissue dissolving ability; thus, their activity is greatly reduced when exposed to organic matter.[13] Several other irrigants have been used for endodontic disinfection, but have been found to be inferior to or equally effective as (with regard to bactericidal properties) NaOCl.[14]

These drawbacks have forced a major research effort to find alternative antimicrobial approaches aimed at killing microorganisms without causing resistance. The concept of light-based disinfection as a means of eliminating the bacterial microflora from within the root canal was described by Foote.[15] Light-activated disinfection (LAD) has emerged as a novel approach toward antimicrobial disinfection within the root canal.[16] It is based on the concept that porphyrins and photosensitizers (PSs) can be activated by light to produce cytotoxic elements that induce the desired therapeutic effect.[16] Light-activated disinfection can act on multiple targets within a bacterial organism. These target sites include the lipid membrane, genomic DNA and various proteins, including enzymes. This, in turn, reduces the ability of the organism to acquire resistance against LAD.[16]

The aim of this review was to develop an understanding of the potential use of LAD in endodontics and to suggest strategies to maximize the antimicrobial effects of this technique.

Methodology

The electronic searches of the PubMed/MEDLINE, Web of Science, Scopus, and Cochrane databases were carried out. A total of 303 studies were evaluated for essential parameters, which included the origin, types/variations, methodology, and application of LAD, along with the potential risk factors reported in in vitro and in vivo studies. The searches were carried out using the combinations of the following keywords: "microbial infections"; "porphyrins"; "photosensitization"; "activated oxygen"; "bacterial infections/therapy"; "phototherapy"; "diode laser"; "blue light"; and "wavelength 450–670 nm". After the initial screening, a total of 80 articles were selected. The electronic searches were complemented by a manual search of various textbooks and articles. A total of 7 articles were identified as a result of the manual search. In total, 87 articles were considered relevant and used for this project (Fig. 1).

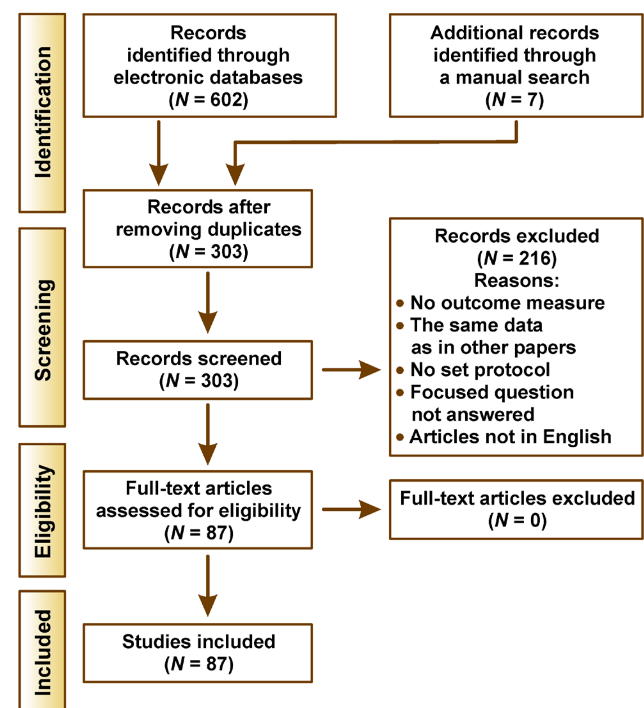

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram showing the literature search and the selection criteria

According to: Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA statement. *PLoS Med.* 2009;6(7):e1000097. doi:10.1371/journal.pmed.1000097. For further information, visit http://www.prisma-statement.org/.

Light-activated disinfection in endodontics

Light-activated disinfection starts when the porphyrins or PSs are exposed to a specific wavelength of light, within the target tissue, leading to the production of singlet oxygen ($^1O_2$), being the main reactive oxygen species (ROS) (Fig. 2).

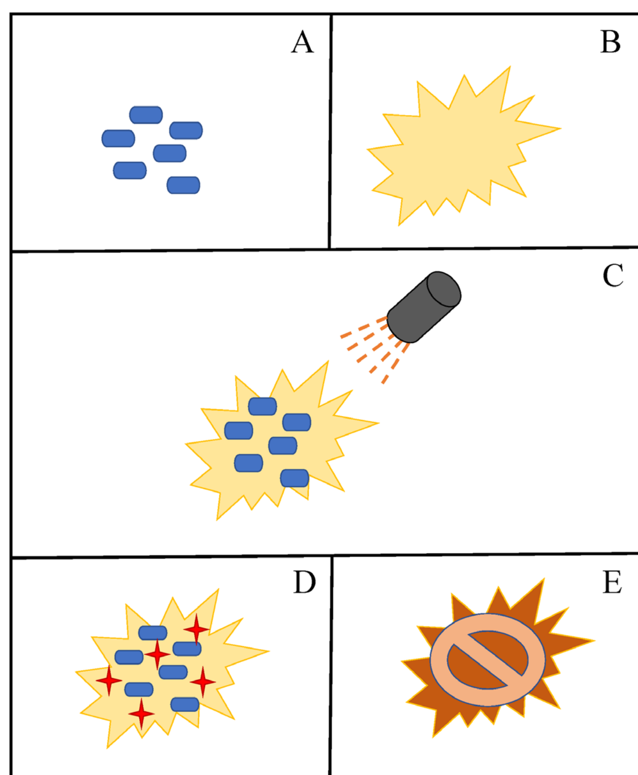

Fig. 2. Mechanism of action of light-activated disinfection (LAD) on a bacterial cell

A – photosensitizer (PS) molecules; B – bacterial cell; C – PS entering the bacterial cell, followed by activation with the use of light of a specific wavelength; D – production of reactive oxygen species (ROS); E – cell death.

Effect on bacterial biofilms

Most of the laboratory and clinical investigations using the LAD technique within the root canal use a PS rather than bacterial porphyrins. Photosensitizers are chemical derivatives of the naturally occurring porphyrins within the specific species. The effective elimination of both *Streptococcus mutans* and *E. faecalis* has been reported with a combination of LAD and either methylene blue (MB) or toluidine blue O (TBO), with a killing efficacy of 97–99.9% for planktonic bacterial loads of up to 10 million organisms at an exposure time of 120 s.[17] Relatively similar results have been reported for the elimination of *Staphylococcus intermedius*, with complete kills for loads of up to 1,000 million organisms within the root canal, using TBO as a dye and a helium-neon (He-Ne) laser of an output power of 35 mW, when exposed for 150 s.[18] When *E. faecalis* is used as the infecting organism, there is a reported 77.5% killing rate with a combination of MB and a diode laser at a fluence level of 60 J/cm^2, 99.9% with TBO and a laser of an output power of 50 mW at an energy level of 6.4 J,[19] and 90% killing ex vivo and 99.99% killing in vitro while using a combination of TBO and a diode laser of an output power of 100 mW at an energy level of 15 J.[20] According to George and Kishen, 99.99% elimination of *E. faecalis* biofilms could be achieved by using MB and a 30 mW diode laser set at 36 J.[21] By applying a dual-stage approach (a modified PS formulation and an irradiation medium), they managed to achieve disinfection without canal enlargement. This procedure was termed "advanced non-invasive light-activated disinfection" or ANILAD.[21] The use of LAD has also been shown to be effective against *Prevotella intermedia*, *Peptostreptococcus micros*, *Fusobacterium nucleatum*, *Porphyromonas spp.*, and *Actinomyces spp.*[21]

Studies have also shown the effectiveness of LAD in eradicating mixed biofilm infections. Fimple et al. suggested that, when combined with MB, diode lasers could cause a 73–80% reduction in multi-species bacterial biofilm loads.[22] An in vitro study by Soukos et al. on planktonic biofilms showed that all microorganisms were eliminated following MB-mediated LAD, except *E. faecalis*, which showed only a 47% reduction.[23] However, the authors did report a 97% reduction of *E. faecalis* on *E. faecalis*-based biofilms afterward. The authors suggested that the variation was due to a difference in susceptibility toward much higher energy fluence for LAD that was being used.[23] In another study, Williams et al. compared the sensitivity of planktonic microorganisms against the biofilms grown in root canals and Perspex® simulated canals.[24] The specimens were exposed to a combination of LAD with TBO. The results indicated that LAD was less effective in root canals than in the suspension form. The study did not run a comparison for single species within the planktonic and biofilm mode of growth.[24] It should also be noted that clinically, most acute exacerbations during endodontic treatment involve the *Porphyromonas* bacterial species,[25] in particular *P. endodontalis*, an anaerobe that is highly susceptible to $^1O_2$.

Use of LAD with the existing irrigation methods

Light-activated disinfection should be used in conjunction with the existing measures of irrigation, such as the use of NaOCl. In a study by Bonsor et al., 14 patients were evaluated to assess the efficacy of TBO and a diode laser in combination with the conventional root canal treatment.[26] The results showed a 96.7% bacterial reduction.[26] Another study by the same authors included 64 patients and used a chelating agent (ethylenediaminetetraacetic acid – EDTA) before the use of LAD.[27] The results also showed

a significant bacterial reduction.[27] Garcez et al. conducted a study on 20 patients and the initial exposure to LAD resulted in a 98.5% bacterial reduction.[28] The treatment was followed up with a calcium hydroxide ($Ca(OH)_2$) dressing for 1 week before another round of LAD exposure, resulting in a 99.9% bacterial killing rate. The authors suggested that the use of LAD before and after $Ca(OH)_2$ was more effective than the initial dosage.[28]

Previous studies have shown that typical LAD parameters for the effective killing of microbes are on the order of 15 J/cm^2 delivered using a visible red diode laser with an output power of up to 100 mW over 60–120 s.[17,29–32] Lee et al. provided certain guidelines for the use of LAD in a clinical environment.[33] They suggest that PS should be placed in direct contact with the infected site for a short period, allowing the microorganism to absorb as much of the reactive agent as possible. This would increase sensitivity to light. Also, the dye must be agitated within the canal to eliminate air bubbles that could impede contact with the bacteria.[33] The photosensitizer must also be applied into a root canal space that is free of blood and saliva, as these can potentially impair the efficacy of photosensitization.[34] In addition, to achieve maximal effects of the laser energy, it should be delivered through a diffuser tip, thus providing a narrow cylindrical pattern of light emission.[33] The emission pattern also follows the shape of the root canal.[33] Diffuser tips reduce power density, which, in turn, reduces the risk of optical injury.[33]

A study measuring a temperature rise in the root canal during LAD reported a value of 0.16 ±0.08°C.[35] This is lower than the reported 7°C safety level for periodontal injury.[35] Another study measuring thermal effects during LAD suggested that a change in temperature was less than 0.5°C.[17] This change was not said to be clinically significant, since the critical threshold levels for irreversible pulpitis is 11 times higher, at 5.5°C.[35] This seems to suggest that, with regard to the concerns about the adverse effects due to a rise in temperature in the root canal, using LAD for endodontic disinfection can be considered harmless to the surrounding periodontal tissues.[35]

Strategies to maximize bacterial killing by LAD

Pre-treatment of cells with membrane permeabilizing agents

Nitzan et al. and other researchers suggested that the application of polycationic polypeptide polymyxin B nonapeptide (PMBN) prior to LAD exposure increased the permeability of the outer cell membrane of various Gram-negative bacteria.[36–39] This treatment allows a greater penetration of the photosensitizing agent in situations where the supply of ROS is low. The application of PMBN does not cause the release of lipopolysaccharides (LPSs) from the cell; rather it causes the outer membrane to expand, resulting in an increased penetration of PS. A study by Walther et al. concluded that following pre-treatment with PMBN, Gram-negative *Yersinia pseudotuberculosis* and *Escherichia coli* had an increased susceptibility to a combination of LAD exposure and protochlorophyllide.[40] In a similar approach, Yonei and Todo showed that the lethal effects of EDTA increased when the *E. coli* samples were exposed to LAD beforehand.[41] This may be due to the presence of chlorpromazine in EDTA.[41] Also, EDTA can stimulate the release of LPSs in *E. coli* treated with calcium chloride ($CaCl_2$) when LAD is used with either rose bengal or hematoporphyrin/zinc phthalocyanines.[42]

Modification of the photosensitizer

In a study by Bezman et al., the authors were able to covalently bind rose bengal to polystyrene beads mixed in a bacterial suspension.[43] The authors concluded that this approach enabled PS to form ROS that could penetrate more easily and efficiently through the outer cell membrane.[43] This is similar to the work by Friedberg et al., who were able to bind PSs to monoclonal antibodies.[44] These antibodies could attach themselves to the surface antigens present on *Pseudomonas aeruginosa*, which resulted in the specific killing of the target bacteria.[44] Wilson applied phenothiazinium TBO and LAD on a variety of both Gram-positive and Gram-negative bacteria, achieving significant eradication rates.[45] Similar results were reported by Usacheva et al.[46] and George and Kishen,[47] where the authors used phenothiazinium dyes to inactivate Gram-positive and Gram-negative bacteria.

Soukos et al. suggested that it might be possible to covalently bond a photosensitizing agent to a poly-L-lysine chain.[48] This delivery vehicle could effectively inactivate a variety of bacterial species. The authors demonstrated that by conjugating chlorine e6 and a poly-L-lysine chain made up of 20 lysine molecules, a killing rate of over 99% for *Actinomyces viscosus* (Gram-positive) and *Porphyromonas gingivalis* (Gram-negative) could be achieved.[48] Similar results were reported by Rovaldi et al., where the authors used a construct of 1 chlorine e6 and a 5-amino acid lysine chain,[49] and by Hamblin et al., where the authors described the effects of a poly-L-lysine-chlorine e6 conjugate of 37 lysines bound with 1 chlorine e6 molecule against both Gram-positive and Gram-negative species, achieving a significantly high killing rate.[50]

5-ALA porphyrin stimulation

Kennedy and Pottier reported the possibility of increasing the amount of porphyrins present in bacterial species that do not have the natural tendency to produce endogenous porphyrins.[51] This was achieved by adding exogenous 5-aminolevulinic acid (5-ALA).[51] The inactivation

of *E. coli* after incubation in 5-ALA and exposure to white light was shown by Gábor et al.[52] However, *Enterococcus hirae* could not be eradicated with this approach.[52]

Alteration of the photosensitizer

Studies have also been conducted to improve the efficacy of the LAD process.[53,54] George and Kishen mixed MB with water, 70% glycerol, and 70% polyethylene glycol (PEG) in a proportion of glycerol:ethanol:water (MIX) of 30:20:50.[47] Their results indicated that the molecules of MB aggregated at a greater rate in $^1O_2$ water as compared to the other aqueous media. The combination of MB with the MIX formulation produced greater bactericidal activity. This is believed to be due to a combined effect of an increased penetration of MIX within the dentinal tubules, the enhanced photooxidation of the model substrate and an increased rate of production of $^1O_2$.[47] A follow-up study suggested that, when compared with water, MIX resulted in an increased level of damage to the cell wall and chromosomal DNA.[55] The same authors also indicted that the alteration of the formula by the addition of an oxidizing agent and O_2 resulted in a more efficient disinfection of the endodontic biofilm.[55] The altered emulsion was composed of perfluoro(decahydronaphtha lene) (oxygen carrier) and hydrogen peroxide (oxidizer) mixed with the detergent Triton®-X100.[55]

Efflux pump inhibitors

Prokaryotic and eukaryotic families have membrane proteins called "efflux pumps", which aid in the removal of amphiphilic molecules from the cell.[56] These molecules combine hydrophobic properties, which facilitate cell penetration, and hydrophilic properties, which allow the distribution of compounds to tissues within the body. Many of the drugs available are amphiphilic; hence, efflux pumps tend to remove these molecules effectively from the cell.[57] Efflux is suggested to be a significant contributor toward bacterial survival (Fig. 3).[58] Inhibiting this process could potentially restore the ability of antimicrobials to decrease bacterial resistance. Kvist et al. indicated that efflux pumps were generally highly active within biofilms, therefore making them good targets to help prevent biofilm formation.[59]

In addition, the amphipathic cations have an inhibitory effect on efflux pumps; therefore, phenothiazinium dyes can act as substrates for the microbial efflux pumps, as they are structurally similar to the amphipathic cations.[60] Indeed, it has been demonstrated that the inhibition of efflux pumps, along with phenothiazinium dyes, increases the efficacy of LAD.[61] However, there are no current clinical applications using these efflux pump inhibitors. This could be due to the increased levels of toxicity observed with these compounds, which has been reported in animal studies.[62]

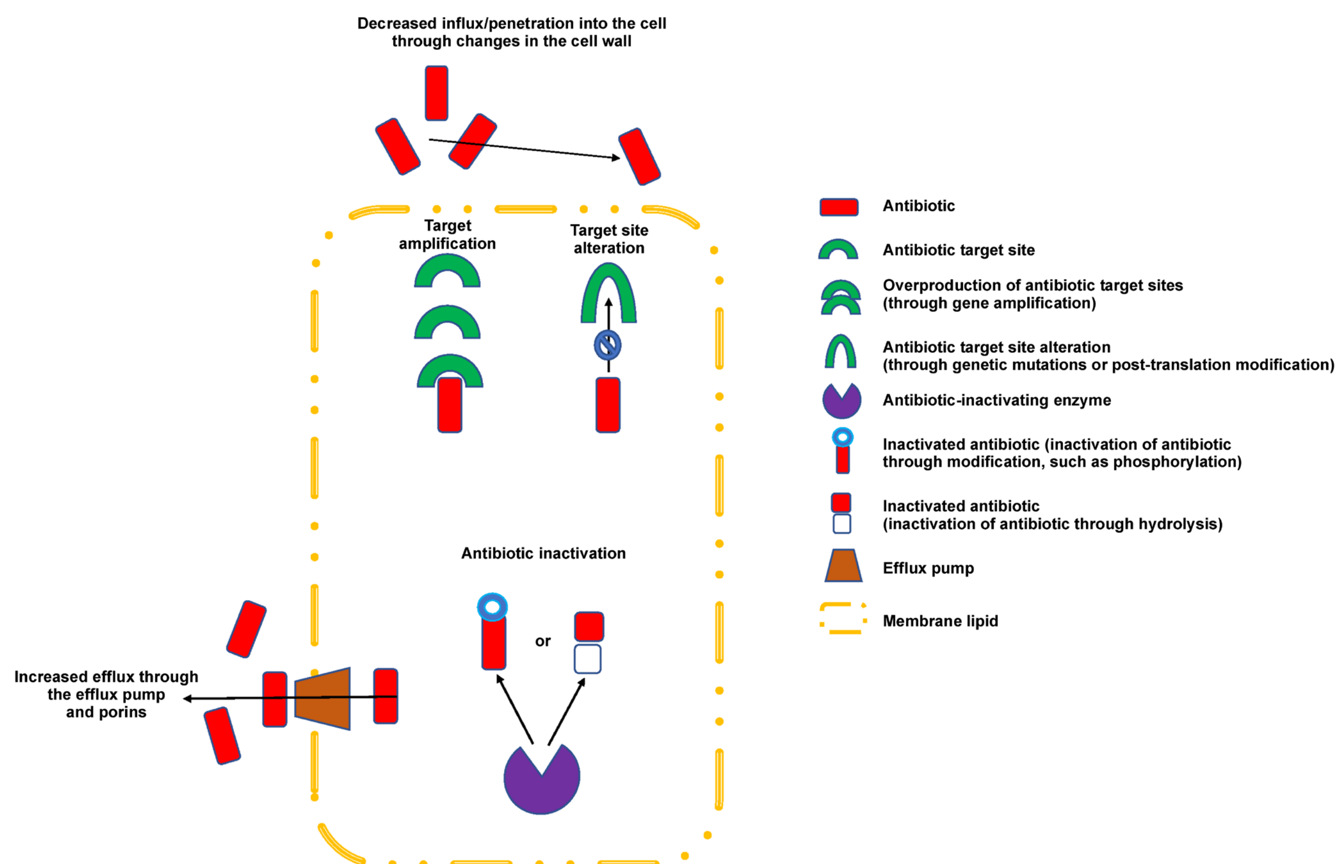

Fig. 3. Schematic diagram highlighting the efflux pump antibiotic resistance mechanisms utilized by bacteria

Factors limiting the efficacy of LAD

The light source is a limiting factor for the penetrative ability of LAD. Light can be either coherent (lasers) or non-coherent (lamps).[63] The type of light required is dependent on the location, dosage, and PSs or porphyrins being used. Lasers provide powerful monochromatic light that reduces the delivery time of LAD. As lasers are monochromatic, the wavelength plays a crucial role in the LAD process, as it should match the absorption bands of PSs or porphyrins.[63] This often means that a combination of different lasers may be required to achieve the desired result. The laser systems used in various LAD studies include argon (Ar)/dye lasers, He-Ne lasers, potassium titanyl phosphate/neodymium-doped yttrium aluminum garnet (KTP/Nd:YAG)/dye lasers, and diode lasers (Table 1 and Table 2). Presently, lasers are the source of choice when used to irradiate areas accessible only with the aid of optical fibers. The beam quality and the output power are characteristics that make lasers highly effective when coupled with optical fiber cores smaller than 500 μm in diameter.[63]

In comparison to lasers, lamps cannot be used in combination with small optical fibers, as their poor beam quality, large beam size and low power densities make them inefficient for use in smaller areas. Lamps, however, can be used directly or coupled with a liquid light guide of 5–10 mm in diameter. Both lamps and lasers have been used in LAD and neither is shown to be better than the other based on their application. Although LAD has been traditionally performed using lasers, the availability of broad-band sources (lamps) is challenging the use of lasers.[63] The scattering of light in tissues has a pronounced effect on light intensity and directionality. Along with refraction, it causes a widening of the light beam, thus lowering the fluence rate (energy per unit area) of the light, which results in a change of the direction of the light. Williams et al. used LAD combined with TBO on S. intermedius with a diode laser at 633 nm and an output power of 80 mW.[24] The organism was irradiated for 30 s, 60 s and 90 s at energy doses of 2.4 J, 4.8 J and 7.2 J, respectively. The authors concluded that the effectiveness of LAD increased with an increase in the dosage of energy.[24] However, care must be taken, as the extensive use of light in this range could be harmful for the host cells.

Conclusions

Light-based disinfection is a promising novel approach for root canal disinfection, as studies have indicated its effectiveness against a vast majority of pathogens, including Gram-negative and Gram-positive bacteria. Light-activated disinfection targets multiple sites within the bacterial cell, therefore limiting the ability of the pathogens to acquire resistance. Moreover, it has been suggested that

Table 1. Light and dye parameters applied in some in vitro studies on the use of LAD in endodontics

Study (year)	Model	Light and irradiation parameters	Photosensitizer (formula)	Results
Seal et al. (2002)[18]	2-day biofilms of *Staphylococcus intermedius*	He-Ne gas laser at 632.8 nm P = 35 mW E = 2.1, 3.2, 4.2, 10.5, or 21 J	TBO ($C_{15}H_{16}N_3S^+$)	maximum of 5 log10 reduction in CFU/mL at 21 J
Soukos et al. (2006)[23]	3-day biofilms of *Enterococcus faecalis*	diode laser at 665 nm PD = 740 mW/cm^2 F = 222 J/cm^2	MB ($C_{16}H_{18}ClN_3S$)	97% reduction in bacterial viability
George and Kishen (2007)[21]	4-day biofilms of *E. faecalis* and *Aggregatibacter actinomycetemcomitans*	diode laser at 664 nm P = 30 mW E = 36 J	MB ($C_{16}H_{18}ClN_3S$)	≥5 log10 reduction in CFU/mL
Fonseca et al. (2008)[19]	2-day biofilms of *E. faecalis*	Ga-Al-As diode laser P = 50 mW E = 6.4 J	TBO ($C_{15}H_{16}N_3S^+$)	≈99.9% reduction in bacterial viability
Fimple et al. (2008)[22]	3-day multi-species biofilm	diode laser at 665 nm PD = 100 mW/cm^2 F = 30 J/cm^2	MB ($C_{16}H_{18}ClN_3S$)	≈73–80% reduction in bacterial viability
Meire et al. (2009)[20]	2-day biofilms of *E. faecalis*	diode laser at 635 nm P = 100 mW E = 15 J	TBO ($C_{15}H_{16}N_3S^+$)	≈1.5 log 10 reduction in CFU/mL
Aydin et al. (2020)[65]	28-day incubation of *E. faecalis*	diode laser at 628 nm P – not mentioned	TBO ($C_{15}H_{16}N_3S^+$)	97.8911% reduction in *E. faecalis* bacterial load
Yoshii et al. (2021)[64]	2–day biofilms of *Lactobacillus acidophilus*	laser at 650 and 940 nm P = 9 mW and 600 mW E – not mentioned	AR ($C_{27}H_{29}N_2NaO_7S_2$) and BB ($C_{37}H_{34}N_2Na_2O_9S_3$)	650-nm laser combined with the BB solution was most effective in sterilizing the dentin plates infected with *L. acidophilus*

He-Ne – helium-neon; P – output power; E – energy; PD – power density; F – fluence; Ga-Al-As – gallium-aluminum-arsenide; TBO – toluidine blue O; MB – methylene blue; AR – acid red; BB – brilliant blue; CFU – colony-forming unit.

Table 2. Light and dye parameters applied in some in vivo studies on the use of LAD in endodontics

Study (year)	Model	Light and irradiation parameters	Photosensitizer (formula)	Results
Bonsor et al. (2006)[27]	64 canals in patients with symptoms of irreversible pulpitis/apical periodontitis	diode laser at 633 nm P = 100 mW E = 12 J	TBO ($C_{15}H_{16}N_3S^+$)	>90% reduction in bacterial viability with the use of a chelating agent along with LAD
Garcez et al. (2008)[28]	20 single-rooted canals in patients with symptoms of necrotic pulp and apical periodontitis	diode laser at 660 nm P = 40 mW E = 9.6 J	PEI ($(C_2H_5N)_n$) and chlorin e6 ($C_{34}H_{36}N_4O_6$) conjugate	≈99.9% reduction in bacterial viability following 2 successive combinations of the conventional endodontic therapy and LAD
Zorita-García et al. (2019)[66]	42 single-rooted teeth in 33 patients with apical periodontitis	diode laser at 630 nm PD = 2,000 mW/cm^2	TBO ($C_{15}H_{16}N_3S^+$)	90.3% reduction in *E. faecalis* bacterial load

PEI – polyethylenimine

LAD can be used either as a substitute or an adjunct to the conventional antimicrobial treatment regimens used for battling polymicrobial biofilms. However, it is the authors' suggestion that further studies be conducted, e.g., incorporating nanocarrier systems for PS to evaluate its effect on various biofilms that are persistent in root canal infections.

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