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Photodynamic therapy of *Enterococcus faecalis* bacteria in endodontic treatment

Jules Yves Jacques Ignace Marie GANDER

Dissertação conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

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Trabalho realizado sob a Orientação de Professor Doutor Paulo Miller

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Agradecimentos

À minha bisavó Manou, que fez 100 anos. Ao que ela me ensinou, me deu e a tudo o que representa.

Aos meus pais, pela incrível vida que me deram, que apenas agora começou.

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Resumo

Objetivo: A revisão sistêmica visa rever a Terapia Fotodinâmica da bactéria *Enterococcus faecalis* em endodontia respeito ao seu mecanismo de ação, fotossensibilizadores e fontes de luz, limitações e procedimentos clínicos.

Materiais e Método: Foi realizada uma pesquisa bibliográfica na base de dados *PubMed* de 2011 a 2022 de artigos em inglês, utilizando as seguintes palavras-chave: “Phototherapy”, “*Enterococcus faecalis*”, “Antimicrobial”, “Agents photosensitizing”, “Endodontics”.

Resultados: Analisando os resultados das diferentes técnicas de terapia fotodinâmica, pode-se ver que, dependendo dos diferentes fotossensibilizadores (Toluidina, Metileno, etc), das diferentes fontes de luz (LASER, LED, etc), dos diferentes protocolos, os resultados só são convincentes quando completam um tratamento endodôntico clássico, e uma irrigação com NaOCl.

Conclusão: A Terapia Fotodinâmica pode ser considerada como uma boa alternativa ao tratamento da bactéria *Enterococcus faecalis* em tratamentos endodônticos, desde que seja utilizada em adição a um protocolo de tratamento convencional, e mais particularmente a irrigação com NaOCl.

Palavras chave: *Endodontics, Photodynamic Therapy, Enterococcus faecalis, Antimicrobial.*

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Abstract

Purpose: The systemic review aims to review the Photodynamic Therapy of the *Enterococcus faecalis* bacteria in endodontics regarding its mechanism of action, photosensitizers and light sources, limitations and clinical procedures.

Materials and Methods: A bibliographic search was conducted in the *PubMed* database from 2011 to 2022 of articles in English using the following keywords: "Phototherapy", "*Enterococcus faecalis*", "Antimicrobial", "Agents Photosensitizing", "Endodontics".

Results: Analyzing the results of the different photodynamic therapy techniques, it can be seen that depending on the different photosensitizers (Toluidine, Methylene, etc), the different light sources (LASER, LED, etc), the different protocols, the results are only convincing when they complete a classic endodontic treatment, and an irrigation with NaOCl.

Conclusion: Photodynamic Therapy can be considered as a good alternative to the treatment of *Enterococcus faecalis* bacteria in endodontic treatments, provided that it is used in addition to a conventional treatment protocol, and more particularly irrigation with NaOCl.

Key words: *Endodontics, Photodynamic Therapy, Enterococcus faecalis, Antimicrobial*

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Acronyms

CFU : Colony Forming Unit

CMI : instrumentação químico-mecânica

DNA: Deoxyribonucleic Acid

GC : Control Group

GT : Test Group

LAI: Laser-Activated-Irrigation

LAD: Light-Activated Disinfection

LASER : Light Amplification by Stimulated Emission of Radiation

LED : Light Emitting Diode

MB : Methylene Blue

MEV : Scanning Electron Microscope (Microscópio Eletrônico de Varredura)

PAD : Photo Activated Disinfection

PAI : Indice Periapical

PEM : Extracellular Matrix of Polymers

PDT : Photodynamic Therapy

PH: Potential of Hydrogen

PIPS: Photon-Induced Photoacoustic Streaming

PS : Photosensitizers

TBO : Toluidine Blue

YAG: Yttrium Aluminum Garnet

1. Introduction

Apical periodontitis is a disease caused by bacteria, and, consequently, successful treatment of this condition depends on the effective elimination of intracanal bacterial populations. Although total eradication of the infection throughout the root canal system is the optimal goal to achieve, in current clinical endodontics, the goal is to reduce bacterial counts to levels that are compatible with periradicular tissue repair.(1,2)

One of the most contentious issues in endodontics today is whether root canal treatment of teeth with apical periodontitis should be completed in one or two visits.(1) The development of treatment methods that can disinfect root canals in one visit predictably has the potential to ease this debate. In this regard, the concept of accelerating root canal disinfection while preserving efficacy sounds intriguing and should be studied. In this context, the use of laser technology in endodontic therapy is a possibility.(3)

In the presence of oxygen, photodynamic treatment (PDT) or photoactivated disinfection employs light of a certain wavelength to activate a nontoxic photoactive dye (photosensitizer). When energy is transferred from an activated photosensitizer to available oxygen, highly reactive oxygen species such as singlet oxygen and free radicals are formed, which can kill bacteria by destroying key cellular components such as proteins, membrane lipids, and nucleic acids.(4,5)

The phenothiazine salts, notably toluidine blue O (TBO) and methylene blue (MB), with absorption wavelengths of 600–660 nm, are the most commonly employed Photosensitizers in recent clinical trials.(6)

The definition of photodynamic treatment (PDT) is "the light-induced inactivation of cells, microorganisms, or molecules." PDT has been referred to by numerous names since its inception, including antimicrobial photodynamic treatment (APD), photo-activated disinfection (PAD), and light-activated disinfection in the dental industry (LAD).(7)

Since the 1960s, photodynamic therapy has grown rapidly in a variety of medical specialties, because it is a selective, noninvasive or, at the very least, minimally invasive modality of treatment for a variety of diseases.(8,9)

In reality, PDT was first created as a treatment for tumors and premalignant disorders, and it now represents a highly promising option for the treatment of localized microbial infections against bacteria, fungi, and viruses.(10)

2. Purpose of the Study

The systemic review aims to review the effect of the Photodynamic Therapy in *Enterococcus faecalis* during endodontic therapy, regarding mechanisms of action, photosensitizers, light sources, limitations and clinical procedures.

3. Materials and Methods

This systematic review was based on clinical trials, clinical analysis, and articles of the past eleven years. The bibliographic research was carried out in the MEDLINE database via *PubMed* (US National Library of Medicine National Institutes of Health).

An advanced research selecting the screening:

"(endodontics[MeSH Terms]) AND (laser therapy[MeSH Terms]) OR (phototherapy[MeSH Terms]) AND (endodontics[MeSH Terms]) OR (enterococcus faecalis[MeSH Terms]) AND (endodontics[MeSH Terms]) OR (agents, photosensitizing[MeSH Terms]) AND (endodontics[MeSH Terms]) OR ((phototherapy[MeSH Terms])) AND (enterococcus faecalis[MeSH Terms]) ".

The first figure demonstrates a total of 424 articles found on *PubMed* with the previous Key words, with a total of 368 duplicates, 74 studies have been kept for the bibliography and 53 articles did not correspond to the inclusion criteria. 10 items were kept for the systematic review and analyzed in the results. Additional research was done on *PubMed* for additional articles and more specific results for a total of 21 additional articles.

4. Results

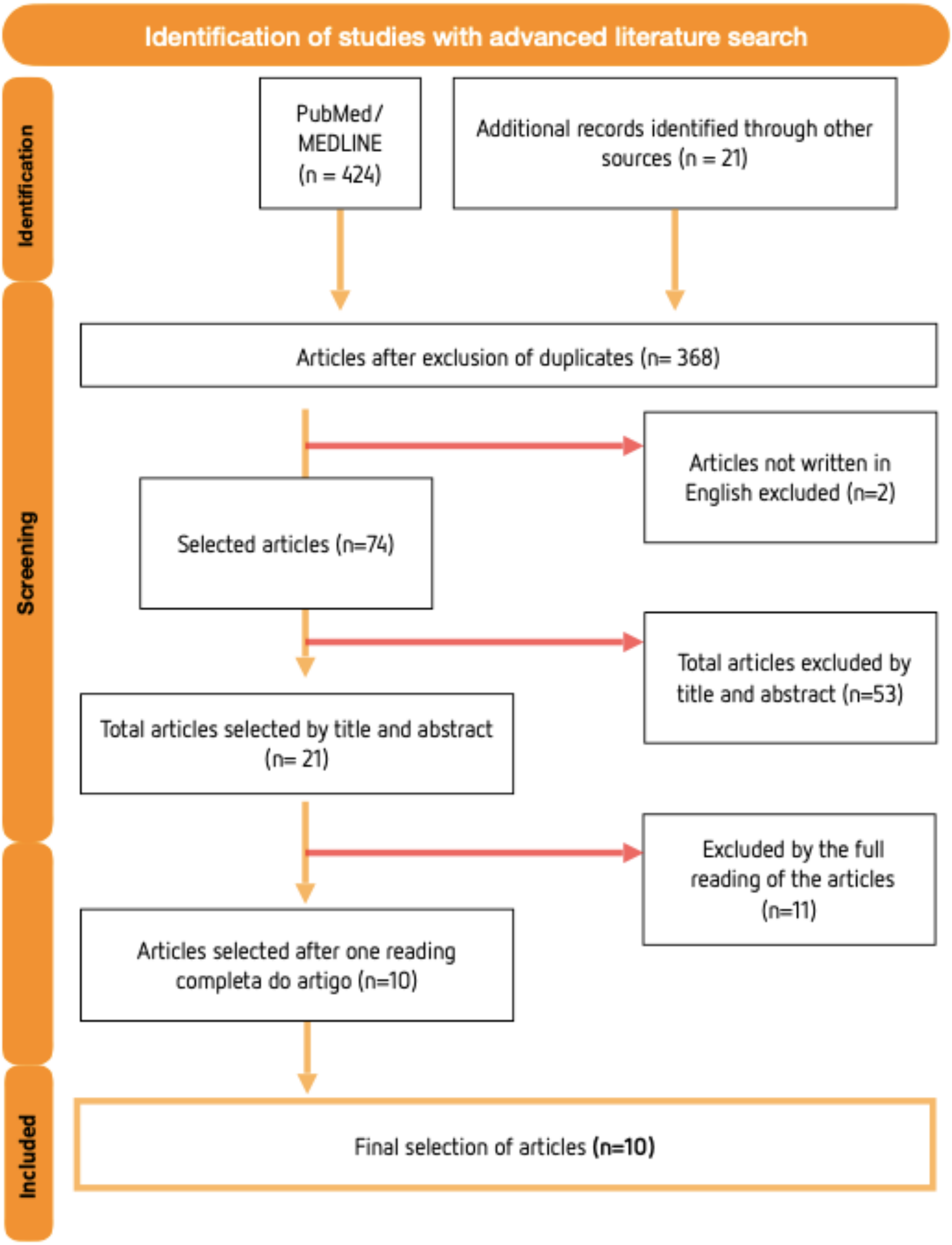

Figura 1: Fluxograma - PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources - estratégia de pesquisa utilizada neste estudo.

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
Asnaashari. *et al.* 2016	Compare the effects of LED photodynamic therapy and calcium hydroxide therapy for root canal disinfection against *Enterococcus faecalis*	Clinical trial n=20 First microbiological samples were obtained on a sterile ProTaper F2 rotary file and 3 paper tips and transferred to a microbiology laboratory. Group 1 (n = 10) specimens were subjected to PAD with photosensitizing solution (PS) (0.1 mg / ml TB) and irradiation with LED Fotosan (635nm, 200mW / cm2) for 60s. Creamy Ca (OH) 2 paste was used in group 2 (n = 10) Data included the number of colony forming units (CFUs) before and after treatments, analyzed by test and analysis of covariance	A significant difference between the results before and after treatment for both groups (calcium hydroxide p = 0.02 <0.05, PAD p <0.0001) indicated the effectiveness of both treatments. The mean numbers for log 10 CFU / mL before calcium hydroxide therapy and PAD with LED irradiation was 10.1968 and 11.3773. After treatment, the mean numbers were 9.4202 and 8.3772, respectively. The difference in results after treatment between the groups was significant (p = 0.01 <0.05) and indicates that PAD was more effective.	DBP and calcium hydroxide therapy, as adjunctive methods to conventional root canal therapy, are effective in disinfecting the root canal. Compared to calcium hydroxide therapy, DAP leads to a greater reduction in the number of *enterococci faecalis* in infected root canals.

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
De Miranda. *et al.* 2017	This short-term randomized controlled clinical trial evaluated the effectiveness of photodynamic therapy (PDT) on clinical success (periapical healing) and the microbiota of primary endodontic infections.	32 patients presenting lower molars with apical periodontitis two therapeutic groups: control (chemo-mechanical debridement [CMD]; n = 16) and PDT (CMD + PDT; n = 16) Calcium hydroxide for 7 days before final obturation. Periapical healing was assessed by the periapical index (PAI).	Significant decreases in IAP scores were observed in both groups over time, although at 6 months, the PDT group showed a significantly better cure score than the control ($p < 0.05$). Most species reduced over time in both groups, and no significant differences in the frequency and levels of the species tested were observed between the groups at any time point assessed.	Conventional endodontic therapy with or without PDT is effective in reducing microbial load, resulting in periapical healing. However, adjuvant PDT provides better periapical healing at 6-month follow-up.

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
Cretella. *et al.* 2017	This study examined the bactericidal effect of diode laser irradiation against intracanal *Enterococcus faecalis*.	n=28 extracted unirradicular and unicanal teeth were treated with ProTaper instruments (Dentsply Maillefer, Ballaigues, Switzerland). Total 120 root canals were inoculated with *E. faecalis* for 21 days, and samples were randomly divided into five groups: Group 1 (n = 24) saline solution (positive controls); Group 2 (n = 24) sodium hypochlorite only 5.25%; Group 3 (n = 24) was irrigated with saline solutions activated by diode laser; Group 4 (n = 24) was treated with sodium hypochlorite 5.25% activated by diode laser; Group 5 (n = 24) was irrigated with saline solution with methylene blue dye activated by Fox diode laser; The residual microbial load was determined with the Uro-Quick system. Data were analyzed by Pearson's chi-square test ($p < 0.001$).	A statistically significant reduction in bacterial count was observed in Group 2 and Group 4 ($p < 0.001$). There were no statistically significant differences between the other groups ($p > 0.001$).	The evidence indicates that the diode laser was no more effective than sodium hypochlorite in reducing free bacteria.

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
Okamoto. *et al.* 2019	This study aimed to evaluate the reduction of bacterial load after conventional endodontic treatment with and without antimicrobial photodynamic therapy (a-PDT) in deciduous teeth.	n=30 anterior deciduous teeth with diagnosis of pulp necrosis. Group I, conventional root canal therapy (n = 15) Group II, conventional root canal therapy combined with PDT (n = 15). PDT: methylene blue at a concentration of 0.005% was used as a photosensitizing agent: application of laser light for 40 s (wavelength: 660 nm, energy density: 4 J/cm2, power: 100 mW). The data were statistically analyzed.	The reduction in the bacterial load was 93% in Group I and 99% in Group II, with no statistically significant difference.	Conventional treatment combined with antimicrobial PDT with the parameters used in this study proved effective, but showed equal effective capacity to conventional endodontic treatment alone.

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
Camacho-Alonso. *et al*. 2017	To evaluate the antibacterial efficacy of photodynamic therapy (PDT) and chitosan against *Enterococcus faecalis* and to assess the possible enhancing effect of chitosan on methylene blue photosensitizer in experimentally infected root canals of human teeth extracted *in vitro*.	n=102 single root extracted teeth were used. The teeth were contaminated with 0.1mL of *E. faecalis* (3,108 cells/mL). 6 groups (n = 17 teeth): Group 1 (2.5% NaOCl); Group 2 (PDT); Group 3 (chitosan 3mg / mL); Group 4 (PDT + chitosan 3mg / mL); Group 5 (positive control, without treatment); Group 6 (negative control, no inoculation, no treatment). Samples were cultured on blood agar plates to determine the number of colony forming units (CFU) / mL. 5 / group analyzed in SEM % area with contamination and debris.	The positive control group showed the highest number of CFU / mL, with statistically significant differences compared to the other treatment groups (p £ 0.05). Group 4 (PDT + chitosan) showed the lowest CFU / mL count, followed by Group 2 (PDT alone), which achieved similar results to Group 1 (NaOCl), but there was no significance between the treated groups. SEM images showed that Group 4 (PDT + chitosan) showed the smallest area of contamination.	The combination of PDT and chitosan showed antibacterial potential against endodontic infection by *E. faecalis.*

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
Da Silva. *et al.* 2017	This study evaluated antimicrobial photodynamic therapy (aPDT) as an adjuvant to endodontic treatment. Ten uni-rooted teeth (control group (CG) = 4 (2 and test group (TG) = 6) with primary endodontic infections, of both sexes, between 17 and 65 years old, were analyzed.	Microbiological samples were collected before and after chemical-mechanical instrumentation (CMI), after aPDT (for the WG) and after removal of the provisional restorations (second session). In the WG, aPDT was performed with 100 lg mL 1 of methylene blue and irradiated with a low-power laser (InGaAIP, 660 nm; 100 mW; 40 s) with a fiber-coupled optical laser.	For GT, one tooth positive for Candida spp. before WCC showed negative results in subsequent samples. *E. faecalis* species was present in four samples before WCC, two after WCC, in one after aPDT and was not detected in the second session. aPDT may be an effective adjunctive therapy, resulting in a reduction (P = 0.0286) of *E. faecalis* incidence before root canal obturation.	APDT can be used as an effective adjunctive therapy in the endodontic treatment of permanent teeth, resulting in a significant reduction in the incidence of *E. faecalis* before root canal obturation at the second session in teeth with primary endodontic infections.

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
Miranda. *et al.* 2012	To evaluate the *ex vivo* efficacy of the EndoVac® system and photodynamic treatment (PDT) as adjuvants to chemomechanical debridement associated with calcium hydroxide (CaOH2) in reducing the levels of intracanal *Enterococcus faecalis*.	n=125 premolars conventionally accessed, prepared and then contaminated with *E. faecalis* for 30 days. 4 groups : 1 : Control (chemomechanical debridement with conventional irrigation); 2 : Endovac (chemomechanical debridement with EndoVac® system); 3 : PDT (chemomechanical debridement with conventional irrigation and PDT) 4 : Endovac + PDT (chemomechanical debridement with EndoVac® and PDT). Samples were obtained before (T1) and after therapeutic procedures (T2) and after intracanal medication (T3), seeded in BHI medium and incubated (37° C, 48h) to determine the colony forming units (CFU mL 1).	The overall mean cell counts (CFU mL 1) of *E. faecalis* were high at initial contamination (T1). A significant ($P < 0.05$) reduction in mean *E. faecalis* counts was observed in all groups from baseline (T1) to both post-therapy samples (T2 and T3); no differences between groups were detected. No significant change in bacterial counts from T2 to T3 was detected.	The coadjuvant use of the EndoVac® system and photodynamic treatment, in combination or not, was as effective as conventional chemomechanical debridement associated with CaOH2 in reducing the count of intracanal *E. faecalis.*

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
Al Ahmad. *et al.* 2012	The aim of this study was to investigate the efficacy of antimicrobial photodynamic therapy (PDT) using visible light together with water-filtered infrared-A (VIS + wIRA) to eradicate single species of planktonic bacteria and microorganisms during initial oral bacterial colonization in situ.	A VIS+ wIRA broadband radiator with a water-filtered spectrum in the range 580-1400 nm was used for irradiation. Toluidine blue (TB) was used as a photosensitizer at concentrations of 5, 10, 25 and 50 mg ml "1. The unweighted (absolute) irradiance was 200 mW cm "2 and was applied for 1 min. Planktonic cultures of Streptococcus mutans and *Enterococcus faecalis* were treated with aPDT. Up to 2 log10 of S. mutans and *E. faecalis* were killed by APDT. Salivary bacteria were killed to a greater extent at 3.7-5 log10.	All TB concentrations tested proved highly effective. The bacterial kill rate at initial oral bacterial colonization was significant (P50.004) at all TB concentrations tested, despite the inter-individual variations found between study participants.	This study showed that APDT in combination with TB and VIS + wIRA is a promising method to kill bacteria during early oral colonization. Taking into consideration the healing effects of wIRA on human tissue, this technique may be useful in the treatment of peri-implantitis and periodontitis.

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
Rios. *et al.* 2011	The aim of this study was to evaluate the antimicrobial effect of DFT using toluidine blue O (TBO) and a low energy light emitting diode (LED) lamp after the conventional 6% NaOCl disinfection protocol.	Extracted single-rooted teeth were cleaned, shaped and sealed at the apex before incubation with *Enterococcus faecalis* for 2 weeks. The roots were randomly assigned into five experimental and three control groups. Dentin chips were collected from the root canals of all groups with a # 50/.06 rotary file, colony forming units were determined and the bacterial survival rate was calculated for each treatment.	The bacterial survival rate of the NaOCl / TBO / light group (0.1%) was significantly lower (P <0.005) than the NaOCl (0.66%) and TBO / light (2.9%) groups.	PDT using TBO and a LED lamp has the potential to be used as an adjunctive antimicrobial procedure in conventional endodontic therapy.

AUTORS	OBJECTIVES	MATERIALS & METHODS	RESULTS	CONCLUSIONS
Pedullà. *et al.* 2012	To evaluate *ex vivo*, the antibacterial efficacy of photon-initiated photoacoustic flux (PIPS) irrigants using an Er: YAG laser equipped with a newly designed, stripped, tapered tip on extracted teeth with infected root canals.	n=148 extracted uni-rooted teeth were prepared for a cone size 25, 0.06. Specimens were sterilized and all teeth except 10 (negative control group) were inoculated with *Ent. faecalis* and incubated in a CO_2 chamber at 37 °C for 15 days in Eppendorf tubes filled with trypticase soy broth medium changed every 2 days. Infected teeth were randomly divided into 4 test groups (n = 32 for each): erbium / YAG laser pulsed in non-ablative settings for 30 s with sterile bi-distilled water (Group A) or 5% sodium hypochlorite (NaOCl) (Group B); no irrigation with sterile bi-distilled water activated by laser for 30 s (Group C) or irrigation with 5% NaOCl for 30 s (Group D); the positive control group received no treatment on infected teeth (n = 10). Colony forming units (CFU) were counted from bacteriological samples collected before (S1) and after treatment (S2).	CFU counts were significantly lower in the 5% NaOCl groups with or without laser activation than in the sterile bi-distilled water without laser activation group ($P < 0.001$). In addition, there was a significant difference between the double-distilled water groups with or without laser activation ($P < 0.001$). The sodium hypochlorite group with laser activation had the greatest reduction in CFU, which was significantly greater than that evident in the double-distilled water groups with or without laser activation ($P < 0.001$). There were no significant differences between the 5% NaOCl groups with or without laser activation ($P > 0.05$). None of the four groups generated negative samples in a predictable manner.	Under the conditions of this *ex vivo* study, there were no significant differences in bacterial reduction between the laser and NaOCl or NaOCl alone groups.

5. Discussion

5.1 Mechanism of action of PDT

Photodynamic Therapy (PDT) has been a popular research topic in medical microbiology, not only because of the increase in antibiotic resistance among bacteria, but also because of its ability to heal sensitive human tissues.(11)

Photodynamic therapy is a two-stage treatment that involves the application and retention of a PS compound in target tissues (first step), which is then activated by exposure to visible light of an appropriate wavelength that is excitatory to this compound and that is applied through a light device that can be directed to reach inner sites or can be directly driven to the target (second step). The PS (photosensitizers) undergoes a shift from a singlet low-energy 'ground state' to a higher-energy 'triplet state' after being irradiated.(6,12)

There are two processes by which the activation of the sensitizer drug to the triple state can enter chemical interactions with biomolecules in the presence of a substrate such as oxygen. Type I reactions result in the creation of free radicals due to the transfer of hydrogen or electrons. After interacting with oxygen, these reactive species may form highly reactive oxygen species like peroxide or superoxide anions, which assault cellular targets.(9)

The activation of free radicals in Type I reactions could cause direct cellular damage. Singlet oxygen is released in type II processes, which is an electrically excited and extremely reactive state of oxygen. Because type II reactions are mediated by singlet oxygen species, this is thought to be the most important mechanism in microbial cell death. However, distinguishing between the two reaction processes is difficult. The fact that both type I and type II processes are involved suggests that the damage mechanism is influenced by both oxygen tension and PS concentration. The presence of these compounds in the treatment site causes oxidative stress, which may cause target cell damage.(6,12)

These cytotoxic species bactericidal's effects are linked to two basic mechanisms: damage to the cellular plasma membrane and/or damage to the cell DNA. Cell death occurs in both scenarios. Cell injury occurs only when reactive oxygen cytotoxic species overwhelm the cell's metabolic defenses, resulting in oxidation of cellular elements such as plasma membranes and DNA, and cell death. This response is cytotoxic and vasculotoxic in humans.(1)

Another form of damage caused by PDT is damage to the bacteria's cytoplasmic membrane caused by cytotoxic species produced by PDT, which results in events such as membrane transport system deactivation, inhibition of plasma membrane enzyme activity, lipid peroxidation, and others. Singlet oxygen species can destroy microorganisms such as bacteria, fungus, viruses, and protozoa.(9,13)

Extensive laboratory studies have shown that an important aspect of this system is that the two components when used independently produce no effect on bacteria or on normal tissue. The effect on the bacteria is exclusively caused by the combination of photosensitizer and light.(14)

5.2 Photosensitizers and light sources

Photosensitizing potential exists in thousands of natural and artificial photoactive substances. However, the most investigated PSs for eradicating microbes are halogenated xanthenes, phenothiazines, acridines, and conjugated chlorins.(4,10,15)

The absence of toxicity and harmful by-products, lack of mutagenesis effect, selective accumulation on the target tissue, adaptability for topical application, low cost, and high absorption coefficient in the spectrum range of the excitation light are all desirable properties for an optimal PS.(14)

It is essential to choose an adequate and effective nontoxic PS capable of high absorption in the light length utilized for PDT to be successful for antimicrobial purposes. Different combinations of PS and light sources have been used to test the role of PDT in endodontic therapy, with varying outcomes. when the same PS and light source were used, the variety of irradiation techniques, as well as variations in PS concentration, irradiation time, and light strengths, make it hard to compare research.(12)

The phenothiazines (synthetic non porphyrin chemicals) methylene blue (MB) and Toluidine blue O (TBO, tolonium chloride) are the most studied and used dyes in PDT at various concentrations. Curcumin, the main component of turmeric powder, which has been used in medicine, as a culinary color, and as a spice for centuries, has lately been employed in dentistry as a PS for PDT.(11)

The wavelength of maximal absorption for MB is 660 nm, while TBO is 630 nm, according to studies. Both MB and TBO have bactericidal properties and can inactivate Gram-positive and Gram-negative bacteria, such as *Enterococcus faecalis*. The PSs that are utilized in dentistry are also determined by the light source. The most basic criterion for PDT light sources is that they fit the PS's activation spectrum (electronic absorption spectrum) and deliver sufficient light potency at this wavelength.(6,16)

The literature describes three main classes of clinical PDT light sources: LASER, light-emitting diodes (LED) and halogen lamps. Helium–neon lasers (633 nm), gallium–aluminum–arsenide diode lasers (630–690, 830 or 906 nm), and argon lasers (488–514 nm) are currently the most commonly used light sources in PDT. These sources' wavelengths range from visible light to the blue of argon lasers, or from the red of helium–neon and gallium–aluminum–arsenide lasers to the infrared of certain diode lasers.(16)

Diode lasers, which are easy to use, cheaper, and more portable than other light sources, have become the dominant light source in PDT. The laser light used in PDT has various advantages, including the ability to provide the correct amount of light through a fiber optic, monochromaticity, high efficiency, high potency, and interstitial light-delivery devices; however, they are expensive. LEDs and other non laser sources have lately been employed in PDT, particularly for irradiating easily accessible tissue surfaces.(6,17,18)

Photosensitizing chemicals derived from phenothiazine, such as methylene blue and toluidine blue, have long been utilized for PDT and have well-established photosensitizing characteristics. MB photo-inactivates several microorganisms, including Gram-positive and Gram-negative bacteria, due to its hydrophilic nature, low molecular weight, and positive charge, which allows it to pass through protein channels in Gram-negative bacteria's outer membrane. Several investigations have found that phenothiazine dyes are phototoxic to target species' DNA and outer membrane.(19)

Methylene blue predominantly interacts with the anionic lipopoly-saccharide macromolecule, resulting in the generation of methylene blue dimers.(9)

The effectiveness of PDT in terms of bacteria and PS interaction is largely based on three elements: I PS ability to interact with the bacterial membrane; (ii) PS ability to penetrate and act inside the cell; and (iii) reactive singlet oxygen formation around the bacterial cell caused by PS illumination. The distinct outer membrane architectures of Gram-negative bacteria, as well as the hydrophobic and charge effects of the PSs, contribute to Gram-negative bacteria's susceptibility to effective death by antibacterial PDT. In fact, the charge of the sensitizer appears to be related to the photosensitivity of bacteria. Both Gram-positive and Gram-negative bacteria can be inactivated by cationic PSs like MB and TBO. Gram-negative species are generally resistant to some commonly used PS in PDT, whereas Gram-positive species are more susceptible, because the relatively porous layer of peptidoglycan and lipoteichoic acid outside the cytoplasmic membrane of Gram-positive species allows the PS to diffuse into sensitive sites.(6,11)

In general, the target bacterium's species should be considered when selecting a PS. Both cationic and anionic dyes can be used if the sample is Gram-positive; cationic dyes are more effective if the sample is Gram-negative.(9)

Besides the PS's ability to bind to bacterial membranes and penetrate bacteria, there have been reports of bacteria inactivation in which the PS does not need to penetrate or even come into contact with the cells to be effective. Some experts believe that if enough singlet oxygen can be created near the bacteria's outer membrane, it will be able to cause harm to important structures.(5,8)

As a result, if the PS is unable to interact with the target bacteria but the therapy's reactive products (such as singlet oxygen) are produced close to the cell, its viability will be affected by the distance to the bacteria. Therefore, reaching the most inaccessible intracanal area should be also important because success may be achieved even without direct contact between the PS and the bacteria.(8,10)

5.3 Pre-irradiation time and irradiation doses

Pre-irradiation time refers to the time between the introduction of the PS into the root canal system and the actual photo-activation. The pre-irradiation time is important in PDT because it permits the PS to penetrate the dentine and exercise its antibacterial effect, as well as keeping the PS inside the bacteria, which allows for more light absorption.(18)

The most critical elements in killing microorganisms using PDT are the energy dose and light irradiation period. PDT's efficacy changed on a statistically significant scale depending on the rise in energy dose, the reduction in bacterial suspension volume, and, most importantly, when the output power was deposited in the form of cycles. The concentration of photosensitizing agent at which there was a greater efficiency in the formation of reactive oxygen species was between 50 and 100 μm, a minimum irradiation energy of 7J promoted a significant reduction in intra-canal bacteria, and the use of optic fiber contributed to the greater formation of reactive oxygen species.(6,18)

The way light is used in the interior of the root canals during aPDT irradiation has an impact on the final outcome. When light delivery technologies like diffusing optic fiber are employed, the distribution of light in the canal is more uniform and intense across a substantially larger area than when a laser tip is used, maximizing the efficiency of root canal disinfection.(17)

5.4 Applications in dentistry

In Oral Surgery and Periodontics, antimicrobial PDT, with its use of a PS in combination with low-intensity laser light enabling singlet oxygen molecules to destroy bacteria, also represents a treatment alternative for alveolar osteitis, post-extraction pain, peri-implantitis.(6,20)

The use of high-power lasers as a light source can be harmful. PDT uses a specific wavelength light for the activation of the nontoxic photoactive dye (photosensitizer). The source of the light in PDT can be an LED lamp, a low-level laser, or a diode laser.(5,21)

LED light serves as a safer alternative light source because it does not generate significant heat. High-power lasers used for PDT generate heat that may injure periapical tissues. Other advantages of LED over lasers are that they are safer, more cost-effective, and easier to handle. LED lamps have a lower thermal productivity and are less harmful to the tissues. They consume less energy and are easy to use. *In vitro* and *in vivo*, the photodynamic method has been utilized to eliminate germs in root canals. These investigations suggested that PDT could be used as a supplement to regular endodontic antibiotic therapy.(18)

5.5 Applications in Endodontics

In cases of persistent bacteria in the root canal system as a result of insufficient disinfection and debridement of the endodontic space, untreated canals, inadequate filling, or coronal leakage, post-treatment endodontic illness can develop. The root canal system cannot be completely cleaned using mechanical equipment alone. A variety of irrigating and disinfecting treatments have been employed to aid in the cleaning and debridement of the canal.(18)

Because photodynamic effects in experimentally infected root canals of extracted teeth led to a 99% reduction in colony-forming unit counts when PDT parameters were optimized, PDT was suggested as a promising effective adjunct to standard antimicrobial intracanal cleaning and shaping for clinical treatment of periapical lesions, in particular for teeth undergoing one-session endodontic treatment or retreatment.(14)

The majority of occurrences of pulp necrosis in primary teeth are caused by anaerobic microbes. According to certain research, after one week, 40% of the original bacterial microbiota re-colonize the root canals, forcing retreatment.(19)

Instrumentation and irrigation with antimicrobial irrigants such as NaOCl do not reliably render root canals bacterium-free, with 40–60% of canals still have cultivable bacteria after chemo-mechanical preparation, according to studies.(22) Irrigation is required before using photodynamics to treat germs.

For reducing endodontic infection and sanitizing root canals, various irrigation solutions have been offered. Because of its broad antibacterial range and ability to dissolve organic residues of necrotic tissue, NaOCl is the most extensively utilized irrigation agent.(12,23)

It dissolves mostly necrotic tissue at low concentrations (0.5–1%), although its dissolving power and antibacterial capabilities improve at higher concentrations (5%), however tissue toxicity increases as well. However, it will only penetrate dentinal tubules to a depth of 130um, whereas bacterial infection can reach the cementum–dentin junction at 1.000um.(2,18)

5.5.1 Enterococcus faecalis :

The gram-positive facultative anaerobic coccus *E. faecalis* has been discovered as a common cause of root canal treatment failure. It is more common in chronic infections than in primary infections. Despite instrumentation, irrigation, and intracanal medicine, the bacteria can persist as a persistent infection in the dentinal tubules.(15,18)

It has a high capacity for survival due to its ability to form biofilm, its resistant cytoplasmic membrane rich in glycerol, its capacity for long-term survival on limited nutrition, its ability to maintain its pH level due to the blocking capacity of the cytoplasm, and its ability to adhere strongly to the dentinal tubule through the production of angiotensin-converting enzyme (which promotes union) and serine protease.(6)

E. faecalis is able to survive for long periods without nutrients and invades dentinal tubules to depths over 300um, characteristics that protect it against the usual irrigating agents. The incidence of positive cultures in primary endodontic infections ranged from 12.5% to 30% for *E. faecalis*.(2)

5.5.2 In Vitro studies : Antimicrobial

Enterococcus faecalis, a Gram-positive facultative anaerobic coccoid, is one of the major etiological factors that plays a role in persistent infections and post-treatment endodontic disease.

A recent systematic review of PDT against *E. faecalis* provides a direct comparison of these investigations, indicating that, as others have said, its usage *in vitro* has shown a promising bactericidal potential.(5,24)

PDT was found to be efficient in reducing the number of *E. faecalis* colonies found in infected root canals of removed human teeth. Compared to endodontic instrumentation/irrigation treatment methods. To improve the efficacy of irrigants, many agitation techniques have been proposed, including agitation with hand files, plastic instruments, acoustic, ultrasonic, and, more recently, laser devices.(14)

The effect of PDT of LED 630-nm bulbs on root canal disinfection against *E. faecalis* was more significant than the effect of an 810nm laser in an *in vitro* examination in the Asnaashari 2016 study. This may be due to the wider emitted light spectrum of LEDs.(14)

PDT at high doses showed antimetabolic and antibiofilm potential effectiveness against *E. faecalis* biofilms of up to 42.8% when indocyanine green was used as the PS, and to a lesser level with MB and TBO.

Rios 2011's study found that root canals treated with PDT for 30 seconds alone had a 2.9% *E. faecalis* survival rate, but root canals treated with NaOCl followed by PDT had a 0.1% survival rate.(20)

After exposure to TBO alone, *E. faecalis* showed considerable sensitivity. This conclusion is consistent with another study that found TBO to be toxic to *E. faecalis*. PDT with TBO and LED light has the potential to be employed as a supplementary antimicrobial procedure in traditional endodontic therapy by reducing bacterial populations further.(18)

5.5.3 In Vivo studies :

Comparisons are challenging due to methodologic differences in the laboratory research that used PDT to target root canal microorganisms. Varied

PSs, including toluidine blue O, azulene, and chlorin e6, as well as different light settings and light-delivery techniques, were used in the laboratory studies.

Recent *in vitro* studies have shown a positive effect of LAI on debris removal.(25,26)

Although germs were not entirely eliminated from root canals, high bactericidal effects were found when irrigants such as sodium hypochlorite or chlorhexidine were activated by an Er:YAG laser. The findings of the Pedulla, 2012 study(27) reveal that photon-induced photoacoustic streaming (PIPS) of irrigants, created by a pulsed Er:YAG laser with a newly designed quartz tip, has a favorable bactericidal impact.(28,29) There was no statistically significant difference between NaOCl irrigation only and with PIPS, suggesting a lack of sufficient additional effect of laser activation when NaOCl is used.

The teeth treated with PDT using methylene blue as PS in the Camacho 2016 study(24) revealed a significant reduction in CFU/mL, with a lower mean value than samples treated with 2.5% NaOCl.

The photodynamic effects of methylene blue on multispecies root canal biofilms, on the other hand, have been demonstrated, with up to an 80% reduction in CFU/mL and the conclusion that PDT can be an effective supplement to traditional antibiotic treatment.(5)

In the randomized trial of Asnaashari 2016's study, after the process, both calcium hydroxide and PDT, reduced *E. faecalis* bacterial load; however, PDT was more efficient than calcium hydroxide.(14,30)

5.5.4 New researches & features :

Recent researches have shown different applications of photodynamic therapy in Medicine and Dentistry. In previous clinical investigations, the combination of standard endodontic therapy followed by aPDT was effective against microorganisms. Different study designs, photosensitizers, and PDT regimens have been used to investigate the efficacy of PDT in endodontic therapy (with different light sources and dosimetry).(19,31)

New PS compounds such as curcumin and others have been tested in the hopes of improving PDT's effect on *E. faecalis* root infection.(6,24)

Antimicrobial activity and derivatives of chitosan, a natural polysaccharide, have been observed against fungus, bacteria, and viruses. PSs in conjunction with chitosan could boost PDT's antibiofilm efficacy even more. New PS are emerging in PDT treatments, such as rose Bengal, methyl blue alone, or erythrosine + chitosan. The antimicrobial activity of PDT looks greater but specific experiments need to be conducted.(24)

Endodontic treatment aims for a considerable reduction in microorganisms in the root canal system. Before obturation, Garcez *et al.* (2008) (31) suggested a second PDT session to achieve even more considerable microorganism decrease.

The use of more efficient methods for delivering the photosensitizer into tubules and branches of the root canal system such as ultrasonic devices or even the EndoVac® system could increase the efficacy of the PDT.(13)

According to Al Amhad's research(11), PDT with the combination of TB and VIS+wIRA (radiator with a water-filtered spectrum) is an efficient approach for killing bacteria during the early oral bacterial colonization. Taking into account the therapeutic effects of wIRA on human tissue, this approach could be useful in the treatment of peri-implantitis and periodontitis, albeit further research is needed.(13)

The single-appointment endodontic treatment of teeth with apical periodontitis versus a two-visit treatment is an area of endodontics that has been largely debated. Introducing treatment protocols that can predictably disinfect the root canals in one visit can resolve this controversy.(15,21)

Despite technological and scientific advancements in endodontics, microbiological variables cause many instances to fail. The development of new technologies to remove these persistent bacteria, has been a driving force for many researchers in recent years. PDT is a minimally invasive procedure that has been shown to be effective in removing bacteria, that are still viable, in the root canal system, as an adjuvant to traditional root canal treatment.

6. Conclusion

Although there is limited data and sometimes contradictory data on the use of antimicrobial PDT in root canal treatment, preclinical data suggests that this treatment option is a promising adjunctive supplement after conventional chemo-mechanical debridement for further reduction in persistent bacteria.

So there are two things that stand out in this review :

- Only certain specific photosensitizers, Toluidine Blue and Methylen Blue showned good results.
- Conventional irrigation with NaOCl, must be used with Photodynamic therapy in order to achieve good results on *Enterococcus faecalis*.

More, *in vivo,* clinical trials are needed to reach more valid results about the use of PDT in endodontics, as well as to define the ideal parameters for PS concentration, design of alternative PS formulations, energy dosage, and optimal time of irradiation.

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