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Photodynamic therapy, a new trend in endodontics for the removal of *Enterococcus faecalis*

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ABSTRACT

Disinfection is achieved through procedures such as instrumentation, irrigation, and intra-canal medication; however, these are not enough since several studies have reported *E. faecalis* as one of the most prevalent persistent microorganisms in root canal treatments that have not achieved healing of the periapical tissues. Efforts have been made to improve disinfection protocols by including different technological tools, as in the case of photodynamic therapy, which uses a light source and photosensitizing substances that favor the process of elimination of the remaining bacteria within the root canal system. The present review of scientific literature delves into the clinical importance of photodynamic therapy and its effect on the disinfection and inhibition of *E. faecalis* within the root canal system, which has become a key element for the success of endodontic treatment.

Keywords: *Enterococcus faecalis*, photodynamic therapy, photosensitizers.

INTRODUCTION

Endodontic treatment is based on the mechanic removal of necrotic pulp tissue and the chemical disinfection of the root canal system (1). Microorganisms and their metabolic by-products are etiological agents of the most frequent endodontic pathologies. Therefore, eliminating or reducing microorganisms within the root canal system should be one of the main objectives for successful treatment (2).

To meet the antimicrobial challenge, solutions are used as irrigants that, during the endodontic procedure, act through continuous contact with target microorganisms. However, these solutions fail to adequately penetrate the dentinal

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tubules, so there is no ideal irrigant solution, as none has all the requirements, including biocompatibility with host tissues, tissue solvent property, antimicrobial effect, and cost (3). On the other hand, penetration of microorganisms into the surrounding dentin occurs through the dentinal tubules, and contamination can reach a depth of approximately 1000 µm. Despite the variety of microorganisms, the dominant species are anaerobes whose microbial load is between 70% and 100% (4).

Enterococcus faecalis is a facultative gram-positive anaerobic microorganism, commonly isolated in primary and secondary endodontic infections. Among pathogenic factors, the most important is the ability to form biofilms, which enhances the resistance of bacteria to antimicrobial agents. Apart from that, the ability to penetrate deep into the dentinal tubules by adhering to the dentinal collagen prevents the antibiotic substances used from making contact (5, 6). Since it is a microorganism frequently found in persistent endodontic infections, the removal of *E. faecalis* from the root canal system is of utmost clinical importance (7).

To obtain better results, efforts have been made to develop novel techniques and devices that boost the disinfection process of the microorganisms present inside the root canal system, such as the use of sonic and ultrasonic tips and, in recent years, light sources inside the canal, as in the case of photodynamic therapy (PDT) (8).

PDT is a disinfection method with a powerful antibacterial action, which is applied against periodontal and endodontic infections and other oral pathologies. This procedure is based on a triad consisting of a non-toxic molecule known as photosensitizer, a light source (lasers, fluorescent lamps or LEDs) and molecular oxygen, where the photosensitizer transfers the energy received to the molecular oxygen and converts it into reactive species, thus causing the death of microorganisms by affecting their membranes, proteins, and nucleic acids.

PDT is mainly used in root canal treatment for root canal disinfection, which is a key procedure in determining its success, thus producing multiple benefits, including high efficiency in reducing bacterial load, reduction of postoperative pain and decrease in the size of periapical lesions. Apart from that, it can increase the efficiency of chemical-mechanical root canal preparation in complex anatomies such as C-shaped canals and in root canal retreatment (9, 10).

The purpose of this review is to investigate, describe and analyze the effectiveness of PDT against *E. faecalis*, as well as its possible impact on the generation of new root canal disinfection protocols.

ENTEROCOCCUS FAECALIS

Bacteria are the main protagonists in the pathogenesis and progression of pulpal and periapical diseases. Therefore, the main objective of a root canal treatment should be to remove microorganisms within the root canal system. *E. faecalis* is the most common strain isolated from teeth with failed root canal treatment and persistent infection (11).

The ability of *E. faecalis* to cause root canal infections has been related to the possession of several characteristics that give it virulence factors. The ability to form biofilms is a dominant characteristic of this bacterium, which extends to resistance to conventional intracanal irrigants (5).

With the advent of proteomic studies, it is revealed that biofilm formation in *E. faecalis* can be managed by a cell-to-cell communication mechanism through signaling molecules called quorum sensing phenomena (5).

The role of the FsrB quorum sensing system as a regulator of pathogenicity, host tissue degradation, and biofilm formation are part of the virulence characteristics of *E. faecalis*. Many characteristics of bacterial biofilms contribute to their increased antimicrobial resistance relative to planktonic cells, including decreased antibiotic penetration, antibiotic sequestration, and the presence of persister cells. Moreover, genetic elements involved in biofilm-associated antimicrobial resistance for *E. faecalis* have been determined. These elements include operon genes encoding two glycosyltransferases (GTF), enterococcal polysaccharide antigen (epa), epaOX and epaI, gelE encoding gelatinase and the fsr quorum sensing system. They also demonstrated that GTFs play additional roles in *E. faecalis*, including cell shape determination, maintaining cell envelope integrity and polysaccharide composition. In addition, an epaOX deletion of *E. faecalis* results in the most notable phenotypic differences in biochemical composition and biofilm architecture (5).

At the same time, it has been shown that wild-type biofilms exhibit a similar architectural arrangement after exposure to daptomycin, a cell membrane-active antibiotic. A connection between biofilm architecture, cell envelope stress and the epa operon is suggested.

Furthermore, with the basic information obtained from FsrB through bioinformatics analysis, it has been shown to be a valid and stable protein with acceptable quality that can be considered as a protein encoded by the target gene for photodynamic disinfection (5).

PHOTODYNAMIC THERAPY

Background

In the 1980s, the foundations of modern phototherapy were laid by Danish scientist Niels Finsen, who worked extensively with light sources ranging from small active rays to ultraviolet radiation. His research enabled other scientists to subsequently use these light sources as a therapeutic modality against Lupus vulgaris and smallpox (12).

In 1990, in a study by German medical student Oscar Raab and Professor Hermann von Tappeiner aimed at finding new drugs against malaria. It was discovered that paramecia incubated with acridine orange (AO) dye died faster right after a thunderstorm. These results were similar to those when AO-treated paramecia were exposed to sunlight from an adjacent window compared to incubation in a darkened room. Therefore, von Tappeiner postulated that light plays a role in the acceleration of the chemical-biological reaction. This phenomenon was called "photodynamics" and its theory was that oxygen was required for the photosensitization process to occur (12).

In 1907, von Tappeiner published a book summarizing the results of his clinical experiments, in collaboration with German dermatologist Albert Jesionek, using the dye xanthene eosin together with illumination to treat basal cell carcinoma, condyloma acuminatum on the female genitalia and lupus vulgaris, with favorable results. This was the first real clinical use of PDT to treat a disease. Subsequently, following the boom in the field of biochemistry and porphyrin compounds, PDT revolutionized. In 1913, the Austrian physician Fredrich Meyer-Betz experimented on himself with an IV injection of 200 mg of hematoporphyrin. Therefore, after exposure to light, he noticed the development of extreme pain and swelling, which was confined to the areas exposed to light. These areas remained photosensitive for several months after the incident. It was thus concluded that hematoporphyrin was a photosensitizing agent and that it also targeted cancer cells more effectively and provided better overall results (12).

In the 1960s, Dougherty et al., following their pioneering studies in both basic science and clinical applications, gained further recognition after conducting clinical trials of PDT on a worldwide scale. They also established the International Photodynamic Association in 1986 and expanded it to several countries around the world. As a result, in 1999, the World Food and Drug Administration approved PDT to treat mainly oncological and dermatological diseases, such as precancerous skin lesions of the face or scalp, cancer and other diseases. It has also been proposed to be useful in almost all specialties of medicine, and potential applications continue to expand every day (12).

In dentistry, PDT is a new disinfection alternative with a powerful antibacterial action, which has a variety of applications, mainly in periodontics and endodontics. In the latter, it is mainly used for root canal disinfection, which is the key point in determining a successful outcome of a root canal treatment. This method has gained popularity in contemporary dentistry due to its various benefits, including high efficiency in reducing bacterial load, reducing postoperative pain and decreasing the size of periapical lesions (10).

Mechanism of action

PDT is a treatment that consists of two stages involving, first, the application and retention of a photosensitizer in the target tissues and, second, activation by exposure to visible light that has an appropriate wavelength and is emitted through a device. This light should be aimed directly at the target. After irradiation, the photosensitizer undergoes a transition from a low energy singlet, ground state, to a higher energy triplet state (9).

There are two mechanisms by which, in the presence of a substrate such as oxygen, sensitizer activation to the triplet state can enter into chemical reactions with biomolecules. Type I mechanisms act through the formation of free radicals by electron or hydrogen transfer. These reactive substances, after interaction with oxygen, can produce highly reactive oxygenated substances, such as peroxide or superoxide anions, which attack target microorganisms. Type I reactions can cause damage to target cell components directly by the action of free radicals (9).

In type II mechanisms, an electronically stimulated and highly oxidizing oxygen state is released, known as singlet oxygen, which would be the main cause of microbial cell destruction. However, it is not easy to distinguish between both PDT reaction mechanisms. A contribution of type I and type II processes indicates that the mechanism of target cell damage will depend on the oxygen tension as well as the concentration of the photosensitizer (9).

Photosensitizers

Photosensitizers are key elements in PDT, which transfer the energy received to molecular oxygen and convert it into reactive species, causing the death of microorganisms by affecting their membranes, proteins and nucleic acids (10).

Photosensitizers are divided into three subgroups, first, second and third generation. Water-soluble porphyrins called hematoporphyrins are characterized as first-generation photosensitizers. And methylene blue, toluidine blue, photosensitizers®, Foscan®, and 5′-aminolevulinic acid (ALA) are examples of second-generation photosensitizers. The latter have higher singlet oxygen quantum yield, chemical purity and selectivity than first generation photosensitizers. Third-generation drugs have recently been investigated with the main objective of reducing damage to healthy cells and increasing bioavailability. These substances generally consist of drug delivery systems, genetically engineered technologies or combinations of monoclonal antibody receptors (13).

There are natural photosensitizers. There are many natural compounds extracted from plants and other organisms that act as photosensitizers and absorb white or UV-A light. There are still many natural photosensitizer compounds to be discovered, so variety cannot be restricted. However, so far, they include coumarins, furanocoumarins, benzofurans, anthraquinones and flavin derivatives. Hypericin and curcumin are two natural compounds that have been extensively studied (14).

An ideal photosensitizer should:

- Have strong absorption in the peak of the red to near-infrared spectral region (between 650 nm and 800 nm).
- Possess substantial triplet quantum yield leading to a good yield of reactive oxygen species after irradiation.
- Have high tissue selectivity.
- Not exhibit obscure toxicity.
- Have ideal solubility to maintain lipophilic ability to cross the phospholipid membrane and avoid self-aggregation.
- Exhibit high stability under storage conditions.
- Kill microorganisms sufficiently without damaging eukaryotic host cells.
- Show optimal absorption, distribution, metabolism, and excretion (ADME).
- Have a small size to allow penetration of the microbial membrane.
- Have low manufacturing costs (13).

Light sources

Light sources used for root canal PDT include helium-neon and argon lasers, neon lasers, metal vapor lasers, and diode lasers. Due to the disadvantages of high-power lasers, such as tooth surface change and thermal damage to periodontal tissues, as well as the lack of antimicrobial activity, low-level lasers are used for the activation of photosensitizing molecules. The application of low-level lasers in endodontics, such as diode lasers, improves periapical tissue healing and reduces post-treatment discomfort and complications (15).

Nowadays, among low-level lasers, the diode laser is preferred due to its low cost and portability. The light emitting diode (LED) has been one of the most favorable disinfection methods recently. It emits narrow-spectrum uncollimated light across the ultraviolet to near-infrared wavelength ranges. Advantages such as low cost, ease of use and lower power consumption compared to laser made it a desirable alternative device. In addition, it is portable, flexible, lightweight and, most importantly, it does not increase tissue temperature, thus avoiding additional tissue damage. LED has been applied in many clinical fields, such as pain relief, skin rejuvenation, wound healing and viral diseases. Recently, it was suggested that LEDs can be used instead of diode lasers as the light source for PDT (15).

Protocol of use

The root canal is filled with 0.5 mL of photosensitizer, with a pre-irradiation period of 5 min. Subsequently, the diode laser fiber is inserted into the root canal with a wavelength of 635 nm, an output power of 220 mW and a power density of 3.05 W/cm^2 or LED fiber with a wavelength of 635 nm and a power density of 2000-4000 mW/cm^2, emitting light in continuous wavelength (CW). Irradiation is performed for 30-90 seconds. Fiber moves into the root canal in apico-cervical helical movements. Finally, the root canal is irrigated once again with 5 mL of sterile saline to remove the photosensitizer (16). When natural photosensitizers are used, the synergistic use of irrigants, such as ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl), is recommended (6, 17).

DISCUSSION

PDT is a disinfection method that has demonstrated antimicrobial capacity against *E. faecalis*, which has become clinically relevant as a persistent organism in primary and secondary infections of root canals (7).

To improve the antimicrobial effectiveness of PDT, the use of photosensitizers is recommended. Afkhami et al. (15) compared the use of photosensitizing substances, such as methylene blue and toluidine blue, demonstrating greater efficacy of the latter due to its amphiphilic characteristics, which generate the elimination of bacteria present in the root canal, which is also due to its high binding capacity to *E. Faecalis*.

Mozayeni et al. (17) compared the efficacy of toluidine blue, methylene blue and a natural compound, such as curcumin, in PDT against *E. Faecalis*, all these compounds used synergistically with NaOCl; and it was concluded that the use of toluidine blue with NaOCl and curcumin with NaOCl are superior when eliminating *E. Faecalis* versus the methylene blue group with NaOCl. Cusicanqui et al. (6) also evaluated curcumin as a photosensitizer, and obtained statistically favorable results when it was combined with EDTA or hydroxyethylidene bisphosphonate (HEBP) against an *E. faecalis* biofilm, as chelators appeared to contribute to the reduction of the vitality of the inner layers of the biofilm.

On the use of other natural compounds, Pourhajibagher et al. (11) demonstrated that the use of Chlorella in PDT against *E. faecalis* was very effective. This reinforces the use of natural photosensitizers that could avoid adverse reactions from any synthetic or mineral compound.

Regarding the effectiveness of the different light sources, currently the most used are LED light and diode laser. Afkhami et al. (15) demonstrated that there is no significant difference between them; however, the use of photosensitizers is necessary to increase their antibacterial capacity.

On the other hand, it is important to mention that PDT is mainly used as a complement to irrigation protocols and in the last disinfection phase of endodontic treatment. De Vasconcelos Neves et al. (16) compared PDT with diode laser and methylene blue, NaOCl plus PDT, PUI with NaOCl plus PDT and XP Endo Finisher with NaOCl plus PDT, and noticed that XP Endo Finisher plus PDT protocol resulted in the highest percentage of inhibition (100%), probably due to the ability to generate infiltration of the irrigant and photosensitizer in areas of difficult access within the root canal system compared to PUI and conventional irrigation. Therefore, developing new final disinfection protocols will benefit the elimination of pathogenic microorganisms within the root canal, generating higher treatment success rates (16).

Mustafa et al. (1) demonstrated in an *in vitro* study that PDT was superior in the removal of *E. faecalis* in C-canals versus hand instrumentation alone due to its ability to enter the complex anatomy of these canals. On the other hand, Maciel Martins et al. (2) demonstrated that, when saline plus PDT and EDTA plus PDT were used, superior results were obtained in the elimination of *E. faecalis*, being 97.6% and 89.8%, respectively, compared to only using saline (68.2%) and only EDTA (76.4%). The same conclusion was obtained by Sarda et al. (3), who demonstrated the superiority of PDT plus NaOCl in the 98% elimination of *E. faecalis*, compared to PDT alone (73%) or 3% sodium hypochlorite alone (76%).

CONCLUSIONS

This literature review on the effectiveness of PDT against E. faecalis concludes that PDT increases the disinfection and inhibition effect when the traditional disinfection protocol is carried out in an adjuvant manner. The use of photosensitizers increases the effectiveness of PDT, with toluidine blue obtaining the best results. On the other hand, in relation to the light source used, such as LED and diode laser, there are no significant differences. The disinfection protocol proposed by De Vasconcelos Neves et. al (16), which used XP Endo Finisher followed by PDT, resulted in the percentage of 100% inhibition *in vitro*. Therefore, complementary clinical studies should be performed in that sense. It is advisable for future PDT research to conduct clinical studies with long-term controls to revalidate the results obtained today.

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