Photodynamic therapy in Endodontics: Use of a supporting strategy to deal with endodontic infection

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ABSTRACT

Endodontic treatment is of paramount importance to abolish infection in teeth with pulp necrosis. The success of this type of treatment depends on efficient elimination of infection in the root canal system (RCS) and correct sealing carried out with root canal filling materials. Due to the anatomical complexity of the RCS, certain areas may be inaccessible to biomechanical preparation (BMP), therefore, the use of intracanal medication enhances the reduction in microorganisms (MO) and their toxic products inside the RCS. MO can still survive even with the scientific and technical advancement of endodontic therapy, being primarily responsible for maintaining endodontic infection. Thus, new treatments should be investigated. Alternative treatments have emerged in the health field with the advent of laser and LED devices, such as photodynamic therapy (PDT), which is a set of physical, chemical and biological procedures that occur after the administration of a photosensitizing agent (PS) activated by visible light of a specific wavelength (laser or LED) to destroy the target cell or assist infection combat. In Endodontics, based on *in vitro* and *in vivo* studies, the use of PDT has proved to act as an adjunct, enhancing the disinfection of the RCS, besides being easy to be applied and not promoting microbial resistance. The aim of this review is to present the current status of photodynamic therapy in Endodontics.

Keywords: Endodontics. Endodontic infection. Photodynamic therapy.

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Introduction

Endodontic treatment is of paramount importance to abolish infection present in the root canal system (RCS).[1] Biomechanical preparation (BMP) with irrigating solutions aims at cleaning and shaping the RCS reducing the number of microorganisms (MO).[2]

Due to the complex anatomical variation of the RCS, certain areas may be inaccessible to BMP,[3] therefore, using calcium hydroxide as intracanal medication increases the opportunity of reducing the MO and their toxic products in the RCS.[4,5]

In recent years, important advances in technology and science occurred with regard to the development of new materials and techniques for endodontic therapy.[2,6] However, even with this progress, there are MO that survive endodontic treatment. These MO are responsible for the persistence of resistant endodontic infections, which contributes to failures[6,7]. Therefore, new therapeutic strategies used during endodontic infection treatment must be constantly investigated.

New alternative treatments in the health field emerged with the advent of laser and LED devices.[8,9] Photodynamic therapy (PDT) is among such therapeutic alternatives. It uses a photosensitizing agent (PS) that is activated by laser or LED and which aims at destroying the target cell or assist infection combat.[8,9,10]

In Endodontics, PDT emerges as an adjunct and innovative therapeutic modality that enhances disinfection of the RCS with a view to suppressing the MO resistant to endodontic treatment.[11,12] PDT is easy to apply, does not promote microbial resistance and can be used after the BMP and before the canal filling, with or without intracanal medication.[11-14]

The aim of this literature review is to present and discuss the use of photodynamic therapy in Endodontics.

Endodontic treatment

The aim of endodontic treatment in teeth with pulp necrosis consists in combating endodontic infection by eliminating bacteria as well as inactivating their toxic products, such as lipopolysaccharide (LPS)[5-16] and the apical biofilm.[6,7,17] When well executed, infection combat associated with adequate root canal filling can provide conditions for tissue repair.[4-7,15,16,17]

However, the complexity of the internal dental anatomy hinders the disinfection of the RCS, requiring the use of intracanal dressing, such as calcium hydroxide, to help combating endodontic infection.[1,4,5,18]

In primary endodontic infections, different bacterial species can propagate in the entire RCS, leading to a polymicrobial infection with a predominance of Gram-negative anaerobic bacteria.[1,6,19] However, in endodontic failures or resistant infections, there are less microbial species and characteristics that differ from those of primary infections. In these cases, endodontic infections have predominance of Gram-positive bacteria.[19,20,21]

The RCS have to be cleaned to eliminate microorganisms and the smear layer in order to allow adequate obturation and post-treatment tissue repair.[4,6,7] The use of sodium hypochlorite and EDTA effectively removes any organic and inorganic components allied to the use of calcium hydroxide as intracanal dressing, thus, promoting disinfection.[5,15] In recent years, advances in endodontic treatment such as nickel-titanium and self-adjusting files (SAF),[2,17] surgical microscope and ultrasonics have transformed the techniques of RCS treatment. However, the anatomical variation of SCR[3] and resistant endodontic infections sustain failures.[21]

Thus, new strategies of endodontic treatment are constantly investigated in order to broaden the spectrum of activity against MO present in endodontic infection.

Using light as a therapeutic agent

In the past, sunlight was widely used in countries such as Egypt, India and China for the treatment of skin diseases (psoriasis, vitiligo), however, empirically.[10] After the technical-scientific advancement of optical techniques, it is now possible to use the light as a therapeutic agent in the health field.[8,9,10]

In the 60s, with the advent of laser and LED devices, new treatment options emerged due to the beneficial therapeutic properties of these devices.[8,9,10,22,23] Laser presents peculiar characteristics such as: monochromaticity, little divergence, intense energy and ultra-short pulses.[24] LED is an acronym for Light Emitting Diode, a low thermal component with a spontaneous radiation mechanism that requires little energy to generate light.[25] Photodynamic therapy is among the applications of laser and LED for therapeutic purposes.[8-12]

Low intensity red lasers are extensively used in PDT because they absorb phenothiazine-based PS and are absorbed by biological tissues.[13,14] LED has been used in optical techniques as an alternative to the use of laser because it provides spontaneous radiation and uses little energy to generate light, with results as good as those of laser.[26]

Photodynamic therapy

Photodynamic therapy was discovered in 1900 by Oscar Raab, who employed a small concentration of acridine dye on protozoa. He observed that after light exposure, a lethal reaction occurred to protozoa, whereas in darkness nothing happened.[10]

PDT is characterized by physical, chemical and biological processes that occur when the light source absorbs PS and destroys or leads to lysis of the target cell (damaged tissues or MO present) by oxidation. PDT has been currently established as a treatment modality for cancer and non-oncological diseases, in addition to acting as an adjunct to other therapies already recognized.[8-10,27]

Studies carried out in the 90s showed that PDT can also be used to combat infections due to its good antimicrobial action. Additionally, PDT has proved to be more advantageous than antibiotics as it does not promote bacterial resistance. This type of treatment is also known as antimicrobial photodynamic therapy (aPDT).[28,29,30]

Photodynamic therapy mechanism of action

When PDT is performed, at the moment of its application and at a molecular level, an energy exchange occurs among the molecules of the photosensitizing agent employed. Such molecules are activated by visible light source (laser or LED) with specific wavelength for the respective PS, which induces destructive reaction towards the target cell in the presence of oxygen.[10]

In PDT, the molecular reactions can be classified into type I and type II. Type I increases the hydroxyl radicals which react with biomolecules resulting in the formation of hydrogen peroxide. The cytotoxic effects cause hydrogen loss in unsaturated molecules, such as phospholipids from cytoplasm membrane, thus, changing membrane permeability and integrity. As for type II reaction, an energy transfer between photosensitizing agent and oxygen occurs, producing a highly reactive singlet oxygen, which temporarily reacts with cellular components (cell wall, nucleic acid, peptides and molecules involved with cell wall structure maintenance). Singlet oxygen oxidizes in a quick, safe and efficient way, promoting local specific necrosis, eliminating the target cells, only.[10,31,32]

PDT mechanism is also effective for inactivation of fungi, as it changes the integrity of cell wall and membrane, allowing photosensitization that determine the changes on cellular organelles and subsequent cell death.[33,34]

Photosensitizing agent (PS)

In order to generate the desired effect of the PDT, the PS must have selectivity and biological stability, good photochemical action and low toxicity to healthy tissues.[31,32] The PS must have a resonant absorption band with spectrum of action of light on a particular wavelength of maximum absorption. The effect of PDT in the tissue depends on the level of PS.[10,31,32] The closer the wavelength of light used in the PS, the more efficient the PDT, provided that this PS has low toxicity and absorption bands that do not cause any injury to adjacent tissues and biological target.[10] The action of PS on bacteria is directly related to the load. PS with positive or neutral charge interacts in a dynamic way, inactivating the layer of peptidoglycan and lipoteichoic acid in the outer membrane of Gram-positive and allows PS diffusion.[10,28,31] In Gram-negative bacteria, PS interacts with their outer membrane, acting as a functional and physical barrier among the cells and the biological environment.[35,36]

The PS most commonly reported in the literature are: methylene blue, toluidine blue, rose bengal, malachite green, erythrosine, rhodamine, porphyrins, and phthalocyanines.[31,32]

In Endodontics, it is possible to employ the PDT with PS of the phenothiazine class: methylene blue (MB) and toluidine blue (TB),[14,37] both activated by red laser or LED. However, in addition to TB and MB, new PS have been investigated in order to assist and improve microbial reduction of the root canal system.[38]

MB is a cationic photosensitizing agent used as a dye to indicate the presence of bacteria. It is soluble in water and alcohol, offering electrocatalytic features in several enzymatic reactions.[39] MB has also good light absorption, reaching 660 nm bands (red light) and demonstrating the ability in generating reactive oxygen species.[10,32]

Fimple et al[13] showed that improving AM concentration and density of light energy (J/cm²) increases bacterial destruction because the major targets of such PS seem to be the components of DNA and the cell membrane of which permeability is intensified.

Pre-irradiation period

The pre-irradiation period comprises the time between the application of PS and the beginning of light use (LED or laser). This pre-irradiation time is of paramount importance for absorption by PS, even before irradiation starts.[10,31,32] In Endodontics, the pre-irradiation period may range from two to five minutes, and it is the time when the PS reaches and crosses the bacteria cell membrane.[12,14] It is noteworthy that the PS should be applied locally or near the target cell to prevent the formation of toxic species in undesired regions.[13,14]

Photodynamic therapy in Dentistry

PDT appears as an adjunct and alternative treatment used along with antimicrobial agents since it does not promote microbial resistance[40]. This fact highlights PDT as an attractive treatment strategy in Dentistry, which aims at eliminating or reducing the microorganisms and oral biofilms responsible for oral diseases[40,41]. The efficacy of PDT in oral bacteria was demonstrated *in vitro* for the first time in 1992, by Wilson et al,[28] who found reduction in *Porphyromonas gingivalis, Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* by using the He-Ne laser with TB and MB as photosensitizing agents. In the same year, the same researchers[29] successfully proved PDT action against *in vitro* biofilms formed by *Streptococcus sanguis, Porphyromonas gingivalis, Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* which were irradiated with He-Ne laser with TB and MB as photosensitizing agents.

Several areas of Dentistry have successfully employed PDT. In Periodontology, for instance, PDT is performed after scaling and root planning in periodontal pockets.[42] In Cosmetic Dentistry, PDT is used for decontamination of carious dentine;[43] in Prosthodontics, PDT is performed for decontamination of stomatitis caused by full or partial prosthesis;[44] in Implantology, for the treatment of peri-implantitis;[45] in Pathology, PDT is used in various oral lesions such as herpes labialis;[46] and in Endodontics, it is used to assist decontamination of the RCS after biomechanical preparation.[47]

Photodynamic therapy in Endodontics

In Endodontics, PDT is indicated for treatment of teeth with necrotic pulp as well as for re-treatments, with a view to assisting and enhancing disinfection of the RCS after BMP, as an attempt to act in endodontic infections combating Gram-negative and Gram-positive bacteria in addition to biofilms.[11-14,47,48] The remaining biofilm can hinder the action of intracanal dressings, in addition to enabling microbial resistance, therefore, its inactivation is extremely important.[11,48]

Different PDT susceptibility of microorganisms have been reported, with a reduction of 97% in *Enterococcus faecalis*.[37,49] However, statistically different results were found by Souza et al[50] who did not obtain significant microbial reduction in *Enterococcus faecalis*. Fungi such as *Candida albicans*, can also be found in endodontic infections, and PDT may be indicated as adjunct treatment in these cases.[49]

The use of light (laser or LED) alone may not be effective against microorganisms in endodontic infection. However, if it were used with the photosensitizing agent, it would allow the release of singlet oxygen, thus, achieving the aimed results. With regard to the advantages of PDT, it can be mentioned that this therapeutic modality generates no thermal effects, and there is no harm or death of cells of healthy tissues adjacent to the RCS.[49]

The most commonly used photosensitizing agents in endodontic photodynamic therapy are synthetic and phenothiazines-based, such as MB and TB[37,51] that, at low concentrations, cause the microorganisms to die without being cytotoxic to the surrounding tissues.[52]

Nevertheless, when used in the root dentin, the photosensitizing agents may stain the dental structures. However, such hypothesis may be discarded provided that low concentrations be used, although antimicrobial activity may decrease in view of PS low concentrations.[53] In spite of that, studies have been conducted to test new photosensitizing agents that could be used at higher concentrations without the risk of staining tooth structures, but also providing favorable results with regard to bacteria control.[54]

Researches have currently been carried out in order to obtain better results regarding irradiation time, power unit, core diameter of optic fiber for intracanal use, different types of photosensitizing agents, as well as different concentrations of these agents. Bouillaguet et al[55] have demonstrated that light (laser or LED) was effective in activating photosensitizing agents and that the blue light was as effective as the red one in the production of singlet oxygen. Moreover, they recommended the use of PDT as an auxiliary technique in Endodontics.

Pagonis et al[38] used *in vitro* nanoparticles of poly (L-lactic-co-glycolic acid) containing MB to potentiate the action of the photosensitizing agent in the production of a more reactive oxygen, allowing the killing of various species of microorganisms. They concluded that the synergism between MB and the nanoparticles promoted a significant reduction in colony-forming units and demonstrated the effectiveness of this technique as adjunct to endodontic treatment.

In an *in vitro* study, Nunes et al[56] showed that there is no significant difference in PDT whether or not optic fiber is used in the root canal space for the reduction of *Enterococcus faecalis* species. However, the authors recommend its use due to its ability in uniformly distributing light and reaching all areas of the main root canal, as well as across all the root canal system.

Rios et al[57] concluded that PDT with toluidine blue as photosensitizer agent can be used as adjunct antimicrobial procedure in conventional endodontic therapy. Similar results were found by Ng et al[14] who found a favorable result of PDT as adjunct in Endodontics. Both authors recommend further research to obtain better scientific basis prior to using the technique.

Silva et al[58] conducted a study using dogs' teeth with periapical lesions. They achieved tissue repair in groups that used PDT after biomechanical preparation and concluded that PDT may be a promising adjunct therapy in endodontic procedures.

Cheng et al[59] conducted *in vitro* studies and showed that the PDT or just the use of one of the laser systems (Nd: YAG, Er: YAG; ERCR: YSGG), when associated with endodontic treatment, reduced the number of *Enterococcus faecalis* bacterial colonies. The authors recommend the technique for clinical application.

Komine and Tsujimoto[60] showed that a small amount of MB was able to generate singlet oxygen and produce bactericidal effects against *Enterococcus faecalis in vitro*.

Researches reporting the possible influence of PDT on the types of irrigating solutions, on the presence or absence of intracanal medication or on the smear layer, have not been found in the literature.

The photodynamic therapy technique in Endodontics

The literature remains controversial with regard to application parameters, but it is noteworthy that, according to the scientific results found to date, PDT in Endodontics is gaining prominence.

In general, PDT is indicated in Endodontics for teeth with pulp necrosis with or without periapical lesion, treated in a single session or in multiple sessions. It can also be used in re-treatments.

After biomechanical preparation, the root canal must be effectively dried with cannulas and absorbent paper points.[11] Afterwards, the PDT is carried out, generally with MB or TB as photosensitizer agents, which can be commercially found. This photosensitizer agent is a liquid that must be inserted into the root canal and that should react between 2 and 5 minutes (pre-irradiation period).[1-14,37,38,47,52,53]

Once the pre-irradiation period is concluded, the light (LED or laser) is applied at a specific wavelength, according to the photosensitizing agent used. In the case of MB or TB, red light in a band ranging from 660nm to 685nm should be applied for approximately 3 to 4 minutes. The use of intracanal flexible optic fiber with diameter core compatible with the root canal size is also recommended. After that, the photosensitizing agent must be removed with irrigating or saline solution and the root canal must be aspirated with a cannula and dried with absorbent paper points.[11-14,37,38,47,52,53]

At this point, the clinician can opt for using an intracanal dressing and fill the root canals in another session, or go straight to root canal filling in one session. It should be noted that PDT does not substitute the intracanal medication.

Biosafety

As any other light-emitting device (laser or LED), the use of protective eyewear with specific wavelength is mandatory for both the clinician and the patient. This protection items are generally part of a kit that is obtained when the light sources are purchased. The optic fiber used in PDT must be sterilized or disinfected according to the manufacturer's instructions.[31,32]

Final considerations

PDT in Endodontics can be an important adjunct that helps reducing and controlling microorganisms and their toxic products that are present in endodontic infection, provided that an efficient photosensitizer agent is selected and appropriate parameters for the application of the laser or LED are attended. However, further studies are needed to improve the clinical application protocols.

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