



# Clinical Approach of High Technology Techniques for Control and Elimination of Endodontic Microbiota



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## Introduction

Microbial agents are considered as the main causative agent of human dental pulp (intraradicular) and periapical (extraradicular or periradicular) lesions.1 Recent culture-independent approaches have suggested that the microbiota in these infections is more complex than previously shown by culture-based methods.<sup>2,3</sup> Molecular biology analysis of the microbial communities in intraradicular and periradicular infection revealed that a mixed consortium of both gram-positive and gram-negative bacteria occurred in most cases and the composition of the bacterial communities varied from individual to individual.4-7 In addition to bacteria, fungi and most recently archaea, viruses have been occasionally found in intraradicular infections.8 Among the fungi afflicting human dental pulp, Candida albicans is the most common.9 Because viruses are unable to generate energy and fully depend on the viable host cells during replication, they cannot survive in a necrotic root canal.<sup>10</sup> Human cytomegalovirus and Epstein-Barr virus may be implicated in the pathogenesis of

#### Abstract

The main goal in endodontic treatment is to eradicate or at least reduce intraradicular microbial population to levels that are more compatible with periapical lesions healing process. Since endodontic infections are polymicrobial in nature, intraradicular survival of endodontic microbiota and their pathogenic properties are influenced by a combination of their virulence factors. The purpose of this article is to review the endodontic microbiota and their respective virulence attributes, as well as perform a literature review of the effects of disinfection procedures in the treatment of endodontic infections to gain best practices. Conventional technique for root canal preparation includes mechanical debridement and application of antimicrobial irrigants. Recently, laser irradiation has been used to enhance the results of root canal treatment through its thermal effect. To reduce thermal side effects, laser activated irrigation (LAI) and photon induced photoacoustic streaming (PIPS) were introduced. Antimicrobial photodynamic therapy (aPDT) by photochemical reaction uses light at a specific wavelength to activate a nontoxic photosensitizer (PS) in the presence of oxygen to produce cytotoxic products. Different PSs are used in dentistry including methylene blue (MB), toluidine blue O (TBO), indocyanine green (ICG) and curcumin. Among different options, ICG could be the best choice due to its peak absorption at wavelength of 808 nm, which coincides with the commercial diode laser devices. Also, this wavelength has more penetration depth compared to other wavelengths used in aPDT.

Keywords: Curcumin; Indocyanine green; Photodynamic therapy.

## intraradicular infections.11,12

Microorganisms colonizing intraradicular and periradicular locations can either be the commensals associated with a healthy oral cavity, or the pathogens associated with oral infections, such as periodontal disease and dental caries.13 Intraradicular and periradicular infections are polymicrobial, predominantly anaerobic infection with little microbial specificity.8,14 The severity of these infections may be related to the microbial community's composition topped off with loads and virulence, as the latter are other important factors with impact on disease management and prognosis.14 Biofilm formation, presence of bacterial modulins, synthesis of enzymes, interbacterial cell-cell coaggregation, nutrient effects, modulation of virulence factors and ability to evade host defenses as virulence factors of intraradicular and periradicular microorganisms, have been reported to contribute to endodontic pathogenesis (Table 1).15-20

An understanding of the various virulence mechanisms used by these endodontic pathogens is crucial for the de-

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velopment of novel treatment modalities.21

The final objective of endodontic treatment is an effort to obtain conditions to promote the cure and health of the root, as well as prevention or resolution of periapical disease, by the eradication of microorganisms and their sources of nutrient supply from the root canal system.<sup>22</sup> Accordingly, endodontic treatment including intra-canal procedures such as chemomechanical preparation (instrumentation with copious antimicrobial irrigants) are supplemented or not by a current interappointment intra-canal medication, followed by obturation and ended with perfect restoration.<sup>23,24</sup> The success rate of this procedure is critically dependent on the management of pulpal space infection, which results in reduction of bacterial numbers.<sup>25</sup> It has been shown that, conventional endodontic treatment can occasionally fail to promote total eradication of microorganisms from root canals, with consequent selection of the most resistant subpopulation of the microbiota and may lead to treatment failure.<sup>26</sup> The purpose of this review was to provide background information on the microbiota and virulence determinants of the intraradicular and periradicular infections, as well as to gain a better understanding of endodontic disinfection procedures.

## **Chemomechanical Debridement**

Mechanical debridement which includes removal of pulpal tissue from the root canal system, making adequate space for irrigation and medication, maintenance of integrity and location of the apical part of the root canal and formation of a suitable form to facilitate root canal filling, is considered a critical step in endodontic treatment. However, studies showed some limitations of this procedure, as a result of deep bacterial penetration to accessory canals, isthmus and dentinal tubules.<sup>27,28</sup> Also, some studies have shown that mechanical instrumentation alone leaves some parts of the root canal untouched.<sup>29</sup> To overcome these deficiencies, the application of antimicrobial irrigants and intra-canal medicaments is necessary. Suitable irrigants should have some characteristics such as broad antimicrobial spectrum, ability to avoid smear layer formation, ability to dissolve necrotic pulpal tissue and should be non-toxic to the periradicular tissue. Until now, no irrigant was found with these characteristics.<sup>30</sup>

## Sodium Hypochlorite

Sodium hypochlorite is one of the most widely used antimicrobial irrigants in endodontic treatment. It has the ability to cause necrosis, hemolysis and skin ulceration due to its cytotoxicity. It shows some bactericidal effects through oxidative action of the undissociated hypochlorous acid on the sulphydryl groups of bacterial enzymes. The inhibition of enzyme interferes with metabolic function of bacteria.<sup>31-33</sup>

#### Ethylenediaminetetraacetic Acid

Ethylenediaminetetraacetic acid (EDTA) is a water solu-

ble solid. It produces decalcified dentin by reacting with calcium ions. It shows little antibacterial activity. Its antibacterial effect is related to chelation of cations from the outer membrane of bacteria. EDTA also has the ability to remove the inorganic portion of the smear layer. It shows some cytotoxic effects.<sup>34,35</sup>

## **Citric Acid**

Citric acid also has limited antibacterial effects compared to its capacity to remove inorganic parts of smear layer.<sup>36</sup>

#### MTAD

MTAD, a common intra-canal irrigant, is a mixture of 3% doxycycline (a tetracycline), 4.25% citric acid, and 0.5% polysorbate 80 detergents that is used for the endodontic therapy. Its antibacterial effect is attributed largely to doxycycline that inhibits bacterial protein synthesis, by preventing the association of aminoacyl-tRNA with the bacterial ribosome.<sup>37</sup> Resistance to doxycycline is not uncommon among the bacteria isolated from root canals.<sup>38</sup> Different mechanisms of acquired resistance to doxycycline have been described with ribosomal protection, efflux of the antibiotic from the bacterial cell and enzymatic inactivation of the antibiotic as major modes of action.<sup>39</sup>

#### Nisin

Nisin, a polycyclic antibacterial peptide was used as a substitute for or in combination with doxycycline, in order to improve the bactericidal activity of MTAD.<sup>40</sup> Nisin, an antimicrobial peptide produced by *Lactococcus lactis*, has been extensively used as a preservative in dairy products. This peptide is composed of 34 amino acid residues, including such unusual amino acids as lanthionine (Lan), methyllanthionine (MeLan), didehydroalanine (Dha) and didehydroaminobutyric acid (Dhb).<sup>38</sup> Nisin inhibits the proliferation of most gram-positive bacteria and is heat-stable, odorless, colorless, tasteless, active at low pH and soluble in water and can be effective at levels nearing the parts-per-billion range.

## MTADN

MTADN (nisin combined with MTAD) had the best antibacterial activity in evaluating the effects of MTAD, MTAN (nisin in place of doxycycline in MTAD), and MTADN on *Enterococcus faecalis* during the exponential growth phase and stress states. Nisin improved the post-antibacterial effect of MTAD and has considerable potential for use as a modification of MTAD and it has potential as an intra-canalirrigant.<sup>40</sup>

#### NanoAg-MTA

To prevent endodontic-periodontal infection after repairing root perforations, an obturation material with robust antimicrobial activity such as silver nanoparticles (NanoAg) –MTA is highly desirable. It has been shown that in the NanoAg–MTA, Nano Ag effectively enhanced

Type of infections	Primary infection Chronic Apical Periodontitis/Acute Apical Abscess		Persistent/Secondary Infections Filling Stage/Treated Teeth	
Community	Mixed		Mixed, sometimes single	
No. taxa/case	10–20		1–5	
Uncultivated bacteria	40%-55%		42%-55%	
Most prevalent groups	Gram-negative/gram-positive	e anaerobes	Gram-positive/gram-negative facultative/anaerobes	
	Most prevalent species (% prevalence)	Virulence factors	Most prevalent species (% prevalence)	Virulence factors
	Filifactor alocis G +/ An (48)	Metal-dependent proteases Trypsin-like proteases Sialoglycoproteases	Enterococcus faecalis G +/ F (64-78)	Hemolysin/cytolysin Gelatinase (GelE) Hyaluronidase Enterococcal surface protein (Esp) <i>E. faecalis</i> regulator (Fsr) Aggregation substance (AS) Microbial surface component recognizing adhesive matrix molecule adhesin of collagen from Enterococci (MSCRAMM Ace) Serine protease (SprE) Sex pheromones Second collegen adhesin of <i>E. faecalis</i> (Scm) Lipoteichoic acid Capsule Cell wall polysaccharide Extracellular superoxide Heat-shock proteins
	Pseudoramibacter alactolyticus G +/An (52)	Chemotaxis	Streptococcus anginosus G +/ F (7-53)	Polysaccharide capsule Pyrogenic exotoxins Hyaluronidase Peptidoglycan Lipoteichoic acid Metabolites
	Parvimonas spp. G +/ An P. micra (formerly Pentostrentococcus	Peptidases Hyaluronidase Capsule H <sub>2</sub> S Capsule Surface proteins Collagenase	Streptococcus mitis G +/ F (7-46)	Choline-binding proteins (CBPs)
	micros) (22-78)		Streptococcus sanguinis G +/ F (7-28)	Hydrogen peroxide
			Filifactor alocis G +/ An (46-57)	Metal-dependent proteases Trypsin-like proteases Sialoglycoproteases
	Treponema spp. G -/ F T. maltophilum (89) T. socranskii (75) T. denticola (36-79) T. parvum (22-39)	Biofilm Motility Cystalysin Outer membrane dentilisin protease Lipoprotein complex Toxin antitoxin systems Transposases Trypsin-like protease Periplasmic flagella Major surface protein (Msp) Chymotrypsin-like protease complex Extracellular or membrane-associated proteolytic and hydrolytic enzymes Lipooligosaccharide Lipoprotein Phospholipases Metabolites (acetic and lactic acids, H <sub>z</sub> S) Heat-shock proteins	Actinomyces spp. G +/ An A. israelii (1-20) A. naeslundii (7-20) A. viscosus (7)	Fimbriae

## Table 1. Main Distinctive Features of the Mcrobiota Associated With Different Types of Endodontic Infections

Table 1. Continued				
	Tannerela forsythia G -/ An (30-64)	Trypsin-like protease SiaHI sialidases NanH sialidases S-layer Lipopolysaccharide Acid phosphatase Metabolites (acetic, propionic, butyric, isovaleric and phenylacetic acids) Apoptosis-inducing factor Heat-shock proteins	Parvimonas micra G +/ An (5-60)	Peptidases Hyaluronidase Capsule H <sub>2</sub> S
	Porphyromonas spp. G -/ An P. endodontalis (15-68) P. gingivalis (30)	Fimbriae Trypsin-like proteinase Cysteine protease Biofilm Lipopolysaccharide Capsule Outer membrane proteins Outer membrane vesicles Acid phosphatase Metabolites (butyric and propionic acids, indole, H <sub>2</sub> S) Lipoproteins Phospholipase DNase Hyaluronidase Fibrinolysin Chondroitin sulfatase Hemolysins Heat-shock proteins	Lactobacillus spp. G +/ F (7-35)	See primary infection
	Fusobacterium spp. G -/ An F. nucleatum (19-86) F. necrophorum (23) F. periodonticum (19)	Heat shock proteins Lipopolysaccharide Outer membrane proteins Capsule Metabolites (butyric and propionic acids, ammonia, indole) Serine protease Synergistic interaction with facultative organisms or other anaerobes	Olsenella spp. G +/ An O. profusa (7-100) O. uli (27-67)	See primary infection
	Prevotella spp. G -/ An P. denticola (77) P. oralis (40) P. baroniae (35) P. buccae (29) P. melaninogenica (26- 29) P. intermedia/nigrescens (22-69) P. tannera (18-35) P. ruminicola (29) P. oris (39-67)	Fimbria Lipopolysaccharide Metabolites (indole, H <sub>2</sub> S,ammonia, acetic and succinic acids) Proteinases Hemolysins Acid phosphatase Phospholipase Heat-shock proteins	Pseudoramibacter alactolyticus G +/ An (50)	Unknown
	Campylobacter spp. G -/ M C. rectus (80) C. gracilis (30)	Extracellular cytotoxin Lipopolysaccharide S-layer Arylsulfatase H <sub>2</sub> S Heat-shock proteins	Propionibacterium spp G +/ F P. propionicum (52) P. acnes (27-52) P. acidifaciens (14)	Biofilm formation Lipase
	Dialister spp. G -/ An D. pneumosintes (48-78) D. invisus (53)	Unknown	Pseudomonas aeruginosa G -/ A (48)	Cell–cell signalling Protease profiles Biofilm

Streptococcus spp. G +/ F S. milleri (31-50) S. constellatus (20-35) S. intermedius (35)	Hyaluronidase Deoxyribonuclease Hydrolytic enzymes	Dialister spp. G -/ An D. pneumosintes (46) D. invisus (30)	Unknown
Bacteroides spp. G -/ An Bacteroidetes clone X083 (36-60)	Lipopolysaccharide Capsular polysaccharide	Solobacterium moorei G +/ An (33-78)	Gelatin hydrolysis
Peptostreptococcus spp. G +/ An P. anaerobius (53-64)	Capsular polysaccharide Synergistic interaction with Facultative organisms or other anaerobes	Pyramidobacter piscolens G -/ An (60)	Unknown
Peptococcus spp. G +/ An (64)	Extracellular enzymes (DNase, RNase, coagulase, and hemolysins)	<i>Bacteroidetes</i> clone X083 (53)	See primary infection
<i>Lactobacillus spp.</i> G +/ An (65)	Adhesion proteins Survive in acidic conditions	Fusobacterium nucleatum (24-40)	See primary infection
Eggerthella lenta G +/ An (47)	Cardiac glycoside reductase (digoxin inactivation enzyme)	Peptostreptococcus stomatis (43)	See primary infection
Anaerococcus prevotii G +/ An (43)	Unknown	T. forsythia (12)	See primary infection
Gemella morbillorum G +/ F (30)	Same pathogenic mechanisms found in viridans streptococci	F. nucleatum (33)	See primary infection
Olsenella uli G +/ An (45)	Biofilm formation	Candida albicans (yeast) F (9)	Mannose-containing proteins Mannan Phospholipase Proteinases Hyaluronidase Acid phosphatase Chondroitin sulfatase Phospholipase Heat-shock proteins
Eikenella corrodens G -/ F (38)	Biofilm formation		
Lachnospiracea spp. G +/ An L. clone 55A-34 (57) L. clone MCE7_60 (43)	Unknown		
Eubacterium spp. G +/ An E. clone BP1-89 (43) E. brachy (56)	Unknown		
Phocaeicola spp. G -/ An (100)	Unknown		

Abbreviations: G -, gram negative; G +, gram positive; F, facultative anaerobic; An, anaerobic; A, aerobic; M, microaerophilic.

the antimicrobial activity of MTA, so the NanoAg–MTA demonstrated a potent antimicrobial activity against four important anaerobic periodontal/endodontic pathogens namely *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis,* and *Prevotella intermedia* in vivo.<sup>41</sup>

Table 1. Continued

# Chlorhexidine

Chlorhexidine is a hydrophobic, positively charged and lipophilic molecule which interacts with lipopolysaccharides on the cell membrane of bacteria. It is used in 2% gel or liquid concentration of 0.2%.<sup>42</sup> It has bactericidal effects against gram-negative and gram-positive microorganisms;

no study has reported the toxicity, teratogenic and cancerogenic effects of chlorhexidine (CHX) on organism. The inability of this irrigant to dissolve necrotic tissue remnants is considered as a disadvantage. Also, 2-min rinse of 2% CHX liquid can remove bacteria from superficial layers of dentinal tubules only up to 100  $\mu$ m.<sup>43,44</sup>

## Passive Ultrasonic Irrigation (PUI)

Ultrasonic irrigation promoted best results in some studies. It shows some advantages like efficiency, time-wise and economical. It improves the effects of intra-canal solution by acoustic streaming. The solution can spread to all parts of the canal even in non-instrumented parts where intact biofilm can be found.<sup>45</sup>

## Lasers

Recently, laser was introduced to endodontics to improve the results acquired by traditional techniques. Different wavelengths have been shown to be effective in reducing bacteria in the root canal system.<sup>46</sup> Among the different wavelengths used in dentistry, neodymium-doped yttrium aluminium garnet (Nd:YAG) laser is considered as the best wavelength for intra-canal disinfection due to its penetration depth close to bacterial penetration in dentinal tubules.<sup>47</sup> This penetration depth can be explained by the ability of enamel prisms and dentinal tubules acting as optical fiber, to propagate the laser to the dentinal periphery of the root.<sup>48</sup>

Diode laser with wavelength range of 800 to 1064 nm is another option for root canal disinfection acting through thermal effect. Diode lasers have gained importance in dentistry based on their affordability and small size.<sup>49</sup> Different studies reported that the application of diode laser for bacterial decontamination produced different success rates dependent on the amount of energy applied and dentin thickness.<sup>50,51</sup> After chemomechanical preparation, irradiation is performed as a final step for decontaminating the root canal system before obturation. An optical fiber with 200 µm diameter and 1mm shorter that the working length is placed inside the canal, starting irradiation with circular movement in the apical-coronal direction.<sup>52</sup> Some studies suggested applying this technique in canals filled with irrigants such as EDTA, citric acid or NaOCl reduces the thermal side effects.53 It should be considered that irradiation of the Nd:YAG and diode laser can create fusion and superficial re-solidification in the dentine with a clear glazed surface.54

We should always keep in mind that it is better to move the fiber inside the canal and not to stay or touch the root canal wall for a long time to avoid producing craters with microcracks. Although there is no statistic correlation between microcracks and a possibility of increase of root fractures in the future, it is better not to create them.<sup>55</sup> The Erbium family laser with two different wavelength including erbium-doped yttrium aluminium garnet (Er:YAG) and erbium, chromium doped yttrium scandium gallium garnet (Er-Cr:YSGG) laser was approved for dental procedure in 1997 by FDA.<sup>56</sup> Laser activated irrigation (LAI) is defined as activation of irrigation by lasers. It seems that the erbium family can be effective in this subject regarding their affinity for water.<sup>57</sup> Cavitation effect caused by this wavelength with formation and implosion of vapor bubble which results in very fast movement of intra-canal solution.<sup>58</sup> These techniques (such as LAI and PIPS) are able to diminish the undesirable thermal effects on the dentinal walls, by application of lower energies accompanied by chemical irrigants.<sup>59</sup> It has been proved that EDTA can be the best solution for the LAI, by enhancing the capability of smear layer removal.<sup>60</sup>

Another technique called the photon induced photoacoustic streaming (PIPS) is based on strong agitation of intra-canal irrigants. The advantages of this technique can be summarized to the reduction of thermal effect and stronger cleaning and bactericidal action, owing to streaming of irrigants by the photonic energy of the laser.<sup>61</sup> The difference between LAI and PIPS can be attributed to the placement of the tip in the coronal part of orifices in the PIPS technique compared to 5 mm above the apex in LAI which eliminates the tip introduction into the root canal.<sup>62</sup> The tip used for this technique has a special design (tapered) with 600 µm in diameter and 9 mm length. It has greater lateral emission rather than in the frontal part.<sup>60</sup> Studies are currently ongoing, for further confirmation of LAI and PIPS techniques.

## Photodynamic Therapy

Photodynamic therapy (PDT) uses light of a specific wavelength to activate a nontoxic photoactive dye (photosensitizer) in the presence of oxygen to produce cytotoxic products that give rise to the preferred therapeutic effects.<sup>63</sup> Various terms are used for PDT such as photoactivated chemotherapy (PACT), photodynamic disinfection (PDD), light-activated disinfection (LAD), and photoactivated disinfection (PAD) in different studies and literature.<sup>64</sup> The successful outcome of PDT depends on the optimal interaction among three elements - light, PS and oxygen.<sup>65</sup>

## Photosensitizers

A photosensitizer (PS) is a chemical compound, which when exposed to light at specific wavelength, reacts with oxygen to produce highly reactive oxygen species (ROS) which results in cell death.

The ideal characteristics of a PS include:

- Chemical purity non-toxic
- Ability to target the tissue, cost-effective, easily available
- Short interval between administration of the drug and peak accumulation in the tissue
- Short half-life
- Rapid elimination from normal tissue
- Activation at wavelength at which penetration into the target tissue is very good
- Ability to produce a large amount of

cytotoxic products.66

## **Methylene Blue**

3,7-bis(dimethylamino)-phenothiazin-5-ium chloride was first synthesized by a German chemist called Henrich Caro in 1876. Then, in 1885 Paul Ehrlich realized its advantages for selective coloring in histology.<sup>67,68</sup> Methylene blue (MB) is a hydrophilic PS with low molecular weight and positive charge. This PS can be used for both gram-positive and gram-negative oral bacteria. The solution penetrates through the porin channels in the outer membrane of gram-negative bacteria and mostly interacts with the anionic macromolecule lipopolysaccharide generating MB dimmers which take part in the photosensitization process. Its peak absorption is at 660 nm.<sup>69</sup>

## **Toluidine Blue O**

Toluidine blue O (TBO) is also a cationic blue coloring agent (3-amino-7-[dimethylamino]-2-methylphenothiazine) used for histological and intravital staining. It is also used for differential diagnosis between benign and malign precancerous leukoplakia. It has a peak abruption at 635 nm.<sup>70</sup> TBO has the ability to have an effect on both gram-positive and gram-negative bacteria due to its physical and chemical properties and hydrophilic characteristics making it pass the bacterial membrane, and it showed attraction to the mitochondria which has negative charge.<sup>71</sup>

The LPS of the outer cell envelope is the site which TBO binds to in gram-negative bacteria but the teichuronic acid residues of the outer wall is the site of binding in gram-positive bacteria.<sup>72</sup>

## **Indocyanine Green**

Indocyanine green (ICG), green coloring agent 1,7-Bis(1,1-dimethyl-3-[4-sulfobutyl]-1H-benz[e]indol-2-yl)heptamethinium-betain-Na, has recently been used as PS. It was proposed that the mechanism of this PS is somehow different from the other PS. The effect of ICG is mainly photothermal therapy (PTT) rather than photochemical.<sup>73</sup> It is an anionic PS. Although its absorption critically depends on the dissolving medium, bonds to plasma proteins and its concentration, its peak absorption is at 810 nm.<sup>74</sup> The two forms of ICG now in the market are one free from iodide (EmunDo, ARC) while the other contains normal quantities of iodide (perio green<sup>®</sup>, elexxion). There is no evidence regarding allergic or anaphylactic reactions following iodide application in dentistry.

#### Curcumin

Curcumin is isolated from *Curcuma longa* L. which is mainly used as a spice. It has some therapeutic effects on liver diseases, wounds, and inflamed joints, as well as for blood purification and microbial effects.<sup>75</sup> Curcumin has shown no toxic effects on a number of cell cultures and animal studies. It has a broad absorption peak in the 300-500 nm range (maximum 430 nm) and produces strong

phototoxic effects. Therefore, curcumin has the capability to be used as a PS.<sup>76,77</sup> This PS shows some restriction for being dissolved in water and requires oil or other synthetic material to make possible its solubility in water.

On the other hand, this PS has some advantages like easy handling, low cost and efficacy.<sup>78</sup> It is important to know that gram-negative bacteria are more susceptible to common PS along with the action of singlet oxygen compared to gram-positive bacteria. This difference is explained by the fact that can be adduced to the structure of gram-negative bacteria consists of lipid bilayers rolling as a physical barrier between the cell and its environment. On the other hand, the LPS in gram-negative bacteria limits the toxic effect of singlet oxygen preventing cell death.<sup>79</sup>

## Light

In photodynamic therapy, the light source which coincides with maximum absorption of the PS applied is required. The light source for PAD can be divided into three main categories namely: (1) broad spectrum lamps, (2) Light emitting diode lamps (LED) and 3) lasers.<sup>80</sup> Among the different sources, lasers show some advantages like monochromacy which allows the laser to best interact with PS due to matching precisely with its peak absorption, which reduces unnecessary tissue heating by bandwidths not effective in PDT reaction.<sup>81</sup> In dentistry, most of the PS are activated by the red light wave lengths between 630-700 nm. Presently, with the introduction of new PS such as ICG, infrared laser like 810 nm is also used.<sup>82</sup>

In PDT endodontic disinfection, blue light (400-500 nm) can be a suitable option for some reasons. First, it is available in nearly all dental offices for curing of dental resin composites. Second, it creates free radicals more efficiently compared to red light. Third, blue light sources produce a much larger spectral emission (400-500 nm) than lasers which are capable of activating PS leading to the production of free radicals. Compared to lasers as light source, LEDs are more cost effective and compact.<sup>83</sup>

#### Mechanism of Photodynamic Therapy

After the activation of PS with appropriate wavelength, electrons are transferred from a low to a higher level of energy which is called the triplet state. Then, the energy is transferred to a biomolecule or to oxygen which leads to the production of cytotoxic species. These products damage the cellular plasma membrane or the cell DNA. Both consequences lead to cell death.<sup>84</sup>

The transfer of electrons in activated PS can be done in two pathways including transfer to the neighboring molecule (type-1 reaction) or to oxygen (type-2 reaction) to produce reactive oxygen species (ROS), typically singlet oxygen. Although, the two pathways can have a role on bacterial killing, type 2 by producing highly reactive singlet oxygen is detected as the main pathway in killing bacteria.<sup>85,86</sup>

This mechanism is different from that of antibiotics; hence the resistance of bacterial strain is not likely, due to acting on multiple targets inside the bacteria.87 The absence of thermal effect in PDT procedure is considered as one of the main advantages in endodontic disinfection, with a lack of thermal side effects in periradicular tissues. The main mechanism of lethal action is based on photochemical reaction.88 Another advantage of PDT is that it contributes to the enhancement of cross-linking of collagen fibrils in the dentin matrix, thereby improving dentin stability. Furthermore, ROS generated by PDT increase the number of intermolecular cross-links between adjacent collagen fibrils and result to higher resistance of collagen to enzymatic degradation at contaminated places. Collagen is the main structural protein of the dentin matrix, increase in collagen cross-linking increases the mechanical properties of dentin in infectious condition inside the canal.89

Two mechanisms have a major role in lethal damage administered by PDT, (*i*) DNA damage, (*ii*) damage to cytoplasmic membrane and cellular contents or inactivation of membrane transport systems and enzymes.<sup>90</sup> It seems that the DNA damage is a more important mechanism for PDT of bacteria. In both gram-positive and gram-negative species, breakdowns of single-stranded and double-stranded DNA occur, as well as fading of the plasmid super-coiled fraction.<sup>91</sup>

One of the main concerns during photodynamic therapy is the photosensitivity of bacteria which seems related to the charge of the PS used. The neutral or anionic PS binds effectively to gram-positive bacteria. They also bind to some degree to the outer membrane of gram-negative bacteria.<sup>92</sup> The porous layer of peptidoglycan and lipoteichoic acid outside the cytoplasmic membrane of gram-positive species allow the PS to penetrate into the sensitive sites. On the other hand, the outer membrane of gram-negative bacteria acts as a physical barrier between cells and its environment. The binding of negatively charged PS to gram-negative bacteria may be improved by linking the PS to a cationic molecule<sup>93,94</sup>.

The success rate of photodynamic therapies depend on the type, dose, incubation time, and localization of the PS, the availability of oxygen, the wavelength of light (nm), the light power density and the light energy fluency.<sup>95</sup> Limitations of this treatment which should be taken into account are the low-oxygenated environment and the diffusion ability of the PS and light to be used.<sup>96</sup>

## Discussion

The chemomechanical technique cannot completely eliminate the bacteria in root canal treatment. Due to the limitation in penetration depth of conventional irrigants such as NaOCl, bacteria remaining in accessory canals cannot be removed.<sup>97</sup> In order to improve the success rate of the root canal therapy (RCT) procedure, other technologies were introduced. Laser as a new technology has the potential to eradicate the bacteria from root canals through photothermal reactions. In this regard, different wavelengths were used. Although the application of laser can enhance the results of treatment, the thermal effects on the dentine are considered to be a main concern.  $^{98}\,$ 

Antimicrobial photodynamic therapy (aPDT) is a recently known technique for disinfection in dentistry; its lethal action is based on photo chemical reaction with no thermal effect compared to laser treatment, which protects the adjacent tissues.<sup>99</sup> On the other hand, it should be kept in mind that the application of some PS like MB can stain the teeth if the appropriate concentration is not used. However, there are evidences suggesting that the application of NaOCl 2.5% is effective in the prevention of tooth discoloration after applying PS. It was suggested that MB in concentrations below 100 µg/ml, reduces the chance of tooth discoloration.<sup>100</sup>

Another advantage of aPDT is its safety. Studies showed that the cytotoxicity produced by aPDT is relatively less than NaOCl when used for RCT.<sup>101</sup> One of the criteria that influence the success rate of aPDT is the species of bacteria and their growth. Silva et al in assessing aPDT on five different strains of *E. faecalis*, concluded that the efficacy of aPDT on *E .faecalis* biofilms was strain dependent due to variability in the phenotype and genotype of clinical strains.<sup>102</sup>

The complexity of the root canal, the oxygen content and the diffusion ability of PS, also have an effect on the outcome. There is a study which reported the efficacy of PDT on anterior and posterior teeth with the same success rate.<sup>103</sup> If the oxygen content in the site of treatment is low, the possibility of cytotoxic oxygen is also reduced, thereby reducing the efficacy of treatment.<sup>104</sup> There can be some restriction on the diffusion of PS inside the canal, such as some irregularities or bacterial biofilm on untouched walls. In order to improve the penetration of PS, some chelating solutions like EDTA or citric acid can be used prior to PDT or the PS can be prepared by these solutions. Also, ultrasonic waves can be used to place the PS deeper.105 Among different PS presented in this article, it seems that ICG can be beneficial due to its peak absorption at 808 nm, which coincides with the commercial diode devices with extensive application such as soft tissue surgery, bleaching, etc. Also, this wavelength has more penetration depth compared to red lasers used for TBO and MB. It is suggested to use PS after application of chelating solution or EDTA or citric acid-based PS prepared by this solution then ultrasonic waves are used to place the PS deeper, then an appropriate wavelength is used to activate the PS.

## Conclusion

Each technique for endodontic disinfection has some advantages and disadvantages. Among these different techniques and due to the development of laser technology in dentistry, the application of PDT has gained special attention. It would be helpful to recognize the best combination of PS and light wavelength through in vitro studies and conduct randomized controlled trials, to investigate the efficacy of PDT on root canal disinfection.

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