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Antibacterial Agents in Dental Treatments

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1. Introduction

Because progressive increase in serious transmissible diseases over the last few decades, every health care specialty that involves contact with mucosa, blood or blood contamination, like dentistry, should regulate regarding sterilization and disinfection. Dental patients and dental health-care workers may be exposed to a variety of microorganisms via blood or oral or respiratory secretions. These microorganisms may include cytomegalovirus, *hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus types 1* and *2, human immunodeficiency virus (HIV), Mycobacterium tuberculosis, staphylococci, streptococci*, and other viruses and bacteria; specifically, those that infect the upper respiratory tract (Blently, 1994). Infections may be transmitted in the dental operatory through several routes, including direct contact with blood, oral fluids or other secretions; indirect contact with contaminated instruments, operatory equipment or environmental surfaces or contact with airborne contaminants present in either droplet spatter or aerosols of oral and respiratory fluids. Infection via any of these routes requires that all three of the following conditions be present (commonly referred to as "the chain of infection": a susceptible host; a pathogen with sufficient infectivity, numbers to cause infection and a portal through which the pathogen may enter the host) (Burkhart, 1970). Effective infection-control strategies are intended to break one or more of these "links" in the chain, thereby preventing infection. A set of infection-control strategies common to all health-care delivery settings should reduce the risk of transmission of infectious diseases caused by blood-borne pathogens such as *HBV* and *HIV*. Because all infected patients cannot be identified by medical history, physical examination, or laboratory tests, it is recommended that blood and body fluid precautions be used consistently for all patients. In dentistry, beside personal protections like eyewear, gloves and gowns, pretreatment mouth rinse, rubber dam and high velocity air evacuation are the other considerations regarding infection control (Hackney, 1989). Suitable sterilization and disinfection of instruments are inseparable parts of infection control puzzle. So, discussion about the techniques and agents used in sterilization and disinfection is very important, nowadays. In this chapter we mention the antibacterial agents used in sterilization and disinfection in dentistry.

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2. Antibacterial agents used in sterilization and disinfection

There are several methods and materials for disinfection. In this chapter, we will discuss the most common antibacterial agents that are used in sterilization and disinfection in dentistry. Disinfectants are substances that are applied to non-living objects to destroy microorganisms that are living on the objects. There are several criteria for Classification of chemical disinfectants that mentioned below (Favero&Bond, 1991):

1. Based on consistency
   a. Liquid (E.g., Alcohols, Phenols)
   b. Gaseous (Formaldehyde vapor, Ethylene oxide)
2. Based on spectrum of activity

Regarding spectrum activity disinfectants have three levels (Table 1).

	Vegetative cells	Mycobacteria	Spores	fungi	viruses	example
High level	+	+	+	+	+	Ethylene Oxide, Glutaraldehyde, Formaldehyde
Intermediate level	+	+	-	+	+	Phenolics, halogens
Low level	+	-	-	+	+/-	Alcohols, quaternary ammonium compounds

Table 1. levels of disinfectants spectrum activity

3. Based on mechanism of action
   a. Action on membrane (E.g., Alcohol, detergent)
   b. Denaturation of cellular proteins (E.g., Alcohol, Phenol)
   c. Oxidation of essential sulphydryl groups of enzymes (E.g., H_2O_2, Halogens)
   d. Alkylation of amino-, carboxyl- and hydroxyl group (E.g., Ethylene Oxide, Formaldehyde)
   e. Damage to nucleic acids (Ethylene Oxide, Formaldehyde)

An ideal disinfectant should have following properties (Crawford, 1983):

1. Should have wide spectrum of activity
2. Should be able to destroy microbes within practical period of time
3. Should be active in the presence of organic matter
4. Should make effective contact and be wettable
5. Should be active in any pH
6. Should be stable
7. Should have long shelf life
8. Should be speedy
9. Should have high penetrating power
10. Should be non-toxic, non-allergenic, non-irritative or non-corrosive
11. Should not have bad odor
12. Should not leave non-volatile residue or stain
13. Efficacy should not be lost on reasonable dilution
14. Should not be expensive and must be available easily

It should be mentioned that the efficacy of disinfectant depends on contact time, temperature, type and concentration of the active ingredient, the presence of organic matter, the type and quantum of microbial load.

2.1 Alcohols

The action mechanisms of this subgroup of disinfectant are coagulation of protein, dehydration of cells and disruption of membranes (Moorer, 2003). Alcohols, usually ethanol or isopropanol, are sometimes used as a disinfectant, but more often as an antiseptic. A 70% aqueous solution is more effective at killing microbes than absolute alcohols. Because water facilitates diffusion through the cell membrane; 100% alcohol typically denatures only external membrane proteins. A mixture of 70% ethanol or isopropanol diluted in water is effective against a wide spectrum of bacteria, though higher concentrations are often needed to disinfect wet surfaces (Brent, 2009). Additionally, high-concentration mixtures (such as 80% ethanol + 5% isopropanol) are required to effectively inactivate lipid-enveloped viruses (such as *HIV, hepatitis B, and hepatitis C*). 70% ethyl alcohol is used as antiseptic on skin. Isopropyl alcohol is preferred to ethanol. It can also be used to disinfect surfaces. It is used to disinfect clinical thermometers. Methyl alcohol kills fungal spores, hence is useful in disinfecting inoculation hoods (Engelenburg, 2002). Alcohols have some disadvantages. They can be a fire hazard. Also, they have limited residual activity due to evaporation, which results in brief contact times unless the surface is submerged, and have a limited activity in the presence of organic material. They are skin irritants and inflammable (Lodgsdon, 1994).

2.2 Aldehydes

The other subgroup of disinfectants is aldehydes that act through alkylation of amino, carboxyl-or hydroxyl group, and probably damage nucleic acids. They have a wide microbiocidal activity and are sporocidal and fungicidal (Crawford, 1983). The most popular of this subgroup are formaldehyde and gluteraldehyde. 40% formaldehyde (formalin) is used for surface disinfection. 10% formalin with 0.5% tetraborate sterilizes clean metal instruments. 2% gluteraldehyde is used to sterilize thermometers, cystoscopes, bronchoscopes, centrifuges, anasethetic equipments etc. An exposure of at least 3 hours at alkaline pH is required for action by gluteraldehyde. 2% formaldehyde at 40^{o}C for 20 minutes is used to disinfect wool and 0.25% at 60^{o}C for six hours to disinfect animal hair and bristles (Favero&Bond, 1991). Disadvantages of these agents are: Vapors are irritating and must be neutralized by ammonia, have poor penetration, leave non-volatile residue, activity is reduced in the presence of protein. Some bacteria have developed resistance to glutaraldehyde, and it has been found that glutaraldehyde can cause asthma and other health hazards; hence ortho-phthalaldehyde is replacing glutaraldehyde (Crawford, 1983).

2.3 Halogens

Halogens for example Chlorine compounds (chlorine, bleach, hypochlorite) and iodine compounds (tincture iodine,iodophores) are oxidizing agents and cause damage by oxidation of essential sulfydryl groups of enzymes. Chlorine reacts with water to form hypochlorous acid, which is microbicidal. Applications of this group are: Tincture of iodine (2% iodine in 70% alcohol) is an antiseptic (Crawford, 1983). Iodine can be combined with

neutralcarrier polymers such as polyvinylpyrrolidone to prepare iodophores such as povidone-iodine. Iodophores permit slow release and reduce the irritation of the antiseptic. For hand washing iodophores are diluted in 50% alcohol. 10% Povidone Iodine is used undiluted in pre and postoperative skin disinfection. 0.5% sodium hypochlorite is used in serology and virology. Used at a dilution of 1:10 in decontamination of spillage of infectious material. Mercuric chloride is used as a disinfectant. This group has some disadvantages like: They are rapidly inactivated in the presence of organic matter. Iodine is corrosive and staining. Bleach solution is corrosive and will corrode stainless steel surfaces (Sattar, 1998).

2.4 Hydrogen peroxide

It acts on the microorganisms through its release of nascent oxygen. Hydrogen peroxide produces hydroxyl-free radical that damages proteins and DNA. Hydrogen peroxide is used in hospitals to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant (Favero&Bond, 1991). It is used at 6% concentration to decontaminate the instruments, equipments such as ventilators. 3%Hydrogen Peroxide Solution is used for skin disinfection. Strong solutions are sporicidal (Sattar, 1998). 1.5-2 % Hydrogen peroxide is used as mouthwashes (Hasturk et al., 2004). It is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. Decomposition in light, breaking down by catalase and reduction of activity by organic matter is their disadvantages (Favero&Bond, 1991).

2.5 Ethylene oxide

It is an alkylating agent. It acts by alkylating sulfydryl, amino, carboxyl and hydroxyl-groups. It is a highly effective chemisterilant, capable of killing spores rapidly. It is the best method for sterilization of complex instruments, delicate materials, and heat labile articles such as bedding, textiles, rubber, plastics, syringes, disposable petri dishes, heart-lung machine, respiratory and dental equipments (Crawford, 1983). Porous and plastic materials absorb the gas and require aeration for 2 hours, before it is safe to contact skin and tissues. It has a sweet odor, readily polymerizes and is flammable. Since it is highly flammable, it is usually combines with CO2 (10% CO2+ 90% EO) or dichlorodifluoromethane. It requires presence of humidity. But, it is highly toxic, irritating to eyes and skin, highly flammable, mutagenic and carcinogenic.

2.6 Phenol

Phenolic materials for example 5% phenol, 1-5% Cresol, 5% Lysol (a saponified cresol), hexachlorophene or chlorhexidine act by disruption of membranes, precipitation of proteins and inactivation of enzymes. They act as disinfectants at high concentration and as antiseptics at low concentrations (Weber et al., 1999).They are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses. They are not readily inactivated by organic matter. Chlorhexidine can be used in an isopropanol solution for skin disinfection, or as an aqueous solution for wound irrigation. It is often used as an antiseptic hand wash. 20% Chlorhexidine gluconate solution is used for pre-operative hand and skin preparation and for general skin disinfection (Favero&Bond, 1991). 0.12 -0.2 % Chlorhexidine are used as mouthwash. It is also used as root canal irrigant which will be discussed later in this chapter. Chlorhexidine gluconate is also mixed with quaternary

ammonium compounds such as cetrimide to get stronger and broader antimicrobial effects (eg. Savlon). Chloroxylenols are less irritative and can be used for topical purposes and are more effective against gram positive bacteria than gram negative bacteria. Hexachlorophene is chlorinated diphenyl and is much less irritative. It has marked effect over gram positive bacteria but poor effect over gram negative bacteria, *mycobacteria*, fungi and viruses. Triclosan is organic phenyl ether with good activity against gram positive bacteria and is effective to some extent against many gram negative bacteria including Pseudomonas. It also has fair activity on fungi and viruses. But it is toxic, corrosive and skin irritant. Chlorhexidine is inactivated by anionic soaps. Chloroxylenol is inactivated by hard water (Crawford, 1983).

2.7 Quaternary ammonium compounds

They are one of the surface active agents and have the property of concentrating at interfaces between lipid containing membranes of bacterial cell and surrounding aqueous medium (Weber et al., 1999). The mechanism of their action is disruption of membrane resulting in leakage of cell constituents. Surface active agents are soaps or detergents. Detergents can be anionic or cationic. Anionics contain negatively charged long chain hydrocarbon .These include soaps and bile salts. If the fat-soluble part is made to have a positive charge by combining with a quaternary nitrogen atom, it is called cationic detergents. Cationic detergents are known as quaternary ammonium compounds (or quat). Typically, quats do not exhibit efficacy against difficult to kill non-enveloped viruses such as norovirus, rotavirus, or polio virus. Newer low-alcohol formulations are highly effective broad-spectrum disinfectants with quick contact times (3–5 minutes) against bacteria, enveloped viruses, pathogenic fungi, and *mycobacteria*. However, the addition of alcohol or solvents to quat-based disinfectant formulas results in the products' drying much more quickly on the applied surface, which could lead to ineffective or incomplete disinfection. Quats are biocides that also kill algae and are used as an additive in large-scale industrial water systems to minimize undesired biological growth. Cetrimide and benzalkonium chloride act as cationic detergents. They are active against vegetative cells, *mycobacteria* and enveloped viruses. They are widely used as disinfectants at dilution of 1-2% for domestic use and in hospitals. This subgroup of disinfectants has several disadvantages as follow: Their activity is reduced by hard water, anionic detergents and organic matter. Pseudomonas can metabolize cetrimide, using them as a carbon, nitrogen and energy source (Favero&Bond, 1991).

3. Antibacterial agents used in dental treatments

Microorganisms are the main cause of pulpal and priapical diaeases. The primary endodontic treatment goal is root canal disinfection and prevention of re-infection of root canal system (Basmadji-Charles et al., 2002; Shahi et al., 2007; Zand et al.,2010). Besides of aseptic principles like rubber dam placement and correct mechanical instrumentation, root canal irrigants are the important aspect to eradication of microbes from root canals. To increase efficacy of mechanical preparation and bacterial removal, instrumentation must be supplemented with active irrigating solutions. Irrigation is defined as washing out a body cavity or wound with water or medical fluid. The objective of irrigation is both mechanical and biologic. The biologic function is related to their antimicrobial effect and mechanical one

is due to flushing out effect (Cheung&Stock, 1993). The ideal irrigant should be germicide and fungicide, nonirritating to tissues, stable in solution, have prolonged antimicrobial effect, not interfere with tissue repair, relatively inexpensive, and non-toxic (Tay et al., 2006). There are several irrigants used in endodontic. In this chapter, we discuss about the properties of routine irrigants used in endodontic field.

3.1 Sodium hypochlorite

Hypochlorite solutions were first used as bleaching agents. Based on the controlled laboratory studies by Koch and Pasteur, hypochlorite then gained wide acceptance as a disinfectant by the end of the 19th century. In World War I, the chemist Henry Drysdale Dakin and the surgeon Alexis Carrel extended the use of a buffered 0.5% sodium hypochlorite solution to the irrigation of infected wounds, based on Dakin meticulous studies on the efficacy of different solutions on infected necrotic tissue (Dakin, 1915). Besides their wide-spectrum, nonspecific killing efficacy on all microbes, hypochlorite preparations are sporocidal, virucidal , and show far greater tissue dissolving effect on necrotic than on vital tissues (Austin & Taylor, 1918) . These features prompted the use of aqueous sodium hypochlorite in endodontics as the main irrigant as early as 1920 (Grossman, 1943). In the endodontic field, NaOCl possesses a broad spectrum antimicrobial activity against microorganisms and biofilms difficult to eradicate from root canals such as Enterococcus, Actinomyces and Candida organisms. Furthermore, sodium hypochlorite solutions are cheap, easily available, and demonstrate good shelf life (Heling et al., 2001; Mahmudpour et al., 2007). Other chlorine-releasing compounds have been advocated in endodontics, such as chloramine-T and sodium dichloroisocyanurate. These, however, never gained wide acceptance in endodontics, and appear to be less effective than hypochlorite at comparable concentration (Dychdala, 1991).There has been controversy over the most suitable concentration of hypochlorite solutions to be used in endodontics. As Dakin original 0.5% sodium hypochlorite solution was designed to treat open wounds, it was surmised that in the confined area of a root canal system, higher concentrations should be used, as they would be more efficient than Dakin solution (Grossman, 1917). The antibacterial effectiveness and tissue-dissolution capacity of aqueous hypochlorite is a function of its concentration, but so is its toxicity (Spyngbergl et al., 1973). However, severe irritations have been reported when 5.25% concentrated solutions were inadvertently forced into the periapical tissues during irrigation or leaked through the rubber dam (Hismann& Hahn, 2000). Furthermore, a 5.25% solution significantly decreases the elastic modulus and flexural strength of human dentin compared to physiologic saline, while a 0.5% solution does not (Sima et al., 2001). This is most likely because of the proteolytic action of concentrated hypochlorite on the collagen matrix of dentin. The reduction of intracanal microbiota, on the other hand, is not any greater when 5% sodium hypochlorite is used as an irrigant as compared to 0.5% (Bystrm&Sundqvist, 1985). From in vitro observations, it would appear that a 1% NaOCl solution should suffice to dissolve the entire pulp tissue in the course of an endodontic treatment session (Sirtes et al., 2005). Hence, based on the currently available evidence, there is no rationale for using hypochlorite solutions at concentrations over 1% wt/vol. This concentration of NaOCl is also used for disinfection of Gutta-percha cones. Reactive chlorine in aqueous solution at body temperature can, in essence, take two forms: hypochlorite (OCL) in pH above 7.6 or hypochlorous acid (HOCl) in pH below 7.6. Both forms are extremely reactive oxidizing agents. Pure hypochlorite

solutions as they are used in endodontics have a pH of 12 , and thus the entire available chlorine is in the form of OCl^-. However, at identical levels of available chlorine, hypochlorous acid is more bactericidal than hypochlorite (Zehnder et al., 2002). One way to increase the efficacy of hypochlorite solutions could thus be to lower the pH. It has also been surmised that such solutions would be less toxic to vital tissues than non-buffered counterparts (Kamburis et al., 2003). However, buffering hypochlorite with bicarbonate renders the solution unstable with a decrease in shelf life to less than 1 week. Depending on the amount of the bicarbonate in the mixture and therefore the pH value, the antimicrobial efficacy of a fresh bicarbonate-buffered solution is only slightly higher or not elevated at all compared to that of a non-buffered counterpart (Costigan, 1936). Another approach to improve the effectiveness of hypochlorite irrigants in the root canal system could be to increase the temperature of low-concentration NaOCl solutions. This improves their immediate tissue-dissolution capacity (Abou-Rass &Oglesby, 1981). Furthermore, heated hypochlorite solutions remove organic debris from dentin shavings more efficiently than unheated counterparts (Cunningham&Balekjian, 1980).

3.2 Chlorhexidine

Chlorhexidine is a strong base and is most stable in the form of its salts. The original salts were chlorhexidine acetate and hydrochloride, both of which are relatively poorly soluble in water (Foulkes, 1973). Hence, they have been replaced by chlorhexidine digluconate. It has a cationic molecular component that attaches to negatively charged cell membrane area and causes cell lysis. Chlorhexidine is a potent antiseptic, which is used as a mouth rinse and endodontic irrigant. The later application is based on its substantivity and long-lasting antimicrobial effect which arise from binding to hydroxyapatite. Aqueous solutions of 0.1 to 0.2% concentrations are recommended for that purpose, while 2% is the concentration of root canal irrigating solutions usually found in the endodontic literature (Zamany et al., 2003). It is commonly held that chlorhexidine would be less caustic than sodium hypochlorite (Spngberg et al., 1973). A 2% chlorhexidine solution is irritating to the skin (Foulkes, 1973). As with sodium hypochlorite, heating chlorhexidine of lesser concentration could increase its local efficacy in the root canal system while keeping the systemic toxicity low (Evanov et al., 2004). Despite its usefulness as a final irrigant, chlorhexidine cannot be advocated as the main irrigant in standard endodontic cases, because: (a) chlorhexidine is unable to dissolve necrotic tissue remnants (Naenni et al., 2004), and (b) chlorhexidine is less effective on Gram-negative than on Gram-positive bacteria (Hennessey,1973). In a randomized clinical trial on the reduction of intracanal microbiota by either 2.5% NaOCl or 0.2% chlorhexidine irrigation, it was found that hypochlorite was significantly more efficient than chlorhexidine in obtaining negative cultures (Ringel, 1982). Most important CHX disadvantage is its inability of to dissolve necrotic tissue remnants and chemically clean the canal system.

3.3 Iodine potassium iodine

Iodine potassium iodine is a traditional root canal disinfectant with wide-spectrum antimicrobial activity. It is used in concentrations ranging from 2% to 5%).The oxidizing agent of this substance, iodine, reacts with free sulfhydryl groups of bacterial enzymes cleaving the disulfide bonds. It was manifested that calcium hydroxide–resistant

microorganisms could be eradicated with combination of IKI and CHX (Baker et al., 2004). It shows relatively low toxicity in experiments using tissue cultures. An obvious disadvantage of iodine is a possible allergic reaction in some patients (Siren et al., 2004).

3.4 MTAD (Mixture of Tetracyclin, Acid, Detergent)

Biopure MTAD was recently introduced in the market as an antibacterial root canal cleanser .MTAD is a mixture of 3% tetracycline isomer (doxycycline), and 4.25% acid (citric acid), and 0.5% detergent (Tween 80). This biocompatible intracanal irrigant is commercially available as a two-part mix (Torabinejad et al., 2005). One of the characteristic of this solution is a high binding affinity of the doxycycline to dentin (Beltz et al., 2003).In this irrigant, doxycycline hyclate is used instead of its free base, doxycycline monohydrate, to increase the water solubility of this broad-spectrum antibiotic. MTAD has been reported to be effective in removing the smear layer due to citric acid action (Torabinejad et al., 2003), eliminating microbes that are resistant to conventional endodontic irrigants and medications (Shabahang& Torabinejad, 2003) and providing sustained antimicrobial activity. With every new product we are always concerned about the cytotoxicity to the underlying tissue. MTAD was compared with commonly used irrigants and medications The results showed MTAD to be less cytotoxic than eugenol, 3 percent H2O2, $Ca(OH)_2$ paste, 5.25 percent NaOCl, Peridex, and EDTA. It is more cytotoxic than NaOCl at 2.63 percent, 1.31 percent, and 0.66 percent concentrations (Zaung et al., 2003).

3.5 Calcium hydroxide

Residual bacteria in the root canal have been held responsible for failures (Sjugren et al., 1990). It is generally believed that the number of remaining bacteria can be controlled by placing an interappointment medication within the prepared canal (Chong&Pitt Ford, 1992; Rahimi et al.,2010). Calcium hydroxide,$Ca(OH)_2$ is the most common interappointment medication used which requires disinfection period of 7 days (Sjugren et al., 1991). However, some microbes such as *Enterococcus faecalis* (George et al., 2005) and *Candida albicans* (Waltimo et al., 1999) are resistant to it. Therefore, alternative intracanal medications have been sought to improve the eradication of bacteria before obturation. Chlorhexidine gluconate is effective against strains resistant to calcium hydroxide (Delany et al., 1989). Recent studies have suggested that CHX could be used in combination with calcium hydroxide to improve antimicrobial efficacy against calcium hydroxide-resistant microbes (Almyroudi et al., 2002). The high pH of calcium hydroxide formulations (pH=12.5) alters the biologic properties of bacterial lipopolysaccharides in the cell walls of gram-negative species and inactivates membrane transport mechanisms, resulting in bacterial cell toxicity (Siqueira & Lopes, 1999). However, as stated above, *E. faecalis* has been reported to be resistant to this effect as a result of its ability to penetrate the dentinal tubules and adapt to changing environment (George et al., 2005).

3.6 Laser irradiation and photodynamic therapy

Novel approaches to disinfecting root canals have been proposed recently that include the use of high-power lasers (Walsh, 2003) as well as photodynamic therapy (PDT) (Hamblin& Hasan, 2004). High-power lasers function by dose-dependent heat generation, but, in addition to killing bacteria, they have the potential to cause collateral damage such as char dentine, ankylosis roots, cementum melting, and root resorption and periradicular necrosis

if incorrect laser parameters are used . Since the introduction of the laser in endodontics in 1971, several lasers were used to eliminating bacteria from root canals. The erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser has highest absorption in water and high affinity to hydroxyapatite, which makes it suitable for use in root canal therapy(Yamazaki et al., 2001; Yavari et al.,2010). Lasers have the ability to clean and effectively disinfect root canals; including eliminating highly resistant species such as *Enterococcus faecalis* (Le Goff et al., 1999). PDT (photodynamic therapy) is a new antimicrobial strategy that involves the combination of a nontoxic photosensitizer and a light source (Demidova&Hamblin., 2004). The excited photosensitizer reacts with molecular oxygen to produce highly reactive oxygen species, which induce injury and death of microorganisms (Wainwright, 1998). It has been established that PS, which possess a pronounced cationic charge, can rapidly bind and penetrate bacterial cells, and, therefore, these compounds show a high degree of selectivity for killing microorganisms compared with host mammalian cells (Maisch et al., 2005). PDT has been studied as a promising approach to eradicate oral pathogenic bacteria (Wilson, 2004) that cause diseases such as periodontitis, peri-implantitis and caries (Walsh, 2003). When PDT followed conventional endodontic therapy, there was significantly more killing and less bacterial growth than was seen after endodontic therapy alone (Garcez et al., 2007).

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