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Irrigantes y selladores-nanopartículas biocompatibles antimicrobianos para uso en Endodoncia

Biocompatible Antimicrobial Irrigants and Nanoparticles-Sealers for Endodontics

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Resumen

El presente trabajo es una revisión bibliográfica de las tendencias actuales y futuras de los cementos selladores y de las soluciones irrigantes en relación a su efecto bactericida, así como del tamaño de partícula del que están constituidos. Son propuestos diversos desinfectantes del conducto radicular con la intención de garantizar la no formación de biopelículas.

Hoy en día los fracasos endodóncicos son más frecuentes y representan un alto riesgo de infecciones en los pacientes más complejos, por lo que está llevando a la mejora continua de los productos utilizados para la limpieza y sellado intraconducto, para asegurar la eliminación completa de los microorganismos. La nanotecnología está ayudando a crear nuevos materiales para aplicaciones biológicas, como biomateriales y dispositivos dentales para aplicaciones a corto o largo plazo que estén en contacto con los fluidos corporales, para los que se exige alto efecto antimicrobiano y biocompatibilidad.

Existen diversos biomateriales disponibles, bajo continua investigación ya que se ha logrado cultivar biofilm hasta en un 10 % de los pacientes con necesidad de retratamiento de conductos. Esto lleva a la mejora continua de los productos utilizados para la limpieza y sellado intraconducto que aseguren la eliminación completa de los microorganismos.

Palabras clave: nanotecnología, biopelículas, desinfectante del conducto radicular, soluciones antimicrobianas.

Abstract

The present paper is a literature review of present and future trends for the antimicrobial irrigant solutions and nanoparticles used in endodontic fillers for root canal disinfection with the intention of ensuring no biofilm formation. Nowadays failures after root canal treatment are increasingly frequent and represent a high risk of more complex infections in patients; so it is carrying a continuous improvement of products used for cleaning and intracanal sealing, that a complete removal of microorganisms can be ensured. In particular, nanotechnology is helping to create novel materials for biological applications, including dental biomaterials and devices for short and long-term applications in contact with body fluids and tissues, for which their antimicrobial effect and biocompatibility are demanded. Current available materials have some disadvantages, which show up after a root canal treatment. This is becoming more frequent and represents a high risk of infections in patients undergoing that treatment. It is known that up to 10% of endodontic treatments fail because of existing bacteria colonies. This leads to continuously improve the products used for cleaning and intracanal sealing to ensure complete removal of microorganisms.

Keywords: nanotechnology, biofilm, root canal disinfection, antimicrobial solutions

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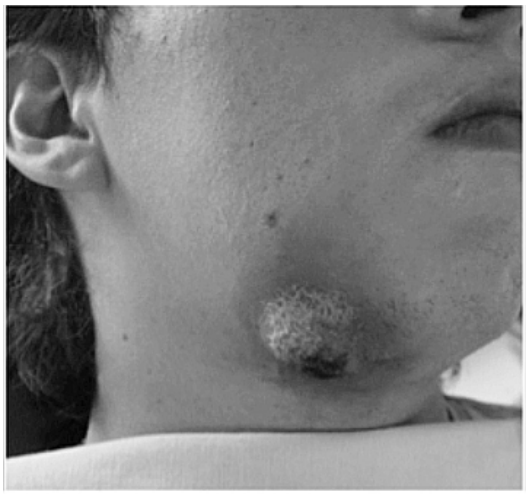
Introducción

The role of microorganisms in the development and maintenance of the pulpal and periapical inflammation has been well documented. Periapical lesions of endodontic origin are common conditions found in the oral cavity presenting pathologic mechanisms that involve interactions between immune cells and bone. Lesions of endodontic origin are associated with bacterial contamination and necrosis of the dental pulp, which typically may progress through four stages: i) exposure of the dental pulp to the oral cavity with subsequent bacterial colonization, ii) inflammation and necrosis of the dental pulp, iii) development of inflammation in the periapical area, and iv) periapical resorption of bone and formation of granulomas or cysts (Image1) (Graves, Oates & Garlet, 2011).

The success of root canal treatment largely depends on the elimination of microbial contamination from the root canal system, although mechanical instrumentation can reduce bacterial population, effective elimination of bacteria cannot be achieved without the use of antimicrobial root canal

Image 1.



Patient with acute alveolar abscess with skin *fistula*, an injury that occurs when the *root* canal *is* full of necrotic *tissue* and bacteria have invaded bone and soft tissues.irrigation and medication (Karim, Kennedy & Hussey, 2007).

With the emergence of nanotechnology to biomaterials, adding different polymeric or metal nanoparticles with shown antimicrobial effect has modified some canal filler and they have been used as adjuvants in the root canal to eliminate the presence of microorganisms (Kim *et al*., 2007).

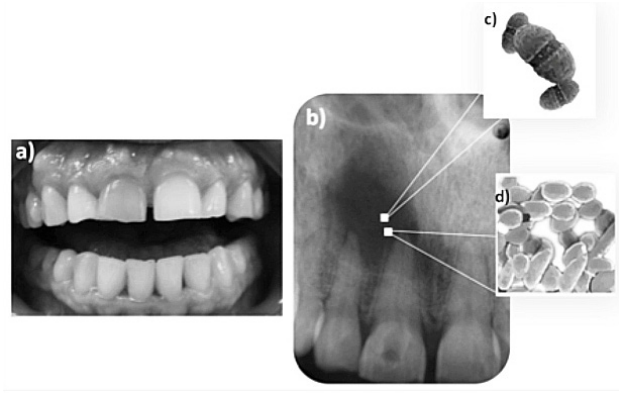
The aim of the present review is to release the current cleaning and sealing intracanal nanomaterials intending to ensure the complete removal of microorganisms.

1. Microorganisms Involved in the Formation of Biofilm

In cases of infected necrotic pulps or root canal treated teeth with apical periodontitis, an intracanal infection is established, and consequently, endodontic procedures should focus on the elimination of the existing microorganisms and also on the prevention of the introduction of new microorganisms inside the root canal. Therefore, endodontic infections can only be treated with professional intervention using chemical and mechanical procedures, being the main steps of the control of infections during the endodontic treatment the chemical-mechanical preparation and intracanal medication (Siqueira and Rocas, 2008; Sjogren, Figdor, Persson and Sundqvist, 1997).

Primary root canal infections are polymicrobial, typically dominated by obligatory anaerobic bacteria. The most frequently isolated microorganisms before root canal treatment include Gram-negative anaerobic rods, Gram-positive anaerobic cocci, Gram-positive anaerobic and facultative rods, *Lactobacillus* species, and Gram-positive facultative *Streptococcus* species. The obligate anaerobes are rather easily eradicated during root canal treatment, while facultative bacteria such as nonmutans Streptococci, Enterococci, and Lactobacilli, once established, are more likely to survive chemical-mechanical instrumentation and root canal medication. In particular *Enterococcus faecalis* has gained attention in the endodontic literature, as it can frequently be isolated from root canals in cases of failed root canal treatments. In addition, yeasts (eg. *Candida albicans)* may also be found in root canals associated with therapy-resistant apical periodontitis (Image 2) (Jaju & Jaju).

Image 2.



Patient with periapical lesion 2 years of evolution. a) Clinical photograph showing dyschromia in the upper right incisor. b) X-ray dento-alveolar pointing to the microorganisms present in the lesion: c) *Enterococcus faecalis* d) *Candida albicans* forming the biofilm.

Apical periodontitis is an inflammatory process on the periradicular tissues caused by the microorganisms present inside the root canal system. It has been shown that the primary etiological agents of apical periodontitis are the anaerobes microorganisms. One of the most important objectives in successful treatment of apical periodontitis is the elimination of intracanal bacteria (Wang, *et al.*, 2007)).

Most intracanal bacteria are sensitive to standard treatment procedures; nevertheless, some bacteria may survive to treatment procedures and their presence at the time of filling the root canal has been detected by culture assays and has been recognized as a risk factor for post treatment apical periodontitis (Siqueira, *et al.*, 2002).

Into the infected root canals, bacteria are often found forming multilayers of dense aggregates known as biofilms; which are matrix-enclosed communities of microorganisms that tightly interact and colonize surfaces in aqueous environment (Monteiro, *et al.*, 2009). Biofilm formation occurs as a result of a sequence of events: microbial surface attachment, cell proliferation, matrix production and detachment (Graves, Oates and Garlet, 2011; Monteiro, *et al.*, 2009); the organization of cells growing in biofilms is determined by specific inter- and intraspecies interactions that will have an influence on their resistance to antimicrobial treatment and consequently on the outcome of diseases (Chavez, 2012). Biofilms can become hundreds of micrometers in depth and display complex structural and functional architecture (Siquiera and Rôças, 2008; Chavez, 2012). Bacteria in biofilm are up to 1,000-fold more resistant than the corresponding bacteria in planktonic form. Consequently, recent studies attempt to evaluate the efficacy of root canal irrigants against biofilms (Arias-Moliz, *et al.*, 2009); where *Enterococcus faecalis* is one of the primary organisms present in patients with post treatment endodontic infection, and *Candida albicans* in the other main microorganisms forming the biofilm.

It has been shown the presence of the microorganisms as biofilm in the dental anatomical complexities and possesses ability to penetrate dentinal tubules (Siqueira and Rôças, 2008; Saunus, Kazoullis and Farah, 2008). In medical microbiology, biofilms have been increasingly studied and estimates indicate that biofilm infections comprise 65 % to 80 % of the human infections in the developed world (Ricucci and Siqueira, 2010). The biofilms formed in rich medium generally showed continuous growth over time, however, in the absence of glucose biofilms showed significantly smaller biovolumes (Chavez, 2012).

The conditioning film, formed by a layer of organic molecules adhered to the surface, is considered a precursor for the initial attachment of planktonic cells. The adhesion of microorganisms then occurs on the surface, which is subsequently facilitated by bacterial signaling. A mature biofilm is characterized when adherent bacteria produce extracellular polymeric substances that aid in rapping nutrients from the surrounding environment. Biofilms create an environment that enhances antimicrobial resistance. The extracellular polymeric substances of biofilms contain considerable amounts of polysaccharides, proteins, nucleic acids and lipids, which are responsible for maintaining the structural integrity of the biofilm and providing an ideal matrix for bacterial cell growth (Monteiro, *et al.*, 2009).

The high pH of the root canal cement alters the biologic properties of lipopolysaccharides in the cell walls of Gram-negative bacteria. However, *E. faecalis* has been reported to be resistant to alkaline stress because of its ability to penetrate the dentinal tubules and possible interspecies communication (Hiraishi, 2010).

Biofilms are heterogeneous communities of microorganisms entrapped in an extracellular matrix, which limits

the penetration of antimicrobial drugs and antibodies; consequently, biofilm infections are rather difficult to treat. Biofilm formation is an important property that helps *C. albicans t*o cause many types of infections. *Candida* biofilms have been reported to be 30–2000 times more resistant to various antifungal agents compared to their planktonic counterparts (Messier, 2011). For *Candida albicans,* biofilm formation is a process that occurs in three stages: (i) an early phase characterized by adhesion of blastospores to the surface; (ii) an intermediate phase where yeast cells proliferate to cover a large surface area and have begun to produce extracellular polymers; and (iii) a maturation phase. Mature *C. albicans* biofilms are matrix entrenched and arranged into layers, with yeast cells attached to the surface with hyphae on the outer surface of the biofilm (Monteiro, 2009; Siqueira, *et al.,* 1997).

Even though a root canal filling might be seen as a state-of-the-art in science and technology, the possibility of a failure cannot be excluded. Studies investigating intraradicular microbiota associated with endodontic treatment failure reported the occurrence of microorganisms in a range from 35 % – 100 % of the cases. This initial adhesion is an essential step for microorganisms to colonize biomaterial surfaces and subsequently form biofilms, which can then lead to infections. In addition, testing the initial adhesion would give insight about the antimicrobial activity of these materials. However, root canal therapy failures caused by persistent microorganisms after therapy or microbial recontamination due to an inadequate coronal seal were shown to correlate with the occurrence of specific microorganisms (Senges, 2011).

Although the concept of apical periodontitis as a biofilm-induced disease has been built upon these observations, the prevalence of biofilms and their association with clinical and histopathologic findings have not yet been reported. In many biofilms, cells were abundant in the deepest layers (Ricucci and Siqueira, 2010). In some instances, bacterial colonization was restricted to the root canal wall surface, and no deep dentinal invasion was observed. This was probably because of the reduced number and small diameter or even the lack of dentinal tubules in certain regions of the apical root segment (Arias-Moliz, *et al.,* 2009). The pattern of bacterial community arrangement in the canal, which adhered to or at least was associated with the dentinal walls with cells encased in an extracellular amorphous matrix and often surrounded by inflammatory cells, is consistent with acceptable criteria to include apical periodontitis in the set of biofilm-induced disease (Sjogren, *et al.,* 1997).

2. Antimicrobial Irrigants: Behavior and Effectively

Irrigation in endodontic treatment serves the following purposes: i) lubrication, ii) dissolving the pulp remnants, iii) washing out debris created by canal instrumentation, iv) removing microorganisms in root canal (planktonic or biofilm) and v) cleaning the smear layer**.** The effectiveness of irrigation depends on the working mechanism(s) of the irrigant and the ability to bring the irrigant in contact with the microorganisms and tissue debris inside the root canal (Gu, *et al.,* 2009).

Numerous products are currently used as endodontic irrigants, such as sodium hypochlorite (NaOCl), chlorhexidine, calcium hydroxide [$Ca(OH)_2$] and saline solution. NaOCl is one of the most popular and widely used endodontic irrigants due to its antibacterial activity and its ability to dissolve necrotic tissue remnants. Hypochlorous acid (HOCl) exerts its effect by oxidation of sulphydryl groups within bacterial enzyme systems, thereby disrupting the microbial metabolism. The chlorhexidine solution causes the disruption of the osmotic balance by binding to bacterial cytoplasmic membranes, resulting in leakage of intracellular material. In addition, chlorhexidine gluconate presents a residual antibacterial effect on the infected canals, which is favorable (Gomes-Filho, 2008).

Chlorhexidine is a cationic biguanide that seems to act by adsorbing onto the cell wall of microorganism resulting in leakage of intracellular components. Chlorhexidine presents bacteriostatic effect at lower concentration; although in higher concentration it is bactericidal because of precipitation and/or coagulation of intracellular constituents. Its optimal antimicrobial activity is at pH 5.5 to 7.0, showing a broad-spectrum antimicrobial activity, targeting both Gram-positive and Gram-negative microorganisms. *In vitro* studies suggested that chlorhexidine and NaOCl have comparable antibacterial effect when used in similar concentration. In addition, chlorhexidine appeared to be a promising agent to be used as a final irrigant, because a clinical study showed that chlorhexidine solution (2 %), used as a final irrigant, significantly decreased bacterial loads in root canals

that had been irrigated with sodium hypochlorite during canal preparation. Additional advantages of chlorhexidine are its retentive character in root canal dentin and its relatively low toxicity. Despite its advantages, chlorhexidine activity is pH dependent and its activity is reduced by contact with organic matter; which does not happen to sodium hypochlorite (Wang, *et al.*, 2007).

When NaOCl is added to water, the HOCl solution is formed, containing active chlorine which is a strong oxidizing agent. Substantial evidence suggests that chlorine exerts its antibacterial effect by the irreversible oxidation of the sulphidrile (–SH) groups of essential enzymes, disrupting the metabolic functions of the bacterial cell. The ultrasonication of NaOCl solution increases its cleaning and antibacterial effects, because it accelerates the chemical reaction and potentiates the bactericidal effect (Gu, *et al.*, 2009). NaOCl proved highly effective in eradicating biofilms of *E. faecalis* after just 1 minute of exposure at a concentration of 0.00625 %, whereas the chlorhexidine solution was determined to be less effective, requiring at least 5 minutes of contact time in concentrations of 2 % or higher (Arias-Moliz, *et al.*, 2009).

Acid irrigating solutions such as ethylenediamine tetraacetic acid (EDTA) and citric and phosphoric acids have been recommended as adjuvants in root canal therapy due to their ability to remove the smear layer and enhance adhesion of resin-based endodontic sealers. In terms of antimicrobial properties, EDTA solution has exhibited minimal activity on *E. faecalis* planktonic and biofilm cultures. The fact that citric and phosphoric acids have shown bactericidal activity against *E. faecalis* suspensions leads to surmise that they might also act on biofilm bacteria, but it has not been widely investigated (Arias-Moliz, *et al.*, 2009).

Finally, silver colloid is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity. Silver diamine fluoride [$Ag(NH_3)_2F$] solution has been used to avoid caries initiation and progression. When silver diamine fluoride is used at 3.8 % as a root canal irrigant or interappointment temporary medication it has potential activity in the reduction of bacterial adhesion. It has been reported that *E. faecalis* was completely killed by silver diamine fluoride and NaOCl after exposure to these agents for 60 minutes (Hiraishi, 2010).

3. Antimicrobial Nanoparticles-Sealers: Evolution and Future

The target in the root canal treatment is to eliminate root canal bacteria, bacterial products and debris from the root canal system. Bacteria present within the dentinal tubules are inaccessible to the commonly used irrigants, medications (eg, calcium hydroxide) and filling cements (Grossman sealer) as a result of their limited fluency into the dentinal tubules. Common failures in endodontic treatments also can be caused by high levels of bacterial adhesion on the frequently used core material guttapercha, in particular when it is not completely covered by a sealer with presumably lower adhesive properties (Senges, 2011); so after a proper cleaning and intracanal irrigation it is necessary to fill the root canal to achieve successful and lasting endodontic treatments. Some nanoparticles have recently been incorporated into the formulation of the cements used in endodontics, highlighting their antimicrobial properties (Arzate-Vázquez, 2012).

4. Medicated Intracanal Sealers

Common filling materials are reported with limited antibacterial activity because most of the cement sealers lose effect in about one week. This factor contributes to the persistence of bacterial biofilm within the root canal system (Shrestha, et al., 2010; Kishen, *et al.*, 2008). The biofilm bacteria show higher antimicrobial resistance as compared with their free-floating planktonic counterparts. The resistance of biofilm bacteria has been attributed to the protective barrier provided by the extracellular polymeric matrix; therefore, agents that possess the ability to disrupt the extracellular polymeric matrix would allow greater penetration of antibacterial agents into the biofilm structure resulting in significant bacterial elimination (Shrestha, *et al.*, 2010).

Antibacterial activity of sealers might help to eliminate residual microorganisms that have survived the chemical-mechanical instrumentation and thereby improve the success rate of endodontic treatment. One of the challenges in endodontic research has been the lack of standardized *in vitro* and *in vivo* protocols for the testing of the antimicrobial effect of sealers. An ideal endodontic

sealer should be biocompatible and dimensionally stable; it should seal well and have a strong, long-lasting antimicrobial effect (Zhang, *et al.*, 2009).

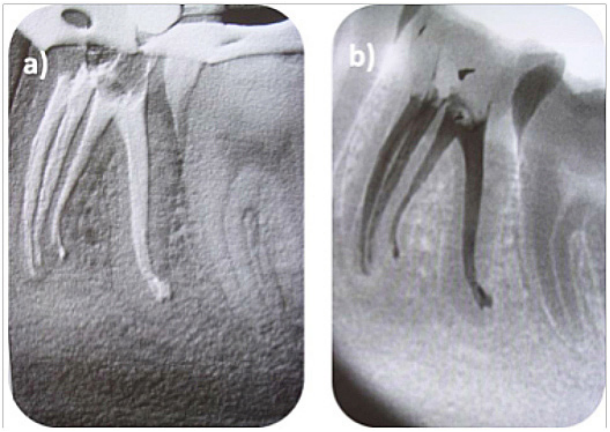
A great variety of endodontic sealers are available commercially, and they are divided into groups according to their chemical composition; eg. Rocanal 2® (zinc oxide eugenol based) (Image 3), AH Plus® (epoxy resin based), and RC Sealer® (polymeric resin based) and Sealapex® (calcium hydroxide-based). Analyses of the sealing ability of endodontic filling materials and techniques have received a great deal of attention. In this connection, leakage studies have been most commonly used. There are some methods for the evaluation of the sealing property of root canal filling materials such as colored dye penetration, bacterial penetration, analysis of radiolabeled tracer penetration, and electrochemical method. However, a universally accepted method for the evaluation of leakage does not exist yet (Kont, 2000).

Zinc oxide– eugenol-based cements are the most commonly used root canal sealers. Eugenol, which is the main constituent of oil of cloves, is weakly ionized and forms a dimeric structure with both inter-and intra-molecular hydrogen bonds. The zinc oxide–eugenol-based sealers display good flow properties compared to other root canal sealers; this also can be related to the considerable creep of zinc oxide– eugenol-based dental cements, compared to other dental cements. The endodontists can prepare the zinc oxide–eugenol-based root canal sealers at their chosen consistency according to the filling technique to use (Camps, Pommel and Bukiet, 2004).

In particular, calcium hydroxide is the main component of the Apexit Plus® Ivoclar Vivadent, Liechtenstein, an intracanal medication which results very effective in decreasing the initial microbial adhesion and shows lower adhesion among different commercial materials. Calcium hydroxide is the most widely used intracanal medication between appointments during the endodontic therapy because its alkaline pH (11-12) increases its antibacterial effect (Senges, *et al.*, 2011).

Currently, it has been observed that some nanoparticles (<100 nm) play an important role in the antimicrobial activity as well as the macro-particles into the endodontic fillers (Shrestha, *et al.*, 2010). It has been found that antibacterial nanoparticles have higher activity because of the higher surface area that enable to achieve a greater degree of interaction with the negatively charged surface of bacterial cells (Kishen, *et al.*, 2008).

Image 3.



Radiovisiography intraoral image where it can be noted: a) root canals filled with zinc oxide eugenol based sealer, b) control image taken with different contrast technique, 6 months after the final filling.

Previous studies have reported significant antibacterial effect of chitosan nanoparticles (CS-Np) and zinc oxide nanoparticles (ZnO-Np) against planktonic *Enterococcus faecalis*. The effectiveness of the CS-Np and ZnO-Np to reduce and disrupt the biofilm structure was evident by confocal microscopy techniques; moreover the antibacterial property of these nanoparticles was retained even after aging for 90 days. Therefore, CS-Np and ZnO-Np possess a potential antibiofilm capability, and further studies with *ex vivo* or *in vivo* models are required to validate its potential application in nonsurgical root canal treatment (Shrestha, *et al.*, 2010).

Chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose] nanoparticles are nontoxic biopolymers derived by the deacetylation of chitin compounds (Shrestha, *et al.*, 2010) and it appears to be economically attractive because chitin, the source of chitosan, is the second most abundant biopolymer in the nature after cellulose (Avadi, *et al.*, 2004). Chitin is a natural polymer occurring in the exoskeleton of the crustaceans and it is a bioadhesive that easily binds to negatively charged surfaces with excellent antimicrobial and antifungal activities (Kishen, *et al.*, 2008). Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi

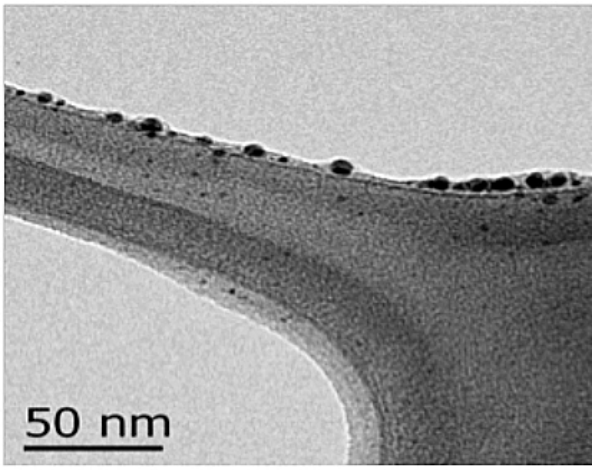
and yeasts. Chitosan has wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria (Kong, *et al.,* 2010); because it acts by altering the bacterial cell permeability resulting in the cell death (Kishen, *et al.,* 2008) and it has shown lower toxicity toward mammalian cells (Kong, *et al.*, 2010). In addition, chitosan exerts an antifungal effect by suppressing sporulation and spore germination (Kong, *et al.*, 2010).

Variations in the bactericidal effect of chitosan have arisen because several reasons: i) microbial factors, related to microorganism species and cell age; ii) intrinsic factors of chitosan, including positive charge density, molecular weight, concentration, hydrophilic/hydrophobic characteristic and chelating capacity; iii) physical state, water-soluble and solid state of chitosan; iv) environmental factors, involving ionic strength in medium, pH, temperature and reactive time (Zhang, *et al.,* 2009; Kong, *et al.*, 2010). Medical chitosan applications require, as a starting material, a low molecular weight chitosan with a high solubility and low viscosity in water at physiologically acceptable pH values (Thikonov, *et al.*, 2006).

Chitosan possesses high chelating capacity for various metal ions (including Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+} and Cu^{2+}) in acid conditions, and it has been widely applied for the removal or recovery of metal ions in different industries. Metal ions that combine with cell wall molecules of microorganism are crucial for stability of the cell wall. Chitosan-mediated chelation of such metal ions has often been implicated as a possible mode of antimicrobial action. Chitosan definitely shows stronger inhibitory effect at lower pH, with inhibitory activity weakening with increasing pH (Kong, *et al.,* 2010). Thus, the antibacterial properties of nanoparticles mixed with zinc oxide–eugenol–based filler have been studied. The ability of nanoparticles-filler in the prevention of the bacterial adherence was examined; the findings showed that the subsequent treatment of dentin with zinc oxide added with chitosan nanoparticles produced a significant reduction in the number of *E. faecalis* cell adhering to dentin. Root canal dentin irrigated with chlorhexidine and later treated with nanoparticles displayed a consistent reduction (97 %) in bacterial adherence (Kishen, *et al.,* 2008)

The large increase in the number and occurrence of antibiotic-resistant bacterial strains has prompted a renewed interest in the use of silver as an antibacterial agent. Silver nanoparticles are known for their broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, protozoa and certain viruses. The antimicrobial activities of silver nanoparticles (AgNPs) onto impregnated dressing and catheters commercially available have been reported (Monteiro, *et al.,* 2009), the lower the particle size, the higher antimicrobial effect it has. (Image 4) (Kishen, *et al.*, 2008).

Image 4.



Transmission electron microscopy showing spherical silver nanoparticles with particle size in 2-10 nm. Silver nanoparticles are added into the root canal cements for improving the antimicrobial effect.

It is possible that AgNPs act similarly to some other antimicrobial agents used for the treatment of bacterial infections showing four different mechanisms of action: interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, and inhibition of a metabolic pathway (Guzman, Dille & Godet, 2012).

The exact antibacterial action of AgNPs is not completely understood. Some researches show that electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is crucial for the activity of nanoparticles as bactericidal materials. Several possible mechanisms have been proposed that involve the interaction of silver with biological macromolecules such as enzymes and deoxyribonucleic acid (DNA) through an electron-release mechanism. It is believed that DNA loses its replication ability and cellular proteins are in-

activated upon Ag^+ treatment (Guzman, Dille and Godet, 2012). Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. It is necessary to emphasize that the silver nanoparticles have bactericidal effects resulting not only in inhibition of bacterial growth but also in killing bacteria (Panáček, *et al.*, 2006)

5. Cytocompatible Nanoparticles

The small size of nanoparticles, results in unique chemical and physical characteristics leading to advanced structural properties compared to the original bulk substance. However, the same characteristics making nanoparticles so attractive for their exploitation in new products have led to concerns that nanoparticles may possess a risk for humans and the environment. For example, the remarkably higher surface to volume ratio of nanoparticles enhances their surface properties thereby increasing the interaction with serum, saliva, mucus, or lung lining fluid components, and makes nanoparticles potentially more reactive than larger particles; nanoparticles may interact in new unpredicted ways with biological systems (Beer, *et al.*, 2012).

Silver nanoparticles were found to induce reactive oxygen species (ROS) and release of cytochrome C into the cytosol and translocation of Bax protein to mitochondria. These observations indicated that AgNPs mediated apoptosis was mitochondria-dependent in fibroblasts. Other researchers further suggested that AgNPs induced a p53 mediated apoptotic pathway through which most of the chemotherapeutic drugs trigger apoptosis (Beer, *et al.*, 2012; Gomes-Filho, *et al.*, 2010).

ROS generation and oxidative stress appear to be two likely mechanisms of AgNPs toxicity. Oxidative stress occurs when generation of ROS exceeds the capacity of the anti-oxidant defense mechanism. Depletion of glutathione and protein bound sulfhydryl groups and changes in the activity of various antioxidant enzymes indicative of lipid peroxidation have been implicated in oxidative damage (Guzman, Dille and Godet, 2012). ROS and oxidative stress elicit a wide variety of physiologic and cellular events including stress, inflammation, DNA damage and apoptosis (Gomes-Filho, *et al.*, 2010; Guzman, Dille and Godet, 2012). A number of investigators reported that cytotoxicity, DNA damage and apoptosis induced by AgNPs were mediated through membrane lipid peroxidation, ROS and oxidative stress.

Studies have shown that the lungs and liver are major target tissues for prolonged AgNPs exposure, in studies of AgNPs on rat liver cells, a significant depletion of the antioxidant glutathione, reduced mitochondrial membrane potential and increased ROS were reported. These findings suggested that AgNPs cytotoxicity is likely mediated through oxidative stress in liver cells (Guzman, Dille and Godet, 2012).

A number of studies have demonstrated that mitochondria are the major targets of AgNPs, and so they are considered as major cellular compartments of relevance for nanoparticles toxicity. Following the structural damage to mitochondria from nanoparticles exposure, a loss of mitochondrial membrane integrity, opening of the permeability transition pore, ROS production and cell death may occur (Guzman, Dille and Godet, 2012; Sharma, Yngard and Lin, 2009; Martinez-Gutierrez, *et al.*, 2010).

6. Irrigants and Nanoparticles-Fillers

The ideal irrigants for root canal should have broad antimicrobial spectrum and high efficiency against microorganisms as well as being: i) systemically nontoxic, ii) non-caustic to periodontal tissues and iii) little potential to cause an anaphylactic reaction (Gu, *et al.*, 2009).

Endodontic irrigants should induce lower or no inflammatory response in surrounded tissues; and in this sense, the 2.0 % NaOCl and 2.0 % chlorhexidine gluconate solutions have been presented in the moderate connective tissue inflammatory reaction. Although it has the largest number of inflammatory cells between days 2 and 14, the presence of inflammatory cells is significantly reduced after 30 days. Evaluating the toxic effect of NaOCl solutions; tissue regeneration occurred at a slower rate than the sites treated with 2.0 % chlorhexidine gluconate solutions; even the NaOCl (5.25 %) solution promotes irritating effect on the periapical tissues with a higher inflammatory response that remained up to the 30-day and in some cases the foreign body granuloma formation occurred (Gu, *et al.*, 2009).

Chitosan is an ideal biopolymer that has received atten-

tion as a material for nanoparticles for the last decade and it is a potentially useful pharmaceutical material owing to its good biocompatibility, low toxicity (Tan, Huang and Zhang, 2007), biodegradability and ability to modify its physical and chemical properties to obtain the desired and appropriate features (Manchanda and Nimesh, 2010). Chitosan-based materials have received much attention in the case of antimicrobial mode of action clarifying the molecular details of the underlying mechanisms and their relevance (Kong, *et al.,* 2010). The interaction of chitosan to the cell was reported when evaluated chitosan nanoparticles 60 nm in size by the cytotoxic assay on primary myoblast cells suggesting no cytotoxic effect when it is used in biological applications (Ichikawa, Iwamoto and Watanabe, 2005); while other cytotoxicity studies done on HEK 293 cells reveals that chitosan nanoparticles have high cell viability (Sharma, Yngard and Lin, 2009)

Nanomaterials may provide solutions to technological and environmental challenges in the areas of medicine (Martinez-Gutierrez, *et al.,* 2010) including dentistry. Some studies have shown that the size, morphology, stability and properties (chemical and physical) of the metal nanoparticles are strongly influenced by the experimental conditions, the kinetics of interaction of metal ions with reducing agents, and the adsorption processes of stabilizing agent with metal nanoparticles (Chua, *et al.,* 2012).

Incorporation of AgNPs with other materials is an attractive method of increasing compatibility for specific applications (Ichikawa, Iwamoto and Watanabe, 2005). Antibacterial activity of the silver-containing materials can be used; for example, in medicine to reduce infections in burn treatment as well as to prevent bacteria colonization on prostheses, catheters, vascular grafts, dental materials, stainless steel materials, and human skin (Guzman, Dille and Godet, 2012). However, the use of medical devices containing silver must be undertaken with caution, since a concentration-dependent toxicity has been demonstrated. It has been reported, that concentrations of silver nanoparticles between 5 g/mL and 10 g/mL induced necrosis or apoptosis of mouse spermatogonial stem cells as the use of silver and the number of available silver-based products has increased, it is becoming important to clarify the efficacy of silver against different microorganisms and biofilms (Monteiro, *et al.,* 2009).

The silver nanoparticles have been added to the endodontic sealers formulation for improving the antimicrobial behavior and it was found that the addition of nanoparticles did not deteriorate the flow characteristics of the sealer but at the same time it decreased viscosity, leading to enhanced flow of the sealer. It is important to note that the original endodontic sealer (zinc oxide–eugenol) did not show any ability to leach out antibacterial components. However, the relevance of this finding in an *in vivo* endodontic milieu is not well established (Kishen, *et al.,* 2008).

Cytotoxicity assays monitoring DNA damage and cell viability were evaluated using human-derived monocyte cell lines. The silver-coated nanoparticles having a size of 20–25 nm were the most effective among all the nanoparticles assayed against the tested microorganisms. In addition, these nanoparticles showed no significant cytotoxicity, suggesting their use as antimicrobial additives in the process of fabrication of ambulatory and nonambulatory medical devices (Manchanda and Nimesh, 2010).

7. Concluding Remarks

The present article reviewed the antimicrobial potential of irrigants and sealers used in the clinical practice and a new proposal of nanomaterials valued in vitro. The used root canal irrigants and sealants are far from ideal, latest enhancements focus on the use of new antimicrobial agents such as chitosan nanoparticles and silver nanoparticles, which have shown broad anti-microbial effect against bacteria and fungi typical of the biofilm and that turn out to be not cytotoxic. Further investigations are required to specify the conditions of size, concentration and ideal morphology of nanoparticles in general to optimize their antimicrobial effect mainly by acting against the resistant biofilm formers microorganisms such as E. faecalis y C. albians.

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