Chlorhexidine and its applications in Endodontics: A literature review

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ABSTRACT

This study aims at presenting the properties of chlorhexidine used as an auxiliary chemical substance for endodontic instrumentation: structure and mechanism of action, substantivity, tissue solvent effect, chlorhexidine x sodium hypochlorite interaction, cytotoxicity, action over biofilm, antibacterial activity, antifungal activity, intracanal dressing, rheological action and allergic reactions. In Dentistry, chlorhexidine has been proved effective and safe against bacterial plaque since 1959. In Endodontics, it has been recommended in liquid or gel form, at different concentrations (usually 2%), as root canal irrigant and dressing (alone or associated with other substances). Additionally, it may be applied as an antimicrobial agent at all stages of root canal preparation, including disinfection of the operative field, removal of necrotic tissues before determining the root length, chemical-mechanical preparation before foraminal clearance and enlargement, disinfection of obturation cones; to shape the main cone with gutta-percha, to remove gutta-percha during retreatment, to disinfect the prosthetic space; etc. It is reasonable to conclude that chlorhexidine, at different concentrations, has an antimicrobial activity against Gram-positive as well as gram-negative bacteria and fungus. Its antimicrobial activity, increased by the substantivity effect, does not have the ability of solving tissues, which is overcome by the rheological action of its gel form that lubricates the endodontic instrumentation used. Its biocompatibility is acceptable with relative absence of cytotoxicity.

Keywords: Chlorhexidine. Microorganism control agent. Endodontics.

How to cite this article: Marion J, Pavan K, Arruda MEBF, Nakashima L, Morais CAH. Chlorhexidine and its applications in Endodontics: A literature review. Dental Press Endod. 2013 Sept-Dec;3(3):36-54.

The authors inform they have no associative, commercial, intellectual property or financial interests representing a conflict of interest in products and companies described in this article.

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Submitted: September 04, 2013. Revised and accepted: September 06, 2013.

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Introduction

Most bacteria found in infected root canals can be removed by the simple mechanical action of endodontic instrumentation. Nevertheless, despite thorough mechanical instrumentation, organic residues and bacteria located deeply inside the dentin tubules cannot be reached due to the anatomic complexity of root canals.[1,2] Irrigation solutions are indicated to aid mechanical preparation and pulp space disinfection. Thus, several substances have been used not only to remove debris and necrotic pulp tissue during and immediately after root canal preparation, but also to help eliminate the microorganisms that could not be reached by mechanical instrumentation.[3] The search for an ideal substance for root canal irrigation has motivated researchers since the beginning of Dentistry. Chemical agents chosen to function as endodontic irrigants have four major properties: antimicrobial activity; organic tissue dissolution that favors debridement of the root canal system; and absence of toxicity against periapical tissues.[1,2,4] Most substances used to irrigate the root canal are liquid: sodium hypochlorite (NaOCl), chlorhexidine gluconate — also known as chlorhexidine digluconate or simply chlorhexidine (Chlorhexidine) —, 17% EDTA, citric acid, MTDA and 37% phosphoric acid solution.[5]

Sodium hypochlorite is the most popular irrigation solution due to its antimicrobial and physicochemical properties.[6,7] The antimicrobial efficacy of NaOCl is due to its high pH (the action of hydroxyl ions) similar to the mechanism of action of calcium hydroxide.[8] The high pH of NaOCl interferes in the integrity of the cytoplasmic membrane with an irreversible enzymatic inhibition that causes biosynthetic alterations in cellular metabolism and destruction of phospholipids, observed during lipid peroxidation. The antimicrobial activity of NaOCl leads to an irreversible enzymatic inhibition of bacteria, which originates hydroxyl ions, as well as to chloramination action.[2] Despite being an effective antimicrobial agent and an excellent organic solvent,[9] NaOCl is known for being highly irritant to periapical tissues,[10] especially at high concentrations.[11] For this reason, the search for another irrigation solution, with lower potential in inducing adverse effects, proves feasible.[2,12]

Thus, irrigation solutions with antibacterial activity and biocompatibility, as it is the case of chlorhexidine, have been recommended to treat infected root canals. The antibacterial effect and long-term action of 2% chlorhexidine digluconate[13-17] led researchers to indicate its use for endodontic treatment.[15,16,18]

Chlorhexidine is a cationic biguanide that acts by adsorption in the bacterial wall of a microorganism, causing leakage of the intracellular components. Due to being a strong base, low-concentration chlorhexidine has a bacteriostatic effect; however, at higher concentrations, it produces a bactericidal effect. Chlorhexidine digluconate has a slightly acidic pH that varies from 5.5 to 6.0, with the ability to donate protons.[19]

Chlorhexidine was first introduced in the late 40s, when scientists, in the search for new agents against malaria, formulated a group of compounds with a broad antibacterial spectrum, known as polibiguanides.[20,21] Chlorhexidine was registered in 1954 by the Imperial Chemical Co. Ltd. (Macclesfield, United Kingdom), under the trademark Hibitane. Due to its biocompatibility and broad antibacterial activity, it was the first antiseptic internationally accepted for skin, wound and mucosa cleansing.[22] Since then, chlorhexidine has been used for several medical purposes, namely: gynecology, urology and ophthalmology, as well as for the treatment of skin burns and disinfection.[23]

In Dentistry, chlorhexidine has been proved effective and safe against bacterial plaque since 1959. In the 70s, it was commercialized in Europe as a 0.2% mouthwash solution and in 1% gel.[21,23]

Chlorhexidine may be applied as an antimicrobial agent at all stages of root canal preparation, including disinfection of the operative field, during root canal instrumentation, removal of necrotic tissues before determining the root length, chemical-mechanical preparation before foraminal clearance and enlargement, as an intracanal dressing (alone or in association with other substances), disinfection of obturation cones; to shape the main cone with gutta-percha, to remove gutta-percha during retreatment, to disinfect the prosthetic space; etc.[5]

Viscous irrigants, such as glycerin-based ones, have low solubility. As a result, they leave residues at the dentin walls, which hinders the final obturation of the root canal system.[12,24] However, Natrosol is a highly efficient non-ionic inert gel that is hydro-soluble and broadly used in cosmetic products based on cationic substances.[2]

Chlorhexidine gel has been widely used in Dentistry. It yields satisfactory results for cavity control, reducing *Streptococcus mutans* and *Lactobacillus*, acting as auxiliary in periodontal therapy, and controlling the growth of Gram-positive and Gram-negative bacteria.[25]

Ferraz et al[12] demonstrated that 2% chlorhexidine gel is highly advantageous in comparison to 2% chlorhexidine solution, even though both of them have similar antimicrobial, substantivity and biocompatibility properties. Chlorhexidine gel lubricates the root canal walls, which reduces friction between the endodontic file and the dentin surface. As a result, it favors instrumentation, improves file performance and reduces the risk of file fracture inside the root canal. Additionally, chlorhexidine gel allows better debridement and, as a consequence, compensates its inability in organic tissue dissolution.[2,26] Chlorhexidine gel leaves the majority of dentin tubules open as a result of its viscosity that keeps debris in suspension and reduces the formation of smear layer, which does not occur with chlorhexidine liquid. Furthermore, the active ingredient of chlorhexidine gel establishes long-term contact with microorganisms and, as a consequence, inhibits their growth.[27] When chlorhexidine gel is used for the mechanical preparation of a root canal, the irrigant solution of choice must be saline solution or distilled water.

In this context, this study aims at conducting a literature review that presents the properties of chlorhexidine used as an auxiliary chemical substance for endodontic instrumentation.

Literature review

Microorganisms have been broadly recognized as the main etiologic factor of periapical bone lesions.[28] Their persistence in the apical area of obturated root canals is responsible for the majority of endodontic treatment failures.[29,30] Thus, microbial control is of paramount importance for an effective endodontic treatment,[28] of which success relies on the elimination of microorganisms from infected root canals.[1]

Most bacteria found in infected root canals can be removed by the simple mechanical action of endodontic instrumentation. However, despite thorough mechanical instrumentation and the several techniques available, organic residues and bacteria located deeply inside the dentin tubules cannot be reached due to the anatomic complexity of root canals.[2,31] For this reason, chemical treatment of the root canal system proves necessary.

According to several authors,[11,32,33,34] the ideal auxiliary substance must have the property of: leaving debris in suspension, lubricating endodontic instruments, dissolving organic tissue, developing antibacterial activity during instrumentation, substantivity, exerting chelating action, promoting cleaning of inaccessible areas, being biocompatible at concentrations that fulfill these properties within a viable clinical time, removing the smear layer formed during instrumentation, having low surface tension, neutralizing action and bleaching effect, having no color alterations, being of easy application, removal, handling and storage, accessible, inexpensive and of extended useful life.

Several substances have been used to irrigate the root canal system, namely: sodium hypochlorite (NaOCl), chlorhexidine gluconate — also known as chlorhexidine digluconate or simply chlorhexidine —, 17% EDTA, citric acid, MTDA and 37% phosphoric acid solution.[5] Sodium hypochlorite, at different concentrations, is the most commonly used substance due to its triple mode of action: necrotic tissue dissolving ability attributed to its high alkalinity; antibacterial properties related to hypochlorous acid formation in chlorine solution; and fat saponification.[35]

Sodium hypochlorite is a halogenated compound of which first use was registered in 1972 under the name of "Javele's water". It was obtained by mixing NaOCl with potassium. In 1820, Labarraque obtained sodium hypochlorite at a concentration of 2.5% of active chlorine. In the early XX century, during World War I, sodium hypochlorite was used to treat infected wounds. In 1915, Dakin[36] proposed a new concentration for the solution (0.5%) because, according to the author, wounds treated with 2.5% sodium hypochlorite took too long to heal due to the high content of sodium hydroxide.[36,37] In Endodontics, its use was first proposed by Coolidge, in 1919; first employed by Walker, in 1936, due to its excellent tissue dissolving ability as well as its antimicrobial efficacy,[39] and disseminated by Grossman.[38,40] It has been employed in Endodontics for more than 60 years as an irrigation solution during chemo-mechanical preparation of the root canal system.[9] Despite NaOCl excellent antimicrobial activity and tissue dissolution ability, it causes irritation to periapical tissues,[41] it is caustic and causes clothes stain and instruments corrosion,[42] especially at high concentrations.[11] According to Ramos and Bramante,[43] biocompatibility is one of the main desirable properties of an irrigation solution. For this reason, the search for another irrigation solution with lower potential in inducing adverse effects proves feasible.[2,12]

Among different alternatives, chlorhexidine has proved to be an effective antimicrobial agent acting inside root canals, showing a great potential to be used as irrigant or intracanal dressing. It is also recommended for cases of incomplete root formation or hypersensitivity to sodium hypochlorite due to its low toxicity. Chlorhexidine is found in the form of liquid (water solution) or gel, at concentrations that vary from 0.2 to 2%.[35,44]

It is characterized as a cationic detergent of the biguanide class. It is available as acetate, hydrochloride and digluconate which is the most used format.[45] Chlorhexidine was first introduced in the late 40s when scientists were searching for new agents against malaria.[20,21] In 1954, it was first used as an antiseptic to treat skin wounds[46] under the trademark Hibitane registered by the Imperial. Ltd. (Macclesfield, United Kingdom).[22]

In Dentistry, chlorhexidine has been proved effective and safe against bacterial plaque since 1959. It was first tested by Löe and Schiott[47] who demonstrated that 0.2% chlorhexidine mouthwash twice a day is effective to decrease biofilm growth and gingivitis development for a period of 21 days.[45] Initially, it was commercialized in Europe, in the 70s, as a 0.2% mouthwash solution and in 1% gel.[21,25]

Due to its broad antibacterial spectrum, it has been widely used in Periodontology. In Endodontics, it has been recommended as digluconate salt, liquid or gel at different concentrations, as well as root canal irrigant[13,15,18,23,48,50,51] or as intracanal dressing.[13,53-57]

In this context, this literature review highlights 11 major points related to chlorhexidine, so as to facilitate understanding. The extensive literature on chlorhexidine determined that discussions should be restricted to factors commonly focused by *in vivo* studies and literature reviews. To this end, the following databases were used for research: MEDLINE, PubMed, BBO, Lilacs, SciELO, websites available on the internet and the library archives of the School of Dentistry / Piracicaba (FOP-UNICAMP).

Structure and mechanism of action

The structural formula of chlorhexidine consists of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain.[22] Classified as a cationic detergent, this biguanide is a strong base which is practically insoluble in water. For this reason, it is prepared in the form of salt,[23] which increases its solubility. In Dentistry, its most commonly used form is chlorhexidine digluconate salt in water solution.[13,22] The bactericidal effect of the drug is due to its cationic molecule binding to extra-microbial complexes and negatively charged microbial cell walls, entering in the cell by active or passive transportation.[58] At high concentrations (2%), chlorhexidine has a bactericidal effect due to precipitation and/or coagulation of thecytoplasm of bacterial cells, probably caused by proteincross-linking, resulting in cell death.[59,60] At lower concentrations (0.2%), chlorhexidine has a bacteriostatic effect, which causes inhibition of the membrane function. This effect remains for several hours after application due to its excellent substantivity (residual effect).[49] Solutions are usually colorless as well as odorless.

When aqueous, chlorhexidine seems to be more stable for pH varying from 5 to 8. pH values above 8 lead to precipitation. In an acidic pH, chlorhexidine solution loses stability and, as a consequence, deterioration of its properties occurs. Its antibacterial effect is excellent for pH values varying from 5.5 to 7.[48,61] Chlorhexidine is found in the form of solution, dentifrices, varnishes and gel.[62]

Tasman et al[63] assessed the surface tension of different irrigation solutions: distilled water; 2.5% sodium hypochlorite; 5% sodium hypochlorite; 17% EDTA; 3% hydrogen peroxide; 3% citanest-octapressin and

0.2% chlorhexidine. The ring method was employed to this end. The authors yielded the following results in ascending order: chlorhexidine; 2.5% hypochlorite; 5% hypochlorite; 17% EDTA; 3% citanest-octapressin; hydrogen peroxide; saline solution and distilled water. The authors concluded that the low surface tension of chlorhexidine favors its penetration into the dentin tubules.

According to Ferraz et al,[2] chlorhexidine gluconate had lower surface tension in comparison to sodium hypochlorite and EDTA. The use of chlorhexidine associated with a gel vehicle provides dentin walls free of waste produced by instrumentation as a result of the mechanical properties of gel.

Substantivity

According to Hortense et al,[64] substantivity is the capacity chlorhexidine has to remain active in the surface where it is applied (tooth, gingiva and oral mucosa surfaces negatively charged). It is slowly released, avoiding salivary flow to neutralize its action. Substantivity is an important property for treatment of dental plaque infections, since antimicrobial agents need some time to neutralize/kill a microorganism.[22]

In Endodontics, the residual antibacterial effect of chlorhexidine is due to its capability to bind to hydroxyapatite.[65] Therefore, a gradual release of chlorhexidine could maintain a constant level of molecules, which is enough to create a bacteriostatic scenario inside the root canal for a long period of time.

Parsons et al[48] conducted one of the first studies recommending the use of chlorhexidine for endodontic purposes. The authors observed the adsorption and release of chlorhexidine solution by bovine pulp and dentin samples, as well as its antibacterial properties after a deliberate contamination caused by *Streptococcus faecalis*. Results revealed that, after the samples were treated with chlorhexidine, no contamination was observed within 48 and 72 hours of bacterial exposure. This confirmed the residual effect of chlorhexidine.

Other studies have been conducted to assess the substantivity of chlorhexidine. Their results showed that this activity can last 48 hours,[18] 72 hours,[16] 7 days (chlorhexidine liquid and gel),[66] 21 days[17] or 4 weeks.[67] Rosenthal, Spangberg and Safavi[68] assessed the substantivity of 2% chlorhexidine in root canal system and its long-term efficacy in comparison to its antimicrobial effect. Their results revealed that chlorhexidine remains in the dentin of root canals with its antimicrobial effect for more than 12 weeks.

According to Messer and Chen,[69] this property differs chlorhexidine from other disinfectants that quickly dissipate and have no residual antibacterial effect. Khademi, Mohammadi and Havaee[67] highlight that only chlorhexidine and tetracycline have the aforementioned property.

Tissue dissolving effect

Several studies have searched for a product that meets the properties necessary for a root canal irrigant: antimicrobial activity, non-toxic to periapical tissues, soluble in water and organic matter dissolving ability.[31] In 1941, Grossman and Meiman[70] demonstrated the importance of tissue dissolving ability of an endodontic irrigant, determining that success of endodontic treatment relies on pulp tissue elimination from the root canal. Zehnder[19] corroborates Grossman and Meiman[70] and asserts that the ideal cleaning of root canals is crucial for endodontic treatment, given that removal of tissues and bacterial residue would prevent the tooth from becoming a source of infection. Therefore, the necrotic tissue dissolving ability of irrigation agents was assessed. An *in vitro* study revealed that 1% sodium hypochlorite had a substantial dissolution capacity, unlike 10% chlorhexidine.[71] According to Moorer and Wesselink,[72] tissue dissolution depends on the frequency of agitation, the amount of organic matter in relation to the irrigant, and on the tissue surface area available for contact with the irrigant. Okino et al[73] assessed the tissue dissolving ability of sodium hypochlorite at different concentrations, 2% chlorhexidine digluconate water solution, chlorhexidine gel and distilled water. Fragments of bovine pulp were submerged in 20 mL of each solution. Both distilled water and chlorhexidine solutions did not dissolve the pulp during the six hours of the experiment.

Considering the experiments performed, it can be concluded that a disadvantage of chlorhexidine is its inability to dissolve tissues.[31]

Interaction between chlorhexidine and sodium hypochlorite

An *in vivo* study conducted by Zamany[74] employed two therapeutic protocols in which, after chemomechanical preparation with NaOCl, a final irrigation with 4 mL of saline solution or 2% chlorhexidine was performed during 30 seconds. Evaluation was carried out by means of culture mediums and biological indicators collected from tooth canals. The chlorhexidine protocol produced a positive culture in one out of 12 cases, whereas the saline solution protocol produced a positive culture in seven out of 12 cases. The use of 2% chlorhexidine digluconate as an extra irrigant used after biomechanical preparation improved the efficiency of endodontic therapy with regard to antimicrobial activity.

For treatment before root canal filling, Zehnder[19] recommends irrigation with sodium hypochlorite to dissolve organic tissue, irrigation with EDTA to eliminate the smear layer and irrigation with chlorhexidine to increase antimicrobial spectrum and substantivity. Despite the visible increase in antimicrobial efficacy produced by the combination of irrigants,[41] chemical interactions, such as precipitation and color change that result from a combination between NaOCl and chlorhexidine, must be taken into account.[19,26,75] This corroborates the study conducted by Basrani et al[76] who sought to determine the minimum concentration of sodium hypochlorite causing pigmentation and precipitation when associated with 2% chlorhexidine. The resultant precipitate was qualified and quantified. All sodium hypochlorite solutions in combination with 2% chlorhexidine digluconate led to color alterations, even with NaOCl at low concentrations (0.023%). The formation of precipitate was also observed until the sixth dilution (0.19%). Both pigmentation and precipitation were directly proportional to the concentration of sodium hypochlorite. By-products were formed in the mixtures with 3% and 6% sodium hypochlorite. One example is the formation of parachloraniline, a fragment that results from hydrolysis of chlorhexidine digluconate. In other words, a by-product that theoretically forms another by-product. Fragmentation occurs in the bond between carbon and nitrogen (guanidine group) of which dissociation requires little energy. The clinical importance of these findings relies on the pathological potential of parachloraniline, as well as on other by-products that result from the mixture. Parachloraniline has a carcinogenic potential and causes methemoglobinemia and cyanoses, being cytotoxic.[77] Other by-products might have pathological action related to their own molecular character, as it is the case of action exerted by higher reactivity (free radicals). The formation of precipitate may be explained by the acid-base reaction that results from mixing sodium hypochlorite and chlorhexidine.[31]

The precipitate that results from mixing sodium hypochlorite and chlorhexidine is also known as fluconation.[78] Basrani et al[76] observed that it produces an orangish-brown solution which, once in the pulp chamber, chemically stains the dentin tubules and, as a consequence, changes tooth color[78-81] and interferes in root canal filling.[28,82] A spectrophotometric analysis revealed the presence of calcium, iron, magnesium, copper, zinc and manganese in the precipitate.[78] According to Heling and Chandler,[83] associating chlorhexidine with EDTA also forms a milky-white precipitate. When combined with saline solution and ethanol, they produce salt. Thus, when sodium hypochlorite is used as an irrigation solution during mechanical preparation, chlorhexidine may be used as a final irrigant or intracanal dressing only after sodium hypochlorite is completely removed from the root canal,[82] so as to avoid interaction between solutions.[5] As complementary irrigation solutions, distilled water and saline solution are recommended.

Cytotoxicity

Chlorhexidine is stable and has low citotoxicity.[6] It is minimally absorbed by the mucosa and skin, it is well tolerated in animals, when administered via parenteral and intravenously, it seems not to cross the placental barrier, it does not cause systemic toxic side effects or alterations in the oral microbiota.[84-88] With regard to the metabolic pathways of chlorhexidine, whenever ingested, it reduces plasma levels and is excreted in feces (90%) and urine (10%). The frequency of metabolic segmentation by oral intake is also low, with no evidence of parachloraniline formation. When carried in the bloodstream of dogs, it is metabolized by the liver and kidney, producing polar metabolites, while chlorhexidine remains intact in the bile.[87]

Tanomaru Filho et al[6] assessed the inflammatory response of different endodontic solutions used in rats. 0.5% sodium hypochlorite, 2% chlorhexidine digluconate and saline solutions were injected in the peritoneal cavity of the animals which were killed after 4h, 24h, 48h and seven days. Results revealed that sodium hypochlorite induced inflammatory response, whereas chlorhexidine digluconate did not provoke any significant response. In 2005, Ribeiro et al[89] assessed the genotoxicity (potential damage to DNA) of formocresol, paramonochlorophenol, calcium hydroxide and chlorhexidine against the ovary cells of Chinese hamsters. The results revealed that none of the agents damaged the DNA. Faria et al[90] assessed the cytotoxicity of chlorhexidine digluconate by means of observing tissue lesions (edema/inflammation) in rats' paws. Assessment was complemented by histopathological examination and analysis of cell death and stress in culture of fibroblasts. Edema (inflammation) was observed as a result of exposing the lesions to chlorhexidine digluconate at different concentrations (0.125; 0.25; 0.5 and 1%). Edema subsided after 14 days at the two lowest concentrations. At 0.125%, no tissue necrosis was observed despite mild inflammation, whereas at 0.25%, small foci of necrosis were found. Edema persisted after 14 days at the two highest concentrations. Inflammation and larger foci of tissue necrosis were also observed. The authors concluded that chlorhexidine digluconate may produce an adverse effect on the resolution of apical periodontitis. Additionally, their results point to higher biocompatibility in concentrations equal to or less than 0.25%. Furthermore, lower concentrations are characterized by promoting cell apoptosis, whereas higher concentrations cause stress and cellular necrosis.

Thus, the concentrations of chlorhexidine clinically used have acceptable biocompatibility,[31] with relative absence of cytotoxicity.[15]

The first studies about the toxicology of chlorhexidine were conducted by Foukes[91] who established the lethal dose of chlorhexidine orally and intravenously taken, and tolerance to chronic administration. The author concluded that chlorhexidine has unusually low toxicity for both, animals and humans. Additional research conducted by Davies and Hull[84] confirmed the findings of other authors, determining the lethal dose of 50 (LD 50) for chlorhexidine applied by intravenous injection (22 mg/Kg/day) , and LD 50 (1800 mg/kg/day) for oral administration. These results were obtained from experiments carried out with species of rodents (rabbits and mice) and ruminants (cattle). Hugo and Longworth[93] found no harmful effect for chlorhexidine digluconate orally taken. To test the carcinogenic potential, four groups with 224 rats each were used. The animals received doses of 5, 25 or 50 mg/kg of body weight and were tested for two years. By the end of the dosage, peak levels dropped by half within one to two weeks. Chlorhexidine levels in the brain, lung, liver, kidney, mesenteric nodes and other lymph nodes, as well as in the blood were determined at regular intervals during the experiment and after the end of administration during three, six and nine weeks. No histological changes were found. The concentration of chlorhexidine in the liver was high in the final controls, but decreased to half after one and two weeks. There was no incidence of neoplasm in the control and treated groups. The extremely low acute oral toxicity found in animals has been confirmed in humans in the last 30 years of experience, with unrestricted use. Pereira[94] conducted a research on acute and chronic toxicity of chlorhexidine digluconate orally taken by mice and found an increase in weight gain in comparison to the control group, significant reduction in the number of deaths attributable to the inhibition of intercurrent infections in the treated groups and absence of teratogenic effects. Case[85] and Rushton[86] concluded that percutaneous absorption is practically null.

Action over biofilm

According to Costerton, Stewart and Greenberg,[95] biofilm is a structured community of microorganisms surrounded by a matrix of polysaccharides produced and adhered to live or inert surfaces. The cells comprising the biofilm structure are phenotypically different from planktonic cells (microorganisms presented in a free and disorganized form), since they are less susceptible to antimicrobial substances.[96]

Biofilm control occurs as a result of the antiseptic property of chlorhexidine associated with adsorption (ability to be retained on an oral surface and be slowly released), assuring an extended antimicrobial environment.[60,97] Adsorption is explained by electrostatic interaction. Due to its cationic characteristic, chlorhexidine has a strong affinity for anions, such as phosphate ions from the cell wall of oral microbiota which normally colonizes the tooth surfaces,[98] thus reducing adherence and colonization of tooth surfaces. This process enhances cell wall permeability and, as a consequence, leads to cytoplasm rupture and causes cell death.[98] Due to its bactericide and bacteriostatic effect, chlorhexidine inhibits the development of microbial plaque development.[64] This anti-plaque effect is probably the most significant property of chlorhexidine.[99]

One of the major mechanisms of resistance of biofilm is associated with failure of agents in penetrating its extension. Polymeric substances, such as those found in biofilm matrix, reduce the diffusion of chemical substances and antibiotics. Solutes tend to diffuse more slowly. The speed of penetration varies according to the type of microorganism and the composition of the exopolysaccharide matrix. A second mechanism of resistance is associated with the ability of a microorganism present in biofilm to survive after long periods of food shortage which decreases its growth rate. Microorganisms with reduced growth rate, or no growth, are less sensitive to chemical substances.[95,99,100,101] Mohammadi and Abbott[31] reported that a microorganism growing in biofilms is two to 1,000 times more resistant than its correspondent planktonic form.

Studies conducted with biofilm composed by a single species[102,103] and apical dentin biofilm[104] revealed that an increase in sodium hypochlorite concentration (varying from 2.25 to 6%) and 2% chlorhexidine solution were effective against the microorganisms tested. Mechanical agitation enhances antimicrobial activities of chemical substances, particularly favoring liquid agents such as 5.25% sodium hypochlorite and 2% chlorhexidine.[102] Chlorhexidine has a significantly lower effect on microbial biofilm in comparison to hypochlorite.[31]

Tyler et al[105] assessed the distribution and transport of chlorhexidine digluconate and glucose in *Candida albicans* biofilm. Their results confirmed the diffusion capacity of chlorhexidine digluconate through biofilm, which is not uniform, thus suggesting that chlorhexidine preferentially binds to sites of microbial cells and/or passes through microcanals present in biofilm. The presence of microcanals suggests that biofilm is somehow organized or at least has a complex structure, since microcanals allow the entrance of nutrients and excreta output. Additionally, the authors concluded that the action of chlorhexidine is directly proportional to concentration that tends to decrease as chlorhexidine goes deeper into the biofilm. Glucose does not diffuse uniformly either, which results in areas with nutrients shortage.

Clegg et al[104] assessed the efficacy of disaggregating and removing polymicrobial biofilm produced by sample collected from teeth of patients diagnosed with periapical lesion 3-mm in diameter associated with pulp necrosis and who were not treated by antibiotic drugs. The samples were seeded in culture medium and evaluated microscopically. 2% chlorhexidine proved not to affect biofilm or eliminate bacteria. Nevertheless, it generated absence of microbial growth (culture medium). 6% sodium hypochlorite was the only substance that favored absence of bacteria, removed biofilm and promoted absence of microbial growth (culture medium).

Antibacterial activity

Its antibacterial activity is explained by the ability of chlorhexidine to be rapidly attracted by the negative charge of bacterial surface, and adsorbed to the cell membrane by electrostatic interactions, probably by hydrophobic bindings or hydrogen bridges. Adsorption is concentration-dependent. In higher concentrations, it causes not only precipitation and coagulation of cytoplasmic proteins, but also bacterial death; whereas in low concentrations, cell membrane integrity is altered, resulting in extravasation of low molecular weight bacteria components.[60,93,106] Thus, the molecule cationic end binds to the pellicle with negative charge (anionic), whereas the other cationic end is free to interact with bacteria that aim at colonizing the tooth.[45] In Endodontics, chlorhexidine is recommended for root canal irrigation during chemo-mechanical preparation,[106] since it inhibits bacterial growth in endodontic infections.[51,56,107] The action of chlorhexidine depends on the susceptibility of microorganisms; Gram-positives have higher susceptibility to chlorhexidine in comparison to Gram-negatives.[107] Some species of *Streptococci* seem to retain an additional amount of chlorhexidine in their extracellular polysaccharide capsules, which might be related to the high sensitivity of *Streptococci* to chlorhexidine.[108]

In 1982, Delany et al[13] conducted an *in vitro* study on the antimicrobial action of 0.2% chlorhexidine gluconate solution used as irrigant and intracanal dressing on root canal microbiota of recent extracted necrotic pulp of human teeth. Bacterial growth was observed by inoculation of dentin debris on agar, which caused a significant reduction in the number of bacteria in both endodontic procedures.

Heling et al[53] conducted an *in vitro* study to assess the antibacterial effect of 2% chlorhexidine gluconate at 20% used, in a in a slow release system, as intracanal dressing in bovine incisors contaminated with *S. faecalis*. The slow release system consisted of strips containing glutaraldehyde as vehicle and 1.2 mg of 20% chlorhexidine as active agent. The microbiological analysis of dentin removed from canal walls revealed that both forms of dressing were effective for depth of 0.5 mm in experimental periods of 24, 48 hours and seven days.

Siqueira Jr. and Uzeda[56] assessed the antibacterial activity of 0.12% chlorhexidine digluconate gel, 10% metronidazole gel, calcium hydroxide with distilled water, calcium hydroxide with PMCC camphorated paramonochlorophenol and calcium hydroxide with glycerin applied on strict and facultative anaerobic bacteria commonly found in endodontic infections. Their results revealed that calcium hydroxide paste with PMCC and chlorhexidine were effective for all species of bacteria tested (strict anaerobic — *Porphyromonas endodontalis, P. gingivalis, Actinomycesisraelis, Fusobacterium nucleatum, Propionibacterium acnes* and *Campylobacter rectus*; and facultative anaerobic — *Staphylococcus aureus, Streptococcus mutans, S. sanguis, S. salivarius, Enterococcus faecalis* and *Actinomyces viscosus*). Metronidazole inhibited the growth of all strict anaerobic species, whereas calcium hydroxide with distilled water or glycerin were ineffective.

Lindskog, Pierce and Blomlöf[57] assessed the effect of 10% chlorhexidine gluconate gel used as intracanal dressing during one month on inflammatory root resorption induced in monkeys. The authors found a reduction in the resorption process due to the antimicrobial action of chlorhexidine inside dentin tubules and on periodontal ligament cells.

Ferraz[51] conducted an *in vitro* research on chlorhexidine gel used as endodontic irrigant in comparison to other irrigants commonly used in Endodontics. The author concluded that 2% chlorhexidine gel or solution showed the highest averages of inhibition halos against all microorganisms tested by the agar diffusion test. Chlorhexidine gel produced, *in vitro*, higher inhibition halos of microbial growth when compared to chlorhexidine solution at equivalents concentrations. However, with no statistically significant differences. Similarly to 5.25% sodium hypochlorite, 2% chlorhexidine solution produced negative cultures after 45 seconds of contact with *Enterococcus faecalis*, acting more rapidly than other irrigants. Teeth irrigated with 2% chlorhexidine gel had a higher number of negative microbiological cultures (80%); after *in vitro* instrumentation, 2% chlorhexidine gel significantly reduced smear layer in comparison to 2% chlorhexidine solution and 5.25% sodium hypochlorite.

Menezes et al[52] conducted an *in vitro* study to assess the efficacy of sodium hypochlorite and 2% chlorhexidine used as irrigation solution. Teeth had been contaminated by *Enterococus faecalis*. The authors concluded that chlorhexidine was more effective.

Haapasalo et al[44] conducted a literature review in which they highlight that the use of chlorhexidine at 0.2 to 2% might offer an additional advantage against resistant microorganisms disseminated by the root canal system. This is due to the ability of chlorhexidine to increase bacterial cell or cell wall permeability; act inside fungi cytoplasm membrane; cause coagulation of intracellular constituents at high concentrations. Other advantages include residual antimicrobial action and substantivity; relatively low toxicity, wide spectrum of action and efficacy against *Enterococcus faecalis* and *Staphylococcus aureus*. According to the authors, chlorhexidine efficacy decreases in contact with organic matter, mycobacteria, bacterial spores and virus, all of which are resistant. Additionally, chlorhexidine has cytotoxicity at high concentrations; chlorhexidine gel is less effective against *Enterococcus faecalis* in comparison to solution; chlorhexidine combinations are so or less effective than its compounds alone; when in contact with tooth dentin (organic compounds), chlorhexidine efficacy decreases, but is not completely neutralized; albumin from bovine plasma neutralizes chlorhexidine action and does not act as a tissue solvent.

Dametto et al[66] conducted an *in vitro* study to assess the antimicrobial activity of 2% chlorhexidine gel against *Enterococcus faecalis* in comparison to other endodontic irrigants (2% chlorhexidine solution and 5.25% sodium hypochlorite). 2% chlorhexidine gel and 2% chlorhexidine solution significantly reduced *E. faecalis* at post-treatment and final phases. 5.25% sodium hypochlorite also reduced *E. faecalis* immediately after root canal instrumentation. However, it did not completely eliminate *E. faecalis* from the root canal. The authors concluded that 2% chlorhexidine gluconate (gel and solution) had higher antimicrobial capacity against *E. faecalis* in comparison to 5.25% sodium hypochlorite seven days after biomechanical preparation.

In 2006, the results of a research conducted by Fachin, Nunes and Mendes[92] agreed with Jeansonne et al[15] who affirmed that 2% chlorhexidine is an effective antimicrobial that produces results statistically similar to 5.25% sodium hypochlorite, and of which substantivity increases antimicrobial performance.

Wang et al[109] assessed the clinical efficacy of 2% chlorhexidine gel with regard to the reduction of intracanal bacteria during root canal instrumentation. The additional antibacterial effect of calcium hydroxide associated with 2% gel used as an intracanal dressing was also assessed. The authors concluded that 2% chlorhexidine gel effectively decontaminates the root canal, and, when used as intracanal dressing, does not produce additional significant effects on bacterial reduction.

Pretel et al[110] concluded that 2% chlorhexidine is a feasible irrigation solution due to its specific characteristics of substantivity and high antibacterial effect. According to the authors, chlorhexidine proves more effective considering its penetration and substantivity inside dentin tubules.

Its bactericidal activity is faster than its fungicide activity and strongly depends on pH. Its maximum activity can only be achieved with pH 8 (Neobrax[111]).

Antifungal activity

Chlorhexidine digluconate has a wide spectrum of action[59,112] with potent antifungal action against *Candida albicans*.[113,114] Fungi (or yeast) represent a small portion of oral microbiota. Candida is the species of fungi most commonly found in healthy (30 to 45%) as well as in medically compromised individuals (95%).[115] These fungi might be involved in cases of persistence and secondary infection associated with relapse of periapical lesions, given that they are microorganisms strongly associated with therapeutic failures.[17,59,65,74,75,114,116-119] For this reason, endodontic irrigants should include these microorganisms in within their spectrum of activity.[31] According to Waltimo et al,[113] the presence of fungi in infected root canals varies between 1 to 17%.

In 1999, Sen, Safavi and Spangberg[120] assessed the antifungal effects of 0.12% chlorhexidine and 1 to 5% sodium hypochlorite on root canals. They performed root sections and removed smear layer in half of the specimens. Root canals were inoculated with *Candida albicans* for 10 days. Subsequently, root sections were treated with 3 mL of the irrigation solution during one, five, 30 and 60 minutes. The authors observed that, in the presence of smear layer, the antifungal activity of all irrigants started after 60 minutes, only. Antifungal activity was higher in teeth of which the smear layer was removed. After 30 minutes, 5% sodium hypochlorite showed antifungal activity of 70% and after one hour, it was totally effective. 0.12% chlorhexidine and 1% sodium hypochlorite proved to be totally effective after an hour.

Waltimo et al[113] assessed the antifungal action of calcium hydroxide, 0.5% chlorhexidine acetate, 0.05% iodinated potassium iodide and sodium hypochlorite, alone and in combination. To this end, they used absorbent paper points contaminated with *Candida albicans*, directly exposed to the disinfectants, for periods of 30 seconds, five minutes, one and 24 hours. In comparison to calcium hydroxide associated with distilled water, 0.5% and 0.05% chlorhexidine proved more effective. After 24h, the association of 0.5% chlorhexidine with calcium hydroxide P.A. was also more effective than calcium hydroxide associated with distilled water and less effective than 0.5% chlorhexidine alone.

Alexandra et al[121] conducted an *in vitro* study in which the efficacy of four chemical substances used as intracanal dressing were compared: calcium hydroxide, chlorhexidine gel, PerioChip (Astra Zeneca) and chlorhexidine gel associated with calcium hydroxide. Saline solution was used as the control group. The substances were tested in three different periods (three, eight and 14 days) using human teeth previously contaminated with *E. faecalis*. Calcium hydroxide eliminated *Enterococcus faecallis* within three to eight days, but it was effective in the 14-day group, probably due to a pH drop. The different formulations of chlorhexidine were effective in eliminating *E. faecalis* from dentin tubules, with chlorhexidine gel showing the best results.

Siqueira Jr. et al[122] assessed the efficacy of four intracanal dressings in decontaminating the root canal of bovine teeth experimentally infected with *Candida albicans*. Infected dentin cylinders were exposed to four different dressings: calcium hydroxide and glycerin; calcium hydroxide and 0.12% chlorhexidine digluconate; calcium hydroxide with camphorated paramonochlorophenol and glycerin; 0.12% chlorhexidine digluconate with zinc oxide. Specimens were in contact with the dressings during 1 hour, 2 and 7 days. *Candida albicans* viability after exposure was evaluated by means of incubating the sample in culture medium to compare the efficacy of the dressing in dentin disinfection. Results revealed that specimens treated with calcium hydroxide associated with camphorated paramonochlorophenol and glycerin, or with chlorhexidine combined with zinc oxide were completely decontaminated after 1-hour exposure. Calcium hydroxide with glycerin eliminated *C. albicans* after 7 days, only. Calcium hydroxide associated with chlorhexidine proved ineffective to disinfect dentin, even after one week of exposure. Calcium hydroxide with camphorated paramonochlorophenol and glycerin, as well chlorhexidine digluconate associated with zinc oxide proved to be the most effective in eliminating *C. albicans*.

Ruff, McClanahan and Babel[123] compared the antifungal efficacy of 6% sodium hypochlorite, 2% chlorhexidine, 17% EDTA and MTDA BioPuro with final rinse as canal preparation, in which teeth were contaminated with *Candida albicans*. Teeth were divided into four groups: Group 1 – 1 mL of 6% sodium hypochlorite for 1 min; Group 2- 0.2 mL of 2% chlorhexidine for 1 min; Group 3 -5 mL of MTDA BioPuro for 5 min, following the manufacturer's instructions; Group 4 – 1 mL of 17% EDTA for 1 min. Results showed that 6% sodium hypochlorite and 2% chlorhexidine were equally effective and significantly superior to the other groups. MTDA was significantly superior to 17% EDTA.

Ballal et al[124] analyzed the antiseptic action of different intracanal dressings. They used *Candida albicans* and *Enterococcus faecalis* as microbiological indicators and conducted an observation on inhibition halos of microbial growth in solid medium culture. All intracanal dressings tested exhibited inhibition halos. Within 24 hours of action against *C.albicans*, calcium hydroxide paste in water proved to be the most effective, whereas against *E.faecalis*, 2% chlorhexidine gel had the best action. After 72 hours, 2.% chlorhexidine gel was the most effective dressing against *C.albicans* and *E.faecalis*, whereas the combination of the two substances yielded the worst results against both biological indicators. The authors concluded that 2% chlorhexidine gel is more efficient than calcium hydroxide paste, whether associated with water or 2% chlorhexidine gel.

Intracanal dressing

Chemo-mechanical preparation significantly reduces microbiota in infected root canals. However, Bystrom, Claesson and Sundqvist;[126] Sjögren et al[127] as well as Ando and Hoshino[125] highlighted the need for intracanal dressing use to prevent those bacteria surviving to chemo-mechanical preparation in a sufficient number and adequate environment from multiplying between treatment sessions. Thus, the need for root canal disinfection through chemo-mechanical preparation is clear. It can be achieved not only by the proper use of an intracanal dressing that has antimicrobial properties and functions as a physical barrier,[3,127-130] but also by proper filling of the root canal system and appropriate coronal sealing.[132] Additionally, intracanal dressing aims at reducing periapical lesions, solubilizing organic matter, neutralizing toxic products, controlling persistent exudate, controlling inflammatory external root resorption and stimulating repair by means of mineralized tissue.[133]

Chlorhexidine has been highly recommended as intracanal dressing due to its immediate antimicrobial action; wide antibacterial spectrum of action against Gram-positive and Gram-negative bacteria, whether anaerobic, facultative and aerobic; yeast and fungi[20,23,59,112] (especially *Candida albicans*);[113,120] relatively absence of toxicity;[49,86] dentin adsorption capacity and slow release of its active substance, which extends its residual antimicrobial activity.[15,16,53,54,134]

Delany et al[13] demonstrated the effect of 0.2% chlorhexidine gluconate used as intracanal dressing on the reduction of remaining antimicrobial population after root canal instrumentation. Due to its wide antimicrobial spectrum, chlorhexidine has been largely used in Endodontics. It has been recommended as digluconate salt, liquid or gel at different concentrations, as well as intracanal dressing.[13,53-57]

Ohara et al[14] assessed the antimicrobial effects of six irrigants against anaerobic bacteria and highlighted that chlorhexidine was the most effective. With regard to the elimination of *E.faecalis* from inside of dentin tubules, chlorhexidine used as intracanal dressing yielded better results than calcium hydroxide.[53]

Lenet et al[135] conducted an *in vitro* study to compare the residual antimicrobial activity of 0.2 and 2% chlorhexidine gel in a system of controlled release, and calcium hydroxide associated with saline solution used as intracanal dressing in bovine incisors, during seven days. After the experimental period, the specimens were inoculated in *E.faecalis* during 21 days. Results revealed that 2% chlorhexidine gel had no viable bacteria in all dentin depths.

According to Vianna,[134] 2% chlorhexidine gel had higher antimicrobial activity. The combination between calcium hydroxide and 2% chlorhexidine gel decreased the antimicrobial activity of chlorhexidine, however, it increased the activity of calcium hydroxide.

Gomes et al[136] assessed the efficacy of 2% chlorhexidine digluconate gel and calcium hydroxide used as intracanal dressing at different time intervals (one, two, seven, 15 and 30 days). To this end, roots from bovine teeth previously infected with *E.faecalis* were used. 2% chlorhexidine gel; calcium hydroxide associated with polyethylene glycol 400; and 2% chlorhexidine gel associated with calcium hydroxide were used as intracanal dressing. The authors observed that 2% chlorhexidine gel inhibited bacterial growth in the infected dentin samples in all periods tested. The combination of calcium hydroxide and polyethylene glycol 400 was inefficient in eliminating bacteria during all periods. Absence of dentin contamination was found in periods of one and two days for samples comprising the association of 2% chlorhexidine gel and calcium hydroxide. As for periods of seven and 15 days, there was a decrease in antimicrobial activity and, after 30 days, all samples from this group were contaminated. In conclusion, 2% chlorhexidine gel has a wide antimicrobial activity against *E.faecalis*. However, the authors highlighted that this property might decrease with time if the medication is used for long periods.

Pinheiro et al[137] conducted an *in vitro* study to assess the antimicrobial activity of 50% calcium hydroxide and 2% chlorhexidine gel used alone or in combination. The following microorganisms were tested: *Enterococcus faecalis*, *Candida albicans*, *Escherichia coli, Sthaphylococcus aureus, Stahphylococcus epidermis* and *Pseudomonas aeruginosa*. After 24 and 48 hours, they assessed the inhibition halos. The halos formed against *E. coli, S. aureus* and S. *epidermis* were discrete and of similar dimension. Calcium hydroxide and 2% chlorhexidine gel used alone showed antimicrobial activity against all microorganisms tested. When combined, the substances showed higher inhibition halos against *E.faecalis* and *C.albicans* in comparison to calcium hydroxide used alone. However, the combination of substances showed smaller halos, for both microorganisms, in comparison with 2% chlorhexidine gel used alone.

In 2006, Montagner et al[138] assessed the antimicrobial action of intracanal dressings on external surface root against different microorganisms. 288 roots extracted from upper canines were divided into two groups, with and without cementum. The following microorganisms were isolated from clinical samples and analyzed: *Enterococcus faecalis*, *Candida albicans*, *Actinomyces viscosus* and *Porphyromonas gingivalis*. 2% chlorhexidine gel; 2% chlorhexidine gel and calcium hydroxide (1:1); 2% chlorhexidine gel, calcium hydroxide and zinc oxide (1:1:1); calcium hydroxide and saline solution; saline solution (positive control) were used as intracanal dressings. The best antimicrobial effect was produced by 2% chlorhexidine gel, followed by 2% chlorhexidine gel and calcium hydroxide; 2% chlorhexidine gel, calcium hydroxide and zinc oxide; and calcium hydroxide and saline solution. *A. viscosus* (2.85 mm) was most sensitive to the medications, followed by *E. faecalis* (1.84 mm), *C. albicans* (0.95 mm) and *P. gingivalis* (0.82 mm). Presence or absence of cementum did not interfere in the substance capacity of reaching the outer root surface and exerting its antimicrobial action. The authors concluded that intracanal dressings associated with chlorhexidine were able to diffuse through the dentin and reach the outer root surface. The combination between calcium hydroxide and saline solution did not show antimicrobial activity in the outer root surface within 72 hours. Conversely, 2% chlorhexidine gel associated with calcium hydroxide and zinc oxide revealed rapid diffusion capacity in root dentin, causing inhibition of bacterial growth.

Gomes et al[139] investigated the antimicrobial activity of intracanal dressings by means of the agar diffusion test as well as by direct contact. The following biological indicators, which represent endodontic infection, were included: *Enterococcus faecalis*, *Candida albicans*, *Staphylococcus aureus*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Agar diffusion and direct contact tests revealed that 2% chlorhexidine digluconate gel (1% Natrosol "hydroxyethil cellulose" with pH 7.0) had the highest efficacy; calcium hydroxide in 2% chlorhexidine digluconate gel, intermediate efficacy; and calcium hydroxide with sterile water as vehicle, the worst. The latter did not produce inhibition halos. There was susceptibility of *Enterococcus faecalis* and *Candida albicans* to intracanal dressings, following the order previously related, as well as inactivity of calcium hydroxide in water in the agar diffusion test. The authors explained that the inability of calcium hydroxide in water to diffuse throughout agar is due to the low solubility of hydroxide, as well as the buffer effect and protein coagulation action occurring in the agar. These effects are liable to occur *in vivo*, which avoids penetration of the intracanal dressing into the dentin tubules and irregularities of the root canal. The antimicrobial action of 2% chlorhexidine digluconate gel is reduced when the substance is associated with calcium hydroxide.

Fachin, Nunes and Mendes[92] assessed the efficacy of four intracanal dressings (camphorated paramonochlorophenol, calcium hydroxide, 2% chlorhexidine gel and 1% sodium hypochlorite) in cases of pulp necrosis with periapical lesion, by means of clinical and radiographic control. All solutions were effective to decrease the size of apical lesions. Initial results reveal that, after three months, the highest percentages of reduction in lesion diameter occurred with 2% chlorhexidine gel.

The results of this research are encouraging with regard to the use of 2% chlorhexidine gel as intracanal dressing in cases of pulp necrosis. Thus, these results corroborate Heling et al,[54] Barbosa et al,[55] Lenet et al[135] and Rosa et al[140] and confirm the efficacy of 2% chlorhexidine used as intracanal dressing.

Ballal et al[124] analyzed the antiseptic action of different intracanal dressings. They used *Candida albicans* and *Enterococcus faecalis* as microbiological indicators and conducted an observation on inhibition halos of microbial growth in solid medium culture. All tested intracanal dressings exhibited inhibition halos. Within 24 hours of action against *C.albicans*, calcium hydroxide paste in water proved to be the most effective, whereas against *E.faecalis*, 2.0% chlorhexidine gel had the best action. After 72 hours, 2.0% chlorhexidine gel was the most effective medication against *C.albicans* and *E.faecalis*, whereas the combination of the two substances yielded the worst results against both biological indicators. The authors concluded that 2% chlorhexidine gel is more efficient than calcium hydroxide paste, whether associated with water or 2% chlorhexidine gel.

Marion et al[141] reported a case conducted by means of a new therapeutic protocol, in which calcium hydroxide was associated with 2% chlorhexidine gel and zinc oxide and used as filling paste for avulsed tooth. The combination between calcium hydroxide, 2% chlorhexidine gel and zinc oxide was also assessed by Souza-Filho et al,[142] Almeida et al[143] and Montagner et al[144] in an *in vitro* study that revealed the antimicrobial action and capacity to keep an alkaline pH of the substance. Other case reports found in the literature[138,145] reveal that this association has a fast diffusing capacity in root dentin, causing inhibition of bacterial growth on the outer surface of the root canal. The case report conducted by Marion et al[141] revealed absence of signs and symptoms in tooth treated with filling paste, which remained after a three-year follow-up, thus proving the efficiency of this medication in the treatment of traumatized permanent teeth.

Rheological action

This action is found in chlorhexidine gel, since it refers to the capacity of maintaining debris in suspension inside the root canal.[5]

When the pulp chamber and root canal are flooded with chlorhexidine gel and mechanical preparation of root canal system is initiated (instrumentation), both inorganic and organic debris (smear layer) — detached from root canal walls — accumulate in the amorphous mass of gel which captures and keep them suspended. Subsequently, active irrigation with saline or distilled water removes the debris, preventing them from accumulating in the root canal walls and, as a result, exposing the entrance of dentin tubules. In other words, it considerably reduces the formation of smear layer, thus improving the efficacy of EDTA as a chelating substance and increasing treatment prognostic.[2,5,27,146,147]

Ferraz et al[2] investigated the antimicrobial action of chlorhexidine gel and solution over *Enterococcus faecalis* and its capacity of cleaning the root canal wall, in comparison to 5.25% sodium hypochlorite. To this end, 70 recently-extracted single-rooted teeth were selected. They were prepared up to the apical foramen with file #40, submitted to a 17% EDTA wash with ultrasound, sterilized and infected. Subsequently, root canals underwent instrumentation with 2% chlorhexidine gel, chlorhexidine solution or 5.25% sodium hypochlorite. Water and Natrosol gel were used as control. As for suppression of bacterial growth, no statistical differences were found between groups. Nevertheless, with regard to cleaning, the highest number of open dentin tubules was found in chlorhexidine gel, followed by chlorhexidine solution and 5.25% sodium hypochlorite, which confirmed the capacity of chlorhexidine gel in preventing smear layer formation, probably as a result of the mechanical action of Natrosol gel.

Allergic reactions

No adverse effects have been published regarding the use of chlorhexidine as irrigant or intracanal dressing.[5] On the other hand, animal studies have shown that 2.0% chlorhexidine used as intracanal dressing did not induce intense inflammatory response when injected into the peritoneal cavity of mice.[148,149] Chlorhexidine has a limited number of adverse effects, such as desquamative gingivitis, tooth and tongue discoloration or dysgeusia (distortion of the sense of taste). Contact sensitivity to chlorhexidine was first described by Calnan.[150] Contact with the conjunctiva may cause permanent damage, whereas accidental contact with the tympanum might cause ototoxicity.[151] It may also cause contact urticaria, photo-sensibility, fixed drug eruption and occupational asthma. Patients with leg ulcers and eczema have particular risks of contact allergy (besides doctors and dentists). Contact sensitivity to chlorhexidine seems to be generally rare. Some studies have demonstrated a high rate of sensitization, around 2%.[152,153] Ohtoshi, Yamauchi and Tadokoro[155] described even rarer reactions caused by chlorhexidine, in which case immediate anaphylactic reactions were observed and IgE antibodies were found in patients' serum.

The major side effects of chlorhexidine are as follows: tooth discoloration (in the cervical third and proximal surfaces),[156] restorations, prosthesis and tongue; dental calculus accumulation, taste alteration (especially to salt), oral desquamation, supragingival calculus formation and occasional parotid glad swelling dyspnea and anaphylaxis.[157-161] Among these effects, tooth discoloration stands out as patients' chief complaint,[162] since it affects 30 to 50% of patients.[88,153] It is considered as the main limiting factor of chlorhexidine when used for long periods of time. Concentration and volume of chlorhexidine interfere in the prevalence and severity of discoloration. Thus, despite having similar efficacy and effectiveness,[164] lower concentrations, in larger volumes, proved to cause less tooth discoloration.[165] However, these unpleasant effects are reversible once the use of chlorhexidine is suspended.[161]

Although sensitivity to chlorhexidine may be rare, the possibility of complications should be kept in mind during its application.[118]

Final considerations

Based on this literature review on the applications of chlorhexidine for endodontic purposes, it is reasonable to conclude that:

» Chlorhexidine, liquid or gel, may be used during all phases of root canal preparation, in which case the concentration of 2% is most frequently used.

- » Its wide antimicrobial spectrum (Gram-positive and Gram-negative bacteria), including fungi, is improved due to substantivity, which may last from 48 hours to 12 weeks.
- » Chlorhexidine does not solve organic tissue, however, chlorhexidine gel may favor it as a result of rheological reaction and lubrication of endodontic instruments during mechanical action.
- » Sodium hypochlorite associated with chlorhexidine results in an orangish-brown solution (parachloraniline) that requires further investigation.
- » Chlorhexidine has been recommended as an alternative to sodium hypochlorite. It is considered a biocompatible solution, however, the possibility of further complications should be taken into account during its application.

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