

Chlorhexidine in Endodontics

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Chemical auxiliary substances (CAS) are essential for a successful disinfection and cleanness of the root canals, being used during the instrumentation and if necessary, as antimicrobial intracanal medicaments. Different CAS have been proposed and used, among which sodium hypochlorite (NaOCl), chlorhexidine (CHX), 17% EDTA, citric acid, MTAD and 37% phosphoric acid solution. CHX has been used in Endodontics as an irrigating substance or intracanal medicament, as it possesses a wide range of antimicrobial activity, substantivity (residual antimicrobial activity), lower cytotoxicity than NaOCl whilst demonstrating efficient clinical performance, lubricating properties, rheological action (present in the gel presentation, keeping the debris in suspension); it inhibits metalloproteinase, is chemically stable, does not stain cloths, it is odorless, water soluble, among other properties. CHX has been recommended as an alternative to NaOCl, especially in cases of open apex, root resorption, foramen enlargement and root perforation, due to its biocompatibility, or in cases of allergy related to bleaching solutions. The aim of this paper is to review CHX's general use in the medical field and in dentistry; its chemical structure, presentation form and storage; mechanism of action; antimicrobial activity including substantivity, effects on biofilms and endotoxins, effects on coronal and apical microbial microleakage; tissue dissolution ability; interaction with endodontic irrigants; effects on dentin bonding, metalloproteinases and collagen fibrils; its use as intracanal medicament and diffusion into the dentinal tubules; its use as disinfectant agent of obturation cones; other uses in the endodontic therapy; and possible adverse effects, cytotoxicity and genotoxicity.

Key Words: chlorhexidine, Endodontics, irrigants, intracanal medicaments, antimicrobial activity.

Introduction

Complete debridement and disinfection of the pulpal space are considered to be essential for predictable long-term success in endodontic treatment. Residual pulpal tissue, bacteria and dentin debris may persist in the irregularities of root canal systems, even after meticulous mechanical preparation (1,2). Therefore, several irrigating substances have been recommended for use in combination with canal preparation, including sodium hypochlorite (NaOCl), chlorhexidine gluconate (also called chlorhexidine digluconate or just chlorhexidine - CHX), 17% EDTA, citric acid, MTAD and 37% phosphoric acid solution. However, if these substances remain in the root canal, they might affect the penetration of the resin sealer in dentin and its polymerization. They might also degenerate dentin if they have a negative effect on the collagen fibers (3,4). It has long been recognized that the antibacterial effects of chemomechanical procedures can be enhanced by the subsequent placement of an antimicrobial intracanal medication, particularly in those cases of exudation, haemorrhage, perforation, root resorption, trauma or incomplete root formation (5-7). Nevertheless, the efficacy of both procedures also depends on the vulnerability of the involved microbial species present in the root canal system. Moreover, in order to avoid re-infection of the cleansed space, not only the placement of a host-compatible root canal filling but also of a permanent coronal restoration must be performed (2).

CHX can be applied clinically as antimicrobial agent during all phases of the root canal preparation, including the disinfection of the operatory field; during the enlargement of the canals orifices; removal of necrotic tissues before performing the root canal length determination; in the chemomechanical preparation prior to the foraminal patency and enlargement; as an intracanal medicament alone or combined with other substances (i.e. calcium hydroxide - CH); in the disinfection of obturation cones; for modeling the main gutta-percha cone; in the removal of gutta-percha cones during retreatment; in the disinfection of prosthetic space; among others. Therefore, the objective of this review was to report relevant aspects of chlorhexidine in Endodontics.

General Use

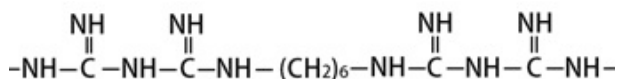
According to Tomás et al. (8), back in 1947, a complex study to synthesize new antimalarial agents led to the development of the polybiguanides (9). These compounds showed significant antimicrobial potential, particularly compound 10,040, a cationic detergent later called chlorhexidine (10). The first salt derived from compound 10,040 that reached the market was chlorhexidine gluconate. It was registered in 1954 by the Imperial Chemical Industries Co. Ltd. of Macclesfield (United Kingdom) under the brand name Hibitane[®], the first internationally accepted antiseptic for cleaning skin, wounds and mucous membranes because of its strong affinity to such areas, with high level of

antibacterial activity and low mammalian toxicity (11). In 1957, only 3 years after coming into the market, the broad antimicrobial spectrum of CHX led to an extension of its indications to include not only skin disinfection, but also use in the fields of ophthalmology, urology, gynecology and otorhinolaryngology. Although CHX started being used to control bacterial plaque in 1959, its use became widespread in dentistry only in the 1970s after the publication of the studies by Loe and Schiött (8,12,13).

CHX is currently considered the gold standard of oral antiseptics and is, along with fluoride, the most extensively researched preventive agent in dentistry (14). In addition to its effects on dental plaque and gingivitis, CHX is effective in the prevention and treatment of caries (15,16); secondary infections to oral surgical procedures, and in the maintenance of the health of peri-implant tissues. CHX reduces the bacterial load of aerosols and reduces bacteremia after dental manipulation. It is also employed in the treatment of recurrent aphthous and denture-related stomatitis. CHX is particularly indicated in certain population groups, such as individuals with orthodontic appliances, disabled people, and immunologically compromised patients (8,17). It also retains its activity in the presence of blood, wounds and burns (11). Soaking dentures in CHX has also been shown to be effective in reducing colonization with *Candida* species (11). It has been used in Endodontics as an irrigating substance (2,6,14,18-25) or intracanal medicament alone or in combination with CH (5,6,19,26-30), among other uses.

Chemical Structure

The structural formula of CHX consists of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain (11), as illustrated in the image below:



Presentation Form

CHX is an almost colorless to pale straw-colored substance or slightly opalescent, odorless or almost odorless substance. The 20% (w/w) CHX salt is the most commonly used. Solutions prepared from all salts have an extremely bitter taste that must be masked in formulations intended for oral use (11). There are a variety of products containing CHX used in dentistry, medicine, veterinary and food, namely Periogard, Peroxidin, Plac Out, Corsodyl, Chlorohex, Descutan, Hibiscrub, Hibitane, Savacol, among others. The most commonly used concentrations in commercially available CHX mouth rinses are 0.12 and 0.20%. The 2%

concentration, used in Endodontics, can be prepared by pharmacies, under prescription (23,24,30,31).

For endodontic purposes, CHX can be used in a liquid or in a gel presentation. CHX gel consists of a gel base (1% natrosol, a hydroxyethylcellulose, pH 6-9) and chlorhexidine gluconate (23,31), in an optimal pH range of 5.5 to 7.0. Natrosol gel is a biocompatible carbon polymer (32) that is a water-soluble substance, and therefore can be easily removed from the root canal with a final flush of distilled water (23,31).

Some studies have shown that the antimicrobial activity of CHX liquid is equal or superior to that of CHX gel when the direct contact was used as a methodology (2,24,33). In other studies, using the agar diffusion test, 2% CHX gel was superior to 2% CHX liquid (34).

Ferraz et al. (23,34) showed that 2% CHX gel has several advantages over 2% CHX solution, in spite of having similar antimicrobial, substantivity and biocompatibility properties. The CHX gel lubricates the root canal walls, which reduces the friction between the file and the dentin surface, facilitating the instrumentation and decreasing the risks of instrument breakage inside the canal. In addition, by facilitating instrumentation, CHX gel improves the elimination of organic tissues, which compensates for its incapacity to dissolve them (23,31). Another advantage of CHX gel is the reduction of smear layer formation (23), which does not occur with the liquid form. CHX gel maintains almost all the dentinal tubules open because its viscosity keeps the debris in suspension (rheological action), reducing smear layer formation. Furthermore, the gel formulation may keep the "active principle" of CHX in contact with the microorganisms for a longer time, inhibiting their growth (24).

It is important to point out that during chemomechanical preparation, before using each file, 1 mL of CHX gel is introduced in the root canal with a syringe (24-gauge needle or smaller) and immediately after each instrumentation, 5 mL of distilled water is used to irrigate the canal. Before the use of EDTA or other chemical substance, a final flush with 10 mL of distilled water is recommended in order to remove traces of CHX inside the root canals.

Storage

A shelf life of at least 1 year can be expected, provided that packaging is adequate, in a dark, refrigerated bottle (11,20). Regarding the gel formulation, it may keep its pH and satisfactory antimicrobial activity for approximately 10 months after the fabrication date. Color alteration has been found in samples 1 year after the fabrication date (unpublished data), probably due to the presence of breakdown products resulting from prolonged shelf life or exposure to high temperatures.

Mechanism of Action

CHX is a strong base and it is more stable in the form of its salts. The salts originally employed were acetate and hydrochlorite, both of which suffer from relatively poor water solubility and were largely replaced by the digluconate in 1957 (35), which is a highly water soluble salt. Aqueous solutions of CHX are more stable within the pH range from 5 to 8. The antimicrobial activity of CHX is pH-dependent, being the optimum range from 5.5 to 7.0, within which is the pH of body surfaces and tissues (11). It readily dissociates at the physiological pH, releasing the positively charged CH component.

The bactericidal effect of the drug is due to the cationic molecule binding to extra-microbial complexes and negatively charged microbial cell walls, thereby altering the osmotic equilibrium of the cells. At low concentrations, low molecular weight substances will leak out, specifically potassium and phosphorous, resulting in a bacteriostatic effect. At higher concentrations, CHX has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm of bacterial cells, probably caused by protein cross-linking, resulting in cell death (14,36), and leaving cell debris in the root canals (37), which can be removed with a vigorous irrigation with distilled water.

Antimicrobial Activity

Regarding the spectrum of activity, CHX is bactericidal and effective against Gram-positive and Gram-negative bacteria, facultative and strict anaerobes (2,14,19,20,23,24,27,38-40), yeasts and fungi, particularly *Candida albicans* (24,33,34,41). It is active against some viruses (respiratory viruses, herpes, cytomegalovirus, HIV) and inactive against bacterial spores at room temperature (42-44). It also retains its activity in the presence of blood (11) and organic matters (45).

In the liquid presentation, CHX kills microorganisms in 30 s or less, while in the gel formulation it takes from 22 s (2% CHX gel) to 2 h (0.2% CHX gel) (24).

Several *in vitro* works using a broth dilution test have shown that 2.0% CHX (in both presentation forms) and 5.25% NaOCl have similar antimicrobial performance against all tested microorganisms (2,21,24,44), while others have shown the superiority of 2% CHX gel or liquid over 5.25% NaOCl (34) using the agar diffusion method.

Clinical investigations have also been performed to compare the antimicrobial activity of CHX and NaOCl, and reported that these two substances had comparable effects in eliminating bacteria (18,25).

Vianna et al. (37), in a clinical study, evaluated the degree of microbial reduction after chemomechanical preparation of human root canals containing necrotic pulp tissue when using two endodontic irrigating reagents, 5.25% NaOCl or 2% CHX gel. Assessment of the bacterial load was

accomplished by use of real-time quantitative-polymerase chain reaction (RTQ-PCR) directed against the small subunit ribosomal DNA using the SYBRGreen and TaqMan formats. The bacterial load was reduced substantially in both groups (over 96%). The bacterial reduction in the NaOCl-group (SYBRGreen 99.99%; TaqMan: 99.63%) was significantly greater ($p < 0.01$) than in the CHX-group (SYBRGreen 96.62%; TaqMan: 96.60%), probably due to the differences between the mechanisms of action of NaOCl and CHX.

Substantivity

The effectiveness of CHX stems from its capacity to absorb to negatively charged surfaces in the mouth (e.g. tooth, mucosa, pellicle, restorative materials), being slowly released from these retention sites and therefore maintaining prolonged antimicrobial activity for several hours (11). This process is known as substantivity, and only CHX and tetracycline have this property so far (46).

Regarding its substantivity, it has been found that the use of CHX as root canal irrigating substance prevented microbial activity from 48 h (22), 7 days (in the liquid and gel formulation) (39), 21 days (47), 4 weeks (46), up to 12 weeks (48).

It seems that the antimicrobial substantivity is related to the CHX molecules available to interact with the dentin (49). Furthermore, the outstanding substantivity of CHX to dentin (evaluated at an interval from 0.5 h to 8 weeks) and its reported effect on the inhibition of dentinal proteases may explain why CHX can prolong the durability of resin-dentin bonds (50), particularly in the presence of collagen (51).

CHX and Biofilms

A biofilm can be defined as communities of microorganisms attached to a surface, embedded in an extracellular matrix of polysaccharides. Within these microcolonies, bacteria have developed into organized communities with functional heterogeneity (52). It constitutes a protected mode of growth that allows survival in a hostile environment. Bacteria in such an environment differ greatly in phenotype when compared with their planktonic counterparts, and are far less susceptible to antimicrobial killing (33,52). It has been reported that microorganisms grown in biofilms could be 2-fold to 1000-fold more resistant than the corresponding planktonic form of the same organisms (49).

Several studies using a single-species biofilm model (33,53) and apical dentin biofilm (54) have reported that higher concentration of NaOCl (varying from 2.25% to 6%) and CHX solution (2%) were effective against the tested microorganisms. The mechanical agitation improved the antimicrobial properties of the chemical substances, favoring the agents in liquid presentation, especially 5.25%

NaOCl and 2% CHX (33). Although CHX is effective against bacterial biofilms, NaOCl is the only irrigation solution with the capacity of disrupting biofilms (49).

Endotoxin Reduction

Lipopolysaccharide (LPS, endotoxin), an outer membrane component of gram negative bacteria predominantly involved in root canal infection is an important mediator in the pathogenesis of apical periodontitis and enhancing the sensation of pain in endodontic infections (55).

Concerning CHX detoxifying activity, Buck et al. (56) reported little or no efficacy on inactivating the biologically active portion of the endotoxin lipid A. Furthermore, *in vitro* studies (57-59) inoculating *Escherichia coli* LPS in root canals showed the low effectiveness of CHX in reducing LPS after chemomechanical preparation. However, Signoretti et al. (60) showed that CHX improved CH properties of reducing the endotoxin content in root canals *in vitro*.

Vianna et al. (6) evaluated clinically the effect of root canal procedures on endotoxins and endodontic pathogens. The canals were instrumented with K-files, 1 mm from the radiographic apex, irrigated with 2% CHX and medicated with either CH, CH plus CHX or 2% CHX gel alone for 7 days. After chemomechanical preparation a mean endotoxin reduction of 44.4% was found, with a mean reduction of bacteria (CFU) of 99.96%. After 7 days of intracanal medicament, endotoxin concentration decreased by only 1.4%. No significant difference was found among different intracanal medicaments. The authors concluded that relatively high values of endotoxin were still present in the root canal after chemomechanical preparation although the majority of bacteria were eliminated. No improvement was achieved by 7 days of intracanal medicament.

Gomes et al. (61), in a clinical study in primarily infected root canals, obtained a higher percentage of endotoxin reduction in the 2.5% NaOCl-group (57.98%), when compared with the 2% CHX-gel-group (47.12%) ($p < 0.05$), using hand K-files for apical preparation 1 mm from the radiographic apex. This result supports the fact that 2.5% NaOCl and 2% CHX have no detoxifying effect on endotoxins. Therefore, it might be argued that the removal of more than 47% of the LPS content from the infected root canals is related to the mechanical action of the instruments in dentin walls accomplished by the flow and backflow of the irrigants.

Gomes et al. (62) in a clinical study comparing the endotoxin levels found in primary and secondary endodontic infections reported that teeth with primary endodontic infections had higher contents of endotoxins and a more complex gram-negative bacterial community than teeth with secondary infections.

Endo et al. (63), in a clinical study with secondarily

infected root canals with post-treatment apical periodontitis, used hand K-files for apical preparation, and 2% CHX gel for root canal irrigation. They found that higher levels of endotoxin were related to larger size of radiolucent area. Chemomechanical preparation was more effective in reducing bacteria (99.61%) than endotoxin (60.6%).

CHX and Coronal Microleakage

Canals medicated with CHX alone or in combination with CH retard the entrance of microorganisms through the coronal portion of the tooth into the root canal system, due to its wide antimicrobial activity and substantivity (64). Such a finding is interesting, especially if the coronal restoration becomes defective or if it is lost. However, even though a temporary seal delays the entrance of saliva and microorganisms into the canal system, it does not prevent microleakage, justifying efforts to incorporate dentin-bonding agents and resin for coronal seal (64).

Regarding coronal microleakage during the intracoronary bleaching (65,66), it was found that CHX used as a vehicle for sodium perborate enhanced its antimicrobial activity and did not affect adversely dentin microhardness (67).

CHX and Apical Fluid Penetration

Canals irrigated or medicated with CHX do not affect negatively the ability of root fillings to prevent fluid penetration into the root canal system through the apical foramen (49,68-70).

Tissue Dissolution Capacity

As far as the use of an auxiliary chemical substance in Endodontics is concerned, several studies have been performed in the search for a substance with major desirable properties for root canal irrigation, which includes the capacity to dissolve organic tissues (49). The tissue dissolution capacity of a substance depends mainly on three factors: the frequency of shaking, the amount of organic matter in relation to the amount of irrigant in the canal system and the surface area of tissue that is available for contact with the irrigant (71).

Chlorhexidine gluconate has been recommended as a root canal irrigant (2) because of its broad spectrum antimicrobial action, substantivity and low toxicity (21). However, CHX's incapacity of tissue dissolution has been pointed out as its major disadvantage. Some attempts have been made to evaluate the activity of CHX to dissolve organic matter, demonstrating that both preparations of this substance, aqueous solution or gel, were not able to dissolve pulp tissues (68,72). Bleeding in case of vital pulp will stop only with the complete removal of the pulp tissue by a full instrumentation of the root canal within its whole extension. Therefore, when CHX is used as an irrigant,

emphasis should be given to full canal instrumentation in order to remove all pulp tissue rests, as CHX does not promote a superficial necrosis.

On the other hand, Ferraz et al. (23) showed that 2% CHX gel produced the cleanest dentin wall surfaces when compared with other irrigants, including NaOCl. Due to its viscosity and rheological action, which keeps the debris in suspension, the gel seems to compensate for CHX's inability to dissolve pulp tissue, by promoting a better mechanical cleansing of the root canal and removing dentin debris and remaining tissues. The mechanical properties of the gel seem to be the main factor for this difference because the same chemical agent in the liquid form showed lower cleaning efficiency, although presenting similar antimicrobial activity (2,24).

Another important fact to be pointed out is that due to the complexity of the root canal system, even irrigation with 5.25% NaOCl does not remove all debris and organic tissues. On the other hand, dentin and organic tissues that get in contact with CHX during irrigation maintain a prolonged antimicrobial activity, as CHX is slowly released from these retention sites (11,73). Furthermore, if CHX is extruded through the apex, it does not induce pain to the patients.

Interaction with Endodontic Irrigants

Due to its wide spectrum antimicrobial activity and its inability of dissolving organic tissues, an irrigation regimen has been proposed, in which NaOCl would be used throughout instrumentation, followed by EDTA, and CHX would be used as a final irrigant (74).

The combination of NaOCl and CHX has been advocated to enhance their antimicrobial properties, and the advantage of using a final rinse with CHX would be the prolonged antimicrobial activity due to the CHX substantivity (75).

Kuruville and Kamath (76) reported that the antimicrobial effect of 2.5% NaOCl and 0.2% CHX used in combination was better than that of either component. However, Vianna and Gomes (75) found that the association of NaOCl and CHX did not improve the antimicrobial activity of CHX alone.

Apart from the antimicrobial aspect, the association of NaOCl with CHX leads to the formation of an orange-brown precipitate, resulting in a chemical smear layer that covers the dentinal tubules and may interfere with the seal of the root filling (31,77). In addition, this precipitate changes the color of the tooth (40,78,79) and is cytotoxic (80).

Heling and Chandler (81) investigated NaOCl and CHX, with and without EDTA, when used in combination as endodontic irrigants against *Enterococcus faecalis*, and verified that combining EDTA with NaOCl or CHX was more effective than using EDTA alone. However, CHX combined

with EDTA also leads to the formation of precipitates, resulting in a chemical smear layer that covers the dentinal tubules.

Prado et al. (4) used electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS) analyses to investigate the byproducts formed with the combinations between the most commonly used endodontic irrigants. Regarding the CHX combinations, 2% CHX gel and solution immediately produced an orange-brown precipitate when combined with 1%, 2.5% and 5.25% NaOCl solutions, and an orange-white precipitate, when combined with 0.16% NaOCl. In combination with EDTA, CHX produced a white-milky precipitate, related to the acid-base reactions. When combined with saline and ethanol, a salt precipitation was produced. No precipitate was observed when CHX was used together with distilled water, citric acid or phosphoric acid.

Regarding the orange-brown precipitate, it occurs due the presence of NaOCl, an oxidizing agent causing chlorination of the guanidino nitrogens of the CHX (4). Basrani et al. (82) detected the presence of *para*-chloroaniline (PCA) in this precipitate. On the other hand, Thomas and Sem (83), Nowicki and Sem (84) and Prado et al. (4) failed to detect it using different methodologies. PCA has been found to be mutagenic in microorganisms (14,85) and cytotoxic (80). Some concern over possible carcinogenicity has also been expressed (82).

Thus, after chemomechanical preparation with NaOCl, the use of CHX as a final irrigant or as an intracanal medicament would require the removal of NaOCl from the canal (77). Do Prado et al. (77) found that with regard to the use of NaOCl with CHX, 10 mL of distilled water in association or not with 17% EDTA and 10% citric acid was not enough to inhibit the formation of the chemical smear layer. In the cases where one wants to associate these substances, the protocol using phosphoric acid did not induce formation of chemical smear layer.

In summary, it is important to remove all traces of the substances used inside the root canals in order to avoid interactions between them.

CHX and Dentin Bonding

Coronal leakage has been extensively demonstrated as a negative contributor to the prognosis of endodontic treatments. Clinical trials have shown that apical periodontal health depends both on the effectiveness of coronal restorations and on the quality of the endodontic therapy (86,87).

Prevention of coronal leakage has usually been accomplished by using temporary restorative materials. However, these products are originally intended for temporary use and therefore have a finite lifetime. Thus, the immediate sealing of endodontically treated teeth using

restorative materials has been considered as a powerful resource in preventing early coronal leakage (88,89). Among non-temporary restorative materials, dentin adhesives have been advocated for use in the pulp chamber in an attempt to work as a durable barrier against microleakage (89).

Bonding to pulp chamber dentin is affected differently by endodontic chemical irrigants. NaOCl have been extensively used in endodontic therapy to provide gross debridement, disinfection, lubrication and dissolution of tissues. Nevertheless, this powerful antimicrobial agent (90) has been shown to jeopardize the polymerization of bonding resins due to its oxidizing action on dentin substrate (91). It is hypothesized that NaOCl might lead to the oxidation of some component of the dentin matrix (91), perhaps demineralized collagen, forming protein-derived radicals (92). These radicals would compete with the propagating vinyl free-radicals generated by the light-activation of resin adhesives, resulting in premature chain termination and incomplete polymerization (91). For this reason, bonding to oxidized dentin has shown to be significantly weak (91,93-97). Furthermore, reductions in the mechanical properties of dentin, such as its elastic modulus, flexural strength and microhardness, have been reported after irrigation of root canals with 5% NaOCl (98-100), which can also contribute to decrease the micromechanical interaction between adhesive resins and NaOCl-treated dentin.

It has been shown that CHX application prior to acid-etching has no adverse effects on immediate composite-adhesive bonds in coronal dentin (101-103), pulp chamber dentin (104), enamel (102,105), or with resin-reinforced glass-ionomer cements (106).

Erdemir et al. (97) reported that endodontic irrigation with CHX solution significantly increased bond strength to root dentin. These authors suggested that adsorption of CHX by dentin may favor the resin infiltration into dentinal tubules, which supposedly explain the high bond strength values obtained. However, the reliability of such concept remains unclear and needs to be tested.

Santos et al. (104) considered that as a non-oxidizing agent, 2% CHX in water solution or in a gel base did not interfere with the interaction of a self-etching adhesive system to pulp chamber dentin. An exception to this tendency was observed when the CHX gel is combined with EDTA. Despite the gel base used with CHX is a water-soluble carbon polymer, which showed to be easily removed from the root canal (31), an occasional presence of residual CHX gel on dentin could react with EDTA, forming products that affect resin infiltration and/or resin polymerization, providing bond strength values slightly lower than those observed for the control group. Therefore, all efforts should be taken to remove traces of the chemical substances inside the canal through intermediate flushes with inert solutions.

De Assis et al. (107) observed that a final flush with CHX favor the wettability of AH Plus and Real Seal SE sealers on dentin surface. Additionally, Hashem et al. (108) verified that the bond strength of ActiV GP was improved by using 2% CHX in the final irrigation after 17% EDTA. Prado et al. (4) found that the irrigation protocols influenced the bond strength of the resin sealers to dentin. In the gutta-percha/AH Plus groups, the bond strength was higher when NaOCl was combined with phosphoric acid or the CHX with EDTA. In Resilon/Real Seal SE groups, the protocol combining CHX with phosphoric acid showed better results. The use of CHX as a final irrigant did not affect negatively the bond strength.

The *in vitro* and *in vivo* application of 2% CHX in cavities after acid etching and before hybridization with adhesive monomers prevents the loss of bond strength with time (109) and preserves the integrity of the hybrid layer (110). In radicular dentin, the use of CHX as an endodontic irrigant may also inhibit the bacteria-related activation of metalloproteinases (111).

CHX, Metalloproteinases and Collagen Fibrils

Matrix metalloproteinases (MMPs) are members of an enzyme family that require a zinc ion in their active site for catalytic activity. MMPs are critical for maintaining tissue allostasis. MMPs are active at neutral pH and can therefore catalyze the normal turnover of extracellular matrix (ECM) macromolecules such as the interstitial and basement membrane collagens, proteoglycans such as aggrecan, decorin, biglycan, fibromodulin and versican as well as accessory ECM proteins such as fibronectin (112).

MMPs are present in sound coronal and radicular dentin and play a role in collagen network degradation in bonded restorations. Collagen is dentin's main organic component, and it has the important function of acting as a matrix for the deposition of apatite crystals (113). It also plays an important role in the bonding between dentin and adhesive systems. During bonding procedures, resin monomers infiltrate demineralized dentin, thus forming a structure named hybrid layer (114,115). The reduction of the bond strength seen between adhesive systems and dentin walls may occur because of the removal of collagen fibrils from the dentin surface by sodium hypochlorite and may impede the formation of a consistent hybrid layer (93). The decrease in bond strength values mentioned in many studies may be caused by collagen degradation and also by structural disorganization of remnant fibrils (116).

In a previous study, 2% CHX gel, whether combined or not with 17% EDTA, did not promote alterations in the morphological structure of dentin organic matrix. It is an auxiliary chemical substance that does not interfere with the collagen present in the organic matrix of root

dentin, and maintains the quality of the dentin substrate for posterior obturation or restoration of the tooth with resin-based materials (116).

CHX has also shown the capacity to preserve the durability of the hybrid layer and bond strength *in vitro* and *in vivo* (109,110), probably to do its effectiveness as a MMP inhibitor (117), resulting in lower degradation of hybrid layer and sub-hybrid layer collagen fibrils. It is a remarkable property because one reason for losing of resin-dentin bonds integrity with time is the degradation of denuded collagen fibrils exposed in incompletely infiltrated hybrid layers (118). This degradation is attributed to an endogenous proteolytic mechanism involving the activity of MMPs present in dentin (119).

CHX as Intracanal Medicament

CH is one of the most versatile medicaments in dentistry, especially for use as an intracanal medicament in vital and non-vital teeth (7). It is believed to have many of the properties of an ideal root canal medicament, mainly due to its alkaline pH (120). It is bactericidal and neutralizes the remaining tissue debris in the root canal system (121). CH also promotes an alkalinizing osteogenic environment on the surrounding tissues through the continuous release of OH⁻ ions (120). Moreover, CH mediates the neutralization of lipopolysaccharides (122), helping in the cleansing the root canal (123). However, CH cannot be considered as a universal intracanal medicament, since it is not equally effective against all bacteria found in the root canal (30). Indeed, several studies (124-126) have reported difficulty in eliminating enterococci effectively, as they tolerate high pH values, varying from 9 to 11 (9).

CHX has been used in endodontics and proposed as both an irrigant and an intracanal medicament. It is active against a wide range of microorganisms, such as Gram-positive and Gram-negative bacteria (including *Enterococcus faecalis*), yeasts and fungi. One of the mechanisms that can explain its efficacy is based on the interaction between the positive charge of the molecule and the negatively charged phosphate groups on the bacterial cell wall, which allows the CHX molecule to penetrate into the bacteria with toxic effects (29). Therefore, its antimicrobial activity is not related to its pH (between 5.5 to 7).

The antimicrobial activity of CHX has also been tested for its use as an intracanal medicament alone (5-7,19,26-30,127-130) or in combination with other substances (5-7,30,127-129,131-133).

When used as an intracanal medicament, CHX is more effective than CH against *E. faecalis* infection in dentinal tubules (5,127,129,134). In fact, the antimicrobial activity of CHX is reduced when combined with other substances, including CH, CH plus zinc oxide, among others

(5,7,30,129,135). However, CHX alone does not act as a physical barrier and does not present radiopacity. The use of CHX gel as intracanal medicament is recommended for a short period of time (3-5 days), particularly in those cases where the canals were fully instrumented but could not be root-filled due to the lack of time. It is also recommended in cases of exudation (unpublished data), as it retains its antimicrobial activity in the presence of blood and other organic matters (11,45). CHX gel is delivered into the canals with a syringe (e.g.: 24-gauge needle), being easily introduced and removed from the root canals.

On the other hand, the antimicrobial activity of CH increases with the combination with CHX (5,6,7,30,64,127-129,131-133). Such combination aims to increase the antimicrobial properties of CH, while maintaining its biological characteristics, mechanical properties, action as a physical barrier (30). It has been reported that the antimicrobial effect of this association is not due to the CHX molecule, but to the action of different byproducts generated by CHX fragmentation. Such byproducts exhibit both antioxidant and pro-oxidant properties, and have a high pH (136). No traces of PCA have been found in the combination of CHX with CH, due to the immediate degradation of CHX (137), even though this mixture liberated reactive oxygen species (ROS) at all time points.

Studies have also shown that CH pastes added with CHX gel, alone or with ZnO, have greater antimicrobial activity than those prepared with distilled water or saline (5,7,30,129,138).

The main advantages of this association are: a) higher antimicrobial than that of CH alone (5,30,129); b) pH around 13 (5,7,30,64), which is greater than that of CHX alone (pH 5.5-7.0) and could help in the control of the inflammatory internal- and external- root resorption (29,139); c) substantivity due to the presence of CHX (30,129); d) physical and chemical barrier better than that of 2% CHX gel alone, preventing root canal re-infection and interrupting the nutrient supply to the remaining bacteria (30); e) the contact angle of CH combined with CHX is lower than that observed when CH is combined with water, increasing the wettability of the medicament, which may explain the increase in the antimicrobial activity of its association with CHX (138); f) CHX improves CH properties of reducing the endotoxin content in root canals *in vitro* (60); g) diffusion through the dentinal tubules (129); h) radiopacity (129).

The paste consistency should be similar to the toothpaste and its radiopacity is similar to that of the root dentin.

To act only as a physical barrier, this medicament can be used for a short period of time. It was observed that to achieve its best antimicrobial activity, it should stay for a period of 15 to 30 days inside the root canal, without being

changed. The immediate antimicrobial action of the paste in the first 7 days seems to be related to the antimicrobial effect of CHX. This effect remains stable up to 14 days. The best action is observed within 30 days, with the diffusion of hydroxyl ions through the dentinal tubules (unpublished data). The use of a temporary sealing with composite ensures an effective seal, preventing contamination of the root canal and solubilization of the medicament by oral fluids, especially in periods longer than 7 days.

Diffusion into the Dentinal Tubules

It has been shown that 2% CHX containing medicaments is able to diffuse into the dentin tubular structure and reach the outer root surface, exerting antimicrobial action. Therefore, the root canal could be considered as a reservoir for the release of intracanal medicament to the whole dentin and to the external root surface (129). The antimicrobial effects of the tested medicaments could be ranked from strongest to weakest as follows: 2% CHX, CH + 2%CHX, CH + 2%CHX +ZnO, CH + sterile saline.

Disinfection of Obturation Cones (Gutta-Percha and Resilon Cones)

The efficacy of NaOCl and CHX as auxiliary chemical substances and their action as disinfectant agents of gutta-percha cones do not involve additional costs to clinicians, since these substances are commonly used in endodontic therapy. CHX has the ability to kill vegetative forms within short periods of time. However, this agent is not able to eliminate some spores, as does NaOCl (43,44).

As a strong oxidizing agent, 5.25% NaOCl is able to cause local changes in surface roughness of gutta-percha cones (140) observed by atomic force microscopy (AFM) (141,142). Moreover, formation of crystals on the surface of gutta-percha cones has been identified after a rapid sterilization with 2.5% and 5.25% NaOCl (43,143) showing that the final rinse with distilled water is essential, especially when NaOCl is used for cone disinfection (143). Other studies showed that 2% CHX did not change gutta-percha cone properties after exposure for up to 30 min, suggesting that this substance is less harmful to the structure of gutta-percha (141,143). It was also found that 5.25% NaOCl and 2% CHX did not produce any changes on Resilon surface (142). Resilon cones exposed to CHX gel presented some residual antibacterial action. The clinical importance of CHX release in endodontic cones might be related to its immediate antimicrobial effect inside the root canal, during the obturation time (140).

CHX and NaOCl lead to an increase in the surface free energy (wettability) of the gutta-percha cones and Resilon surfaces, thereby interfering positively with the adhesion mechanism. This change can be due to chemical

modifications on the surface of these materials caused by the action of these solutions. Comparing the two solutions, CHX was a better disinfectant compared with NaOCl, that is, presented high values of surface free energy. Cones disinfected with CHX presented smaller contact angles than NaOCl, favoring the interaction between the solid surface (cone) and the liquid, in this case, the sealer (144).

Other Uses in the Endodontic Therapy

Before 1990's, CHX gluconate was used in Endodontics as an irrigating solution, but always in a liquid form. One of the first reports of its use in Endodontics dates back to 1964 (145), demonstrating its effectiveness in enhancing radicular dentin permeability. Kennedy et al. (146), in 1967, recommended the use of 14.6% EDTA and 0.005% Hibitane, as separate solutions or combined, for irrigation of vital and non-vital teeth. They reported that these solutions not only reduce the number of microorganisms in the root canals, but also have the advantage of being well tolerated by soft tissue or wounds, provided the contact is not prolonged. However, according to them, chlorinated soda solution should never be used with Hibitane, as it forms a brown precipitate that stains the teeth. CHX in a gel presentation was evaluated by Siqueira and de Uzeda (27) as an intracanal medicament, demonstrating good performance. In 2001, Ferraz et al. (23) proposed the use of CHX gel as endodontic irrigant.

CHX can be used during all phases of the root canal treatment, including in the disinfection of the operatory field (125), due to its antimicrobial and substantivity properties. CHX has been recommended as an alternative to NaOCl, especially in cases of open apex, root resorption, foramen enlargement and root perforation, due to its biocompatibility, or in cases of allergy related to bleaching solutions (24).

Clinical investigations have been performed using 2% CHX gel for root canal preparation in the full extension of the root canal, with foraminal patency and enlargement, followed by root canal filling in the same visit. The results showed that approximately 93% of the patients did report postoperative pain (unpublished data). With foramen enlargement, the risk of irrigant extrusion through the apex increases, favoring the use of CHX, for being less irritating to the periapical tissues than NaOCl and not inducing pain. Irrigation with 17% EDTA for a better smear layer removal is recommended after instrumentation of the root canals with CHX, which should be previously removed with distilled water.

CHX gel can also be used for modeling gutta-percha cones, which improves their adaptation to the apical dentin wall (unpublished data).

The use of CHX gel during retreatment has also been

investigated. *In vitro*, groups that used CHX gel with manual or rotary instrumentation showed smaller debris extrusion as well as the cleanest root canal walls than the ones where solvents were used (unpublished data). A clinical investigation of retreatment cases has reported that chemomechanical preparation with 2% CHX gel was more effective in reducing bacteria (99.61%) than endotoxin (60.6%) (63).

Adverse Effects

No adverse effects have been published regarding CHX use as irrigant or intracanal medicament. However, the direct effect in an *in vitro* test on human stem cells of apical papilla showed lack of viable cells after its use (147). The *in vitro* cytotoxic effect of the CHX on human osteoblastic cells seems to be dose dependent (148). There is a consensus that all irrigating substances when applied direct to cells would impact to a certain degree on cell viability (149). On the other hand, animal studies have shown that 2.0% CHX did not induce intense inflammatory response when injected into the peritoneal cavity of mice (58) or in root canals of dogs, when used as intracanal medicament (59).

CHX adverse effects are usually more related to its topical or oral application. The use of CHX dental gel dentifrices and mouthwashes has been associated with reversible discoloration of the tongue, teeth, and silicate or composite restorations (11,42). Removal of the brownish discoloration can be done with abrasive pastes or instruments (14). However, it should not be used concomitantly with dentifrices, as CHX interacts with detergents and fluoride in toothpaste. The CHX products should be used 30 min after brushing.

Transient taste disturbances and a burning sensation of the tongue may occur on initial use. Oral desquamation and occasional parotid gland swelling have been reported with the use of mouthwash (11). The incidence of skin irritation and hypersensitivity is low when CHX is applied at its recommended concentrations. Strong solutions may cause irritation of the conjunctive and other sensitive tissues, such as brain, meninges and middle ear (11,42). Syringes and needles that have been immersed in CHX solutions should be thoroughly rinsed with sterile water or saline before use. A recent study demonstrated that immediate hypersensitivity to CHX has increased in the United Kingdom (150), therefore it is important to investigate previous allergy to CHX during the history taking and prior its clinical application or prescription.

Cytotoxicity and Genotoxicity

Cytotoxicity is the degree to which an agent has specific destructive action on certain cells, while genotoxicity is related to the potential damage of certain substances to

the DNA, which is not proof of their dangerousness to humans, but does render them potentially mutagenic or carcinogenic.

Results of a previous study have shown that bactericidal concentrations of CHX diacetate were lethal to canine embryonic fibroblasts *in vitro*, whereas non-lethal concentrations allowed significant bacterial survival (151). Moreover, higher concentration of CHX induces necrosis and lower concentration is associated with apoptosis (152).

CHX is cytotoxic in cell culture with different cell lines and its cytotoxicity is not cell type specific (153). CHX showed cytotoxic effects in human gingival fibroblasts (154), human periodontal ligament cells (155), human alveolar bone cells (156), human osteoblastic cells (157).

Gianelli et al. (153) investigated the *in vitro* cytotoxicity of CHX on osteoblastic, endothelial and fibroblastic cell lines. They reported that CHX affected cell viability in a dose and time-dependent manners, particularly in osteoblasts. Its toxic effect consisted in the induction of apoptotic and autophagic/necrotic cell deaths and involved disturbance of mitochondrial function, intracellular Ca²⁺ increase and oxidative stress. These findings agree with those of Li et al. (152), who studied the cytotoxicity of CHX in RAW264.7 murine macrophage cells. The genotoxicity of CHX in RAW264.7 cells had shown DNA damage in a dose-dependent manner.

However, Ribeiro et al. (158) evaluated the genotoxicity of formocresol, paramonochlorophenol, CH and CHX at final concentration ranging from 0.01% to 1% against Chinese hamster ovary cells. Results showed that none of the mentioned agents contributed to DNA damage.

The mechanisms of the cytotoxicity of CHX are still unclear and it is important to understand that the cytotoxic effects of CHX on cell culture are directly dependent on the exposure dose, frequency and duration, and also depend on the composition of the exposure medium (159).

It has been reported that PCA, an industrial chemical, is found in CHX products as a trace contaminant (11). PCA has been shown to be mutagenic in microorganisms (85). However, no evidence of carcinogenicity was found in rats after 2 years of up to 40 mg/kg/day CHX plus 0.6 mg/kg/day p-chloroaniline (11). No detrimental effects were caused by CHX application in man over a 2-year period was found (20). Therefore, the human safety experience with CHX supports its suitability for long-term oral use. However, the development of tooth staining, in a topical or oral application, imposes a practical cosmetic limitation to such use (160). Although sensitivity to CHX is rare, it should be kept in mind during CHX application (49).

In conclusion: 1) CHX is effective in the control of dental plaque and gingivitis, in the prevention and treatment of caries, and in the maintenance of implants; after dental

manipulation, in the treatment of recurrent aphthous and denture-related stomatitis. It is particularly effective in individuals with orthodontic appliances, disabled people, and immunologically compromised patients. In Endodontics, it is used as an irrigating substance, intracanal medicament, among others. 2) Its structural formula consists of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain. 3) For endodontic purposes, CHX can be used in a liquid or gel presentation. The concentration most frequently used is 2%. 4) A shelf-life of at least 1 year can be expected, provided that the packaging is adequate and in the dark bottle. 5) The bactericidal effect of the drug is due to the cationic molecule binding to extra-microbial complexes and negatively charged microbial cell walls, thereby altering the cell's osmotic equilibrium. 6) CHX is bactericidal and effective against Gram-positive, Gram-negative, facultative and strict anaerobes, yeasts and fungi, particularly *Candida albicans*. It is active against some viruses (respiratory viruses, herpes, cytomegalovirus, HIV) and inactive against bacterial spores at room temperature. 7) CHX shows substantivity up to 12 weeks. 8) Although CHX is effective against bacterial biofilms, NaOCl is the only irrigation solution with the capacity of disrupting biofilms. 9) 2% CHX have no detoxifying effect on endotoxins, but it improves CH properties of reducing the endotoxin content in root canals *in vitro*. 10) Canals medicated with CHX alone or in combination with CH delay the entrance of microorganisms through the coronal portion of the tooth into the root canal system. Coronal microleakage is also delayed when CHX is used as a vehicle for sodium perborate during the intracoronal bleaching. 11) Canals irrigated or medicated with CHX do not affect adversely the ability of root fillings to prevent fluid penetration into the root canal system through the apical foramen. 12) CHX does not dissolve organic tissues. 13) CHX in contact with NaOCl, EDTA, saline and ethanol forms precipitate. However, no precipitate was observed when CHX was combined with citric acid, phosphoric acid or distilled water. It is important to remove all traces of the substances used inside the root canals through intermediate flushes with distilled water in order to avoid interaction between them. 14) The *in vitro* and *in vivo* application of 2% CHX in cavities after acid etching and before hybridization with adhesive monomers prevents the loss of bond strength with time and preserves the integrity of the hybrid layer. Irrigation with CHX increases the bond strength to root dentin. 15) CHX does not interfere with the collagen present in the organic matrix of root dentin and inhibits MMPs. 16) CHX increases the antimicrobial activity of CH. 17) 2% CHX containing medicaments is able to diffuse into the dentin and reach the outer surface, exerting antimicrobial action. 18) CHX is effective in disinfecting gutta-percha and Resilon

cones, although it does not eliminate bacterial spores. 2% CHX does not change the properties of gutta-percha and Resilon cones. CHX and NaOCl lead to an increase in the surface free energy (wettability) of the gutta-percha cones and Resilon surfaces. 19) CHX can be used during all phases of the root canal preparation, including the disinfection of the operative field, during the enlargement of the canals orifices and removal of necrotic tissues before root canal length determination; in the chemomechanical preparation: alternating its use with an irrigation with an inert solution (i.e. distilled water, sterile saline); prior to the foraminal patency and enlargement; as intracanal medicament alone or combined with other substances (i.e. CH); in the disinfection of gutta-percha cones; for modeling the main gutta-percha cone; in the removal of gutta-percha cones during retreatment; in the disinfection of prosthetic space; among others. If it extrudes through the apex, during instrumentation and foramen enlargement, it does not induce pain, for being less irritating to the periapical tissues than NaOCl. CHX has been recommended as an alternative to NaOCl, especially in cases of open apex, root resorption, foramen enlargement and root perforation or in cases of allergy related to bleaching solutions. 20) No adverse effects have been published regarding CHX use as an irrigant or intracanal medicaments. CHX adverse effects are usually related to its topical or oral application, including reversible discoloration of the tongue, teeth, and silicate or composite restorations, transient taste disturbances and a burning sensation of the tongue. The incidence of skin irritation and hypersensitivity is low and the biocompatibility is acceptable.

Resumo

Substâncias químicas auxiliares (SQA) são essenciais para o processo de limpeza e desinfecção dos canais radiculares, sendo utilizadas durante a instrumentação dos canais radiculares e, se necessário, como medicamentos intracanaís. Diferentes SQA têm sido propostas e utilizadas, entre elas: hipoclorito de sódio (NaOCl), clorexidina (CHX), EDTA 17%, ácido cítrico, MTAD e solução de ácido fosfórico a 37%. CHX tem sido usada na endodontia como SQA ou medicação intracanal. CHX possui uma ampla gama de atividade antimicrobiana; substantividade (atividade antimicrobiana residual); menor citotoxicidade que NaOCl, demonstrando desempenho clínico eficiente; propriedades de lubrificação; ação reológica (presente na apresentação gel, mantendo os detritos em suspensão); inibe metaloproteinases; é quimicamente estável; não mancha tecidos; é inodora; solúvel em água; entre outras propriedades. CHX tem sido recomendada como uma alternativa ao NaOCl, especialmente em casos de ápice aberto, reabsorção radicular, perfuração radicular e durante a ampliação foraminal, devido à sua biocompatibilidade, ou em casos de alergia ao NaOCl. O objetivo deste trabalho é fazer uma revisão do uso da clorexidina na medicina e na odontologia; sua estrutura química; forma de apresentação e armazenamento; mecanismo de ação, atividade antimicrobiana, incluindo, substantividade, efeitos sobre biofilmes e endotoxinas; efeito sobre infiltração microbiana coronal e apical; capacidade de dissolução do tecido; interação com os irrigantes; efeitos sobre a união à dentina, metaloproteinases e fibrilas de colágeno; a sua utilização como medicamento intracanal e difusão nos túbulos dentinários; a sua utilização como agente desinfetante de cones de

obturação; seus outros usos na terapia endodôntica, possíveis efeitos adversos, citotoxicidade e genotoxicidade.

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