REVIEW

The properties and applications of chlorhexidine in endodontics

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Abstract

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Microorganisms and their by-products are considered to be the major cause of pulp and periradicular pathosis. Hence, a major objective in root canal treatment is to disinfect the entire root canal system, which requires that all contents of the root canal system be eliminated as possible sources of infection. This goal may be accomplished using mechanical instrumentation and chemical irrigation, in conjunction with medication of the root canal system between treatment sessions. To reduce or eliminate bacteria, various irrigation solutions have been advocated. Chlorhexidine is a cationic molecule, which can be used during treatment. It has a wide range of antimicrobial activity. Its cationic structure provides a unique property named substantivity. The purpose of this paper is to review the structure and mechanism of action of CHX, its antibacterial and antifungal activity, its effect on biofilm, its substantivity (residual antibacterial activity), its tissue solvent ability, its interaction with calcium hydroxide and sodium hypochlorite, its anticollagenolytic activity, its effect on coronal and apical leakage of bacteria, its toxicity and allergenicity and the modulating effect of dentine and root canal components on its antimicrobial activity. A Medline search was performed from 1981 to the end of March 2008 and was limited to English-language papers. The keywords searched on Medline were 'chlorhexidine AND endodontics', 'chlorhexidine AND root canal therapy', 'chlorhexidine AND substantivity' and 'chlorhexidine AND toxicity'. The reference lists of each article were manually checked for additional articles of relevance.

Keywords: chlorhexidine, endodontics, irrigants, medicaments, substantivity.

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Introduction

The major causative role of microorganisms in the pathogenesis of pulp and periapical diseases has clearly been demonstrated (Kakehashi *et al.* 1965, Möller *et al.* 1981, Sundqvist 1992). The elimination of microorganisms from infected root canal systems is a complicated task involving the use of various instrumentation techniques, irrigation regimens and intracanal medicaments. Mechanical instrumentation alone does not result in a bacteria-free root canal system and when the complex anatomy of the root canal system (Hess 1925) is considered, this is not surprising. On the other hand, *ex vivo* and clinical evidence has shown that mechanical instrumentation leaves significant portions of the root canal walls untouched (Peters *et al.* 2001) and complete elimination of bacteria by instrumentation alone is unlikely to occur (Byström & Sundqvist 1981). It is assumed, but not demonstrated,

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that any pulp tissue left in the root canals can serve as a nutrient source for any remaining microorganisms. Furthermore, tissue remnants also impede the antimicrobial effects of root canal irrigants and medicaments. Therefore, some form of irrigation and disinfection is necessary to remove residual tissue and to kill microorganisms. Chemical treatment of the root canal can be arbitrarily divided into irrigants, canal rinses and inter-appointment medicaments. Chlorhexidine (CHX) is used widely as an endodontic irrigant and medicament, but there has not been an adequate review of the literature regarding CHX. Hence, the purpose of this paper is to review different aspects of CHX of relevance to endodontics. The literature review was performed using a Medline electronic search. The search was performed from 1981 to the end of March 2008 and was limited to English-language papers. The keywords searched on Medline were 'chlorhexidine AND endodontics', 'chlorhexidine AND root canal therapy', 'chlorhexidine AND substantivity' and 'chlorhexidine AND toxicity'. The reference lists of each article were manually checked for additional articles of relevance.

Structure and mechanism of action

Chlorhexidine is a synthetic cationic *bis*-guanide that consists of two symmetric 4-cholorophenyl rings and two biguanide groups, connected by a central hexamethylene chain (Greenstein *et al.* 1986). CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism (Athanassiadis *et al.* 2007). Its efficacy is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls (Gomes *et al.* 2003a,b), thereby altering the cells' osmotic equilibrium. This increases the permeability of the cell wall, which allows the CHX molecule to penetrate into the bacteria. CHX is a base and is stable as a salt. The most common oral preparation, CHX gluconate, is water-soluble and at physiologic pH, it readily dissociates and releases the positively charged CHX component (Greenstein *et al.* 1986). At low concentration (0.2%), low molecular weight substances, specifically potassium and phosphorous, will leak out of the cell. On the other hand, at higher concentration (2%), CHX is bactericidal as precipitation of the cytoplasmic contents occurs, which results in cell death (Gomes *et al.* 2003a).

Antibacterial activity

Delany *et al.* (1982) evaluated 0.2% CHX-gluconate in infected root canals. Bacteriologic samples were obtained before, during, immediately after and 24 h after instrumentation, irrigation and medication either with CHX-gluconate or with sterile saline. There was a highly significant reduction in the number of microorganisms in the CHX-treated specimens after instrumentation and irrigation. Basson & Tait (2001) compared the *ex vivo* effectiveness of calcium hydroxide [$Ca(OH)_2$], iodine potassium iodide (IKI) and a CHX solution in disinfecting root canal systems that were infected with *Actinomyces israelii*. The root canals were exposed to either IKI, calcium hydroxide or 2% CHX for periods of 3, 7 and 60 days. CHX was the only disinfectant that was able to eliminate *A. israelii* from all samples at all time periods whilst 25% of the specimens treated with IKI and 50% of the specimens treated with $Ca(OH)_2$ still had viable *A. israelii* after treatment. Onçağ *et al.* (2003) evaluated the antibacterial properties of 5.25% sodium hypochlorite (NaOCl), 2% CHX and 0.2% CHX plus 0.2% cetrimide [Cetrexidin (GABA Vebas, San Giuliano Milanese, Italy)] after 5 min and 48 h in extracted human teeth after the canals had been infected by *Enterococcus faecalis*. The 2% CHX and Cetrexidin were significantly more effective against *E. faecalis* than the 5.25% NaOCl at both time periods. Two studies (Gomes *et al.* 2001, Vianna *et al.* 2004) have investigated the *ex vivo* antimicrobial activity against endodontic pathogens of three concentrations (0.2%, 1% and 2%) of two forms of CHX (gel and liquid) and compared them with five concentrations of NaOCl (0.5%, 1%, 2.5%, 4% and 5.25%). Both the 2% gel and 2% liquid formulations of CHX eliminated *Staphylococcus aureus* and *Candida albicans* within 15 s, whereas the gel formulation killed *E. faecalis* within 1 min. All of the tested irrigants eliminated *Porphyromonas endodontalis*, *Porphyromonas gingivalis* and *Prevotella intermedia* within 15 s. The time required for 1.0% and 2.0% CHX liquid to eliminate all microorganisms was the same as the time required for 5.25% NaOCl. These studies confirm that the antimicrobial action is related to the type, concentration and presentation form of the irrigants as well as the microbial susceptibility to the formulation used.

Zamany *et al.* (2003) examined the effects of adding a 2% CHX rinse to the conventional treatment protocol. Their results showed that cultivable bacteria were retrieved at the conclusion of the first visit in one of the CHX cases, whereas seven of the 12 control cases

without CHX showed growth; this difference was statistically significant. Siqueira *et al.* (2007) compared the effectiveness of 2.5% NaOCl and 0.12% CHX as irrigants in reducing the cultivable bacteria in infected root canal systems of teeth with apical periodontitis. They found that the two solutions had comparable effects in eliminating bacteria and they suggested that both could be used as irrigants.

In a randomized clinical trial, Manzur *et al.* (2007) assessed the antibacterial efficacy of intracanal medication with $Ca(OH)_2$, 2% CHX gel and a combination of both [$Ca(OH)_2$/CHX] in teeth with chronic apical periodontitis. Bacteriological samples were obtained from the operative field and the root canals before and after instrumentation in the first treatment session. Further samples were taken from the canals at the commencement of the second appointment 1 week later. They concluded that the antibacterial efficacies of $Ca(OH)_2$, CHX and a mixture of $Ca(OH)_2$/CHX were comparable.

Zerella *et al.* (2005) investigated the effect of a slurry of $Ca(OH)_2$ mixed in aqueous 2% CHX versus aqueous $Ca(OH)_2$ alone on the disinfection of the root canal system of root filled teeth that required root canal re-treatment because the canals had become infected again. Twelve (30%) of the 40 samples were positive for bacteria before root filling. The control medication disinfected 12 (60%) of 20 teeth including two of four teeth that had been originally diagnosed with *enterococci*. The experimental medication resulted in disinfection of 16 of 20 (80%) teeth at the beginning of the third appointment. None of the teeth originally containing *enterococci* showed remaining growth. They concluded that a mixture of 2% CHX and a $Ca(OH)_2$ slurry is as efficacious as aqueous $Ca(OH)_2$ on the disinfection of infected root filled teeth.

Ercan *et al.* (2004) evaluated the antibacterial activity of 2% CHX and 5.25% NaOCl in infected root canals of incisors and premolars. They concluded that both CHX and NaOCl were effective irrigants for reducing the number of microorganisms in teeth with a necrotic pulp, periapical pathosis or both.

Tanomaru *et al.* (2003) evaluated the effect of biomechanical preparation with 5% NaOCl, 2% CHX and physiological saline irrigating solutions and $Ca(OH)_2$ dressing in the root canals of dogs' teeth that contained bacterial endotoxin. They found that biomechanical preparation with the irrigating solutions did not inactivate the endotoxin, but the calcium hydroxide intracanal dressing did inactivate the effects induced by the endotoxin *in vivo*.

Another interesting topic is the additive effect of CHX and hydrogen peroxide. Heling & Chandler (1998) studied the antimicrobial effect of irrigant combinations within dentinal tubules *ex vivo* against *E. faecalis* and found that a specific combination of 3% hydrogen peroxide (H_2O_2) and CHX was superior in its antibacterial activity in dentine compared with other regimens, such as CHX alone and NaOCl. Steinberg *et al.* (1999) challenged *E. faecalis* suspensions in trypticase soy broth (a culture medium rich in peptides) with various combinations of CHX and H_2O_2. The experiments demonstrated that the combination of the two substances totally killed *E. faecalis* at concentrations much lower than that required for each component alone. According to that study, the bactericidal effect of CHX is derived from its ability to denature the bacterial cell wall whilst forming pores in the membrane, whereas H_2O_2 is effective against intracellular organelles, such as DNA. Although the exact synergistic mechanism of CHX and H_2O_2 is not known, it can be postulated that the exposure of bacteria to CHX leads to a more permeable cell wall that the H_2O_2 can easily penetrate and hence damage the intracellular organelles (Steinberg *et al.* 1999). Shabahang *et al.* (2008) evaluated the antibacterial efficacy of the substitution of CHX for doxycycline in MTAD against a strain of *E. faecalis ex vivo*. Findings showed that the presence of doxycycline in the concentration included in the MTAD formulation was effective in eliminating *E. faecalis*. Furthermore, the addition of 0.2% CHX did not adversely affect the antibacterial action of doxycycline. On the other hand, the substitution of 0.2% CHX did not allow the same disinfection efficacy on *E. faecalis* as MTAD.

On the whole, although studies comparing the antibacterial effect of CHX and NaOCl have produced somewhat conflicting results, it seems that when used in identical concentrations, their antibacterial effects *ex vivo* (in infected dentine) and *in vivo* (in the root canal system) are similar.

Antifungal activity

Fungi (or yeasts) constitute a small proportion of the usual oral microbiota with *Candida* species being the most common of the fungi present in both healthy (30–45%) and medically compromised (95%) individuals (Siqueira & Sen 2004). Fungi have occasionally been found in infected root canals that have not had any previous endodontic treatment, but they are more common in filled root canals in teeth that have become infected some time after treatment or in those that have

not responded to endodontic treatment (23). Overall, the occurrence of fungi reported in infected root canals varies between 1% and 17% (Waltimo *et al.* 2004).

Fungi may be involved in cases of persistent and secondary infections associated with recalcitrant peri-iradicular lesions and therefore the spectrum of anti-microbial activity of endodontic medicaments and irrigants should include these organisms. Thus, medicaments that have antifungal effectiveness may assist in the successful management of persistent or secondary endodontic infections caused by fungi (Siqueira & Sen 2004, Waltimo *et al.* 2004). To try and improve antisepsis in single-appointment endodontic treatment regimes, it has been suggested to irrigate and/or 'soak' the root canals with either CHX or iodine-IKI solutions following irrigation with NaOCl. Aqueous CHX solution has a wide spectrum of antimicrobial activity at low concentrations and is especially effective against *C. albicans*. Furthermore, it binds to surrounding tissues and can then be released again slowly over extended periods of time, a phenomenon known as substantivity. Interestingly, it appears that CHX can efficiently inhibit the initial adherence and perhaps further accumulation and biofilm formation of fungi and other micro-organisms. A recent clinical study has shown that canals that received a final rinse with a 2% CHX solution were significantly more often free of cultivable microorganisms than controls irrigated with NaOCl alone (Siqueira & Sen 2004, Waltimo *et al.* 2004).

Sen *et al.* (1999) evaluated the antifungal properties of 0.12% CHX, 1% NaOCl and 5% NaOCl against *Candida albicans* using a cylindrical dentine tube model. They reported that *C. albicans* was more resistant to these irrigant solutions when the smear layer was present than when it was absent. When smear layer was absent, the NaOCl started to display antifungal activity after 30 min. Waltimo *et al.* (1999) evaluated the susceptibility of seven strains of *C. albicans* to four disinfectants, namely IKI, CHX-acetate (0.5%), NaOCl (5% and 0.5%) and Ca(OH)$_2$. Each solution was tested individually as well as in pairs using all possible pairs of these four disinfectants. All *C. albicans* strains tested showed similar susceptibility to these medicaments. They were highly resistant to Ca(OH)$_2$, but the NaOCl and IKI killed all cells within 30 s and the CHX-acetate showed complete killing after 5 min. Combinations of disinfectants were either equally or less effective than the more effective component of the pair tested.

Siqueira *et al.* (2003) evaluated the effectiveness of four intracanal medications in disinfecting root dentine in bovine teeth experimentally infected with *C. albicans*.

Infected dentine cylinders were exposed to four different medications: Ca(OH)$_2$/glycerin; Ca(OH)$_2$/0.12% CHX; Ca(OH)$_2$/camphorated monoparachlorophenol/glycerin and 0.12% CHX/zinc oxide. The specimens treated with the Ca(OH)$_2$/camphorated monoparachlorophenol/glycerin paste or with the CHX/zinc oxide paste were completely disinfected after 1 h of exposure whilst the Ca(OH)$_2$/glycerin paste consistently eliminated the *C. albicans* after 7 days of exposure. Calcium hydroxide mixed with CHX was ineffective in disinfecting dentine even after 1 week. In another study, Siqueira *et al.* (2001) investigated the antifungal activity of several medicaments against *C. albicans*, *Candida glabrata*, *Candida guilliermondii*, *Candida parapsilosis* and *Saccharomyces cerevisiae*. Calcium hydroxide mixed with CPMC/glycerin as a paste showed the most pronounced antifungal effects. Calcium hydroxide in glycerin, Ca(OH)$_2$ with CHX and CHX in detergent had less antifungal activity. Ferguson *et al.* (2002) sought to determine the *in vitro* susceptibility of *C. albicans* to various irrigants and medicaments. The minimum inhibitory concentrations of NaOCl, hydrogen peroxide, CHX-digluconate and aqueous Ca(OH)$_2$ were determined. Their results revealed that NaOCl, hydrogen peroxide and CHX-digluconate were effective against *C. albicans* even when significantly diluted. However, aqueous Ca(OH)$_2$ had no anti-fungal activity. Taken together, it can be concluded that CHX is an effective antifungal agent, but its efficacy is significantly less than NaOCl.

CHX and biofilms

The term biofilm was introduced to designate the thin-layered condensations of microbes that may occur on various surface structures in nature. Free-floating bacteria existing in an aqueous environment, the so-called planktonic form of microorganisms, are a pre-requisite for biofilm formation (Bowden & Hamilton 1998). Biofilms may thus become established on any organic or inorganic surface substrate where plank-tonic microorganisms prevail in a water-based solution. In dental contexts, a well-known and extensively studied biofilm structure is established during the attachment of bacteria to teeth to form dental plaque. Here, bacteria free in saliva (planktonic organisms) serve as the primary source of organisms for the organization of this specific biofilm (Bowden & Hamilton 1998). In endodontics, the biofilm concept was initially discussed mainly within the framework of bacteria on the root tips of teeth with necrotic and

infected pulps or pulpless and infected root canal systems. Such bacterial aggregations have been thought to be the cause of therapy-resistant apical periodontitis (Tronstad & Sunde 2003). Although not described in as much detail, bacterial condensations (that is, biofilms) on the walls of infected root canals have been observed.

Antimicrobial agents have often been developed and optimized for their activity against fast growing, dispersed populations containing a single microorganism. However, microbial communities grown in biofilms are remarkably difficult to eradicate with antimicrobial agents and microorganisms in mature biofilms can be notoriously resistant for reasons that have yet to be adequately explained (Bowden & Hamilton 1998). There are reports showing that microorganisms grown in biofilms could be twofold to 1000-fold more resistant than the corresponding planktonic form of the same organisms (Svensater & Bergenholtz 2004). Spratt *et al.* (2001) have evaluated the effectiveness of 2.25% NaOCl, 0.2% CHX, 10% povidone iodine, 5 ppm colloidal silver and phosphate buffered solution (PBS) as a control against monoculture biofilms of five root canal isolates including *P. intermedia*, *Peptostreptococcus miros*, *Streptococcus intermedius*, *Fusobacterium nucleatum* and *E. faecalis*. They reported that NaOCl was the most effective antimicrobial agent followed by the iodine solution. Clegg *et al.* (2006) evaluated the *ex vivo* effectiveness against apical dentine biofilms of three concentrations of NaOCl (6%, 3% and 1%), 2% CHX and a commercially available mixture of a tetracycline, an acid and a detergent known as BioPure MTAD (Dentsply, Tulsa Dental, Tulsa, OK, USA). They reported that the 6% NaOCl and 3% NaOCl were capable of disrupting and removing the biofilm, but the 1% NaOCl and the MTAD were capable of disrupting the biofilm, but did not eliminate the bacteria. The 2% CHX was not capable of disrupting the biofilm. Viable bacteria could not be cultured from specimens exposed to 6% NaOCl, 2% CHX or 1% NaOCl followed by BioPure MTAD. Dunavant *et al.* (2006) evaluated the efficacy of 6% NaOCl, 1% NaOCl, Smear Clear™ (SybronEndo, Orange, CA, USA), 2% CHX, REDTA (Roths International Ltd, Chicago, IL, USA) and BioPure™ MTAD™ against *E. faecalis* biofilms using a novel laboratory testing system. Biofilms grown in a flow cell system were submerged in test irrigants for either 1 or 5 min. There was a significant relationship between the test agent and the percentage kill of the bacteria in the biofilm. No significant relationship between time and percentage kill was found. The percentage kills of the bacteria were: 6% NaOCl (>99.99%), 1% NaOCl (99.78%), Smear Clear™ (78.06%), 2% CHX (60.49%), REDTA (26.99%) and BioPure™ MTAD™ (16.08%). There was a significant difference between NaOCl (both concentrations of 1% and 6%) and all other agents. Therefore, both 1% NaOCl and 6% NaOCl were more efficient in eliminating *E. faecalis* biofilm than the other solutions tested. In another study, Lima *et al.* (2001) assessed the effectiveness of CHX-based or antibiotic-based (clindamycin and metronidazole) medications in eliminating 1- and 3-day *E. faecalis* biofilms. Each biofilm-containing membrane was thoroughly covered with 1 mL of the test medications and incubated for 1 day at 37 °C. The treated biofilms were then aseptically transferred to vials containing a neutralizing agent in saline solution and vortexed. Suspensions were diluted 10-fold, seeded onto *Mitis salivarius* agar plates and the colony-forming units counted after 48 h of incubation. There were significant differences between the formulations tested. The association of clindamycin with metronidazole significantly reduced the number of cells in the 1-day biofilms. However, of all medications tested, only 2% CHX-containing medications were able to thoroughly eliminate most of both the 1-day and 3-day *E. faecalis* biofilms. On the whole, it seems that although CHX is somewhat effective against bacterial biofilms, NaOCl is the only irrigation solution with the capability of disrupting the biofilms.

Substantivity

Chlorhexidine as well as tetracyclines have a unique feature in that dentine medicated with it acquires antimicrobial substantivity (Khademi *et al.* 2006). The positively charged ions released by CHX can adsorb into dentine and prevent microbial colonization on the dentine surface for some time beyond the actual the period of time of application of the medicament (Athanassiadis *et al.* 2007).

The antimicrobial substantivity of CHX has been assessed in several periodontal and endodontic studies. In an *in vivo* periodontal study, Stabholz *et al.* (1993) evaluated the substantivity of the human root surface after *in situ* subgingival irrigation with tetracycline HCL and CHX. They found that the substantivity of tetracycline at 50 mg mL^{-1} was significantly greater than that of CHX for 12 days and greater than saline for 16 days.

In a laboratory study, White *et al.* (1997) evaluated the antimicrobial substantivity of a 2% CHX solution as an endodontic irrigant. Findings showed that the

substantivity lasted 72 h. In an *in vivo* study, Leonardo *et al.* (1999) evaluated the antimicrobial substantivity of 2% CHX used as a root canal irrigating solution in teeth with pulp necrosis and radiographically visible chronic periapical lesions. They reported that the CHX prevented microbial activity with residual effects in the root canal system for up to 48 h after application. However, some other studies have reported that the substantivity of CHX can last for longer periods of time. Khademi *et al.* (2006) found that 5 min application of 2% CHX solution induced substantivity for up to 4 weeks. Rosenthal *et al.* (2004) evaluated the substantivity of 2% CHX solution within the root canal system after 10 min of application and they reported that the CHX was retained in the root canal dentine in antimicrobially effective amounts for up to 12 weeks.

Antimicrobial substantivity depends on the number of CHX molecules available to interact with the dentine. Therefore, medicating the canal with a more concentrated CHX preparation should result in increased resistance to microbial colonization. The antibacterial substantivity of three concentrations of CHX solution (4%, 2% and 0.2%) after 5 min of application has been evaluated. Results revealed a direct relationship between the concentration of CHX and its substantivity (Mohammadi *et al.* 2008). On the contrary, Lin *et al.* (2003a) attributed the substantivity of CHX to its ability to adsorb on to the dentine during the first hour. They stated that it is only after the saturation point is reached after the first hour that the antimicrobial capability of CHX increases with time. Furthermore, Komorowski *et al.* (2000) revealed that 5 min application of CHX did not induce substantivity and that the dentine should be treated with CHX for 7 days. Taken together, it seems that residual antimicrobial activity of CHX in the root canal system remains for up to 12 weeks.

Modulating effect of dentine on CHX

The root canal milieu is a complex mixture of a variety of organic and inorganic compounds. Hydroxyapatite, the main component of dentine, is the major representative of inorganic components present. In addition, inflammatory exudate, entering the apical root canal in purulent infections, is rich in proteins, such as albumin. The relative importance of the various organic and inorganic compounds in the inactivation of root canal disinfectants have been studied restrictively (Haapasalo *et al.* 2000). Difficulties in designing experiments that will give reliable and comparable data have been some of the greatest challenges for researchers for many

years. Haapasalo *et al.* (2000) introduced a new dentine powder model for studying the inhibitory effect of dentine on various root canal irrigants and medicaments. They investigated the modulating effect of dentine on the antibacterial activity of saturated $CA(OH)_2$ solution, 1% NaOCl, 0.5% and 0.05% CHX acetate and 2/4% and 0.2/0.4% IKI. Dentine powder had an inhibitory effect on all medicaments tested. The effect was dependent on the concentration of the medicament as well as on the length of time the medicament was pre-incubated with the dentine powder before adding the bacteria. Similarly, 0.2/0.4% IKI lost its effect after pre-incubation for 1 h with dentine before adding the bacteria. The effect of 0.05% CHX and 1% NaOCl on *E. faecalis* was reduced, but not totally eliminated by the presence of dentine. No inhibition could be measured when full strength solutions of CHX and IKI were used in killing *E. faecalis*. Portenier *et al.* (2001) evaluated the inhibition of the antibacterial effect of saturated $Ca(OH)_2$ solution, CHX-acetate and IKI by dentine, hydroxylapatite (HA) and bovine serum albumin (BSA). Calcium hydroxide was totally inactivated by the presence of 28 mg of dentine powder or BSA. CHX (0.05%) was strongly inhibited by BSA and slowed down by dentine. However, HA had little or no inhibitory effect on CHX. The antibacterial effect of 0.2/0.4% IKI on *E. faecalis* was totally inhibited by dentine (28 mg), but was practically unaffected by HA or BSA. A stepwise reduction of dentine from 28 to 2.8 mg 150 μL^{-1} was followed by a similar reduction of the inhibition of the antibacterial activity of CHX. IKI was not inhibited at all with dentine amounts <28 mg. However, the effect of saturated calcium hydroxide solution was totally eliminated by dentine at all four concentrations tested. It could be assumed that inhibition by dentine of the antibacterial activity of $Ca(OH)_2$, CHX and IKI occurs by different mechanisms (Portenier *et al.* 2001). Surprisingly, $Ca(OH)_2$ was sensitive to the inhibitory effect of all three materials tested. The inhibition of $Ca(OH)_2$ by dentine and by the other compounds is, of course, dependent on their quantitative relationships (Portenier *et al.* 2001). One major mechanism for resistance of survival of *E. faecalis* in the root canal filled with $Ca(OH)_2$ may be the buffering effect of dentine against the pH rise. Inorganic HA had little or no inhibitory activity against CHX as compared with dentine, whereas BSA was the strongest inhibitor of CHX, with more than 10% of *E. faecalis* cells still viable after 24 h of incubation with the medicament. This indicates that periapical inflammatory exudate entering the root canal is a greater threat to the activity

of CHX than the dentine walls. Dentine powder totally eliminated the antibacterial effect of IKI; whereas the major component of dentine, HA did not affect the antibacterial effect of IKI, nor did BSA. In addition, it is generally known that blood rapidly inactivates the antibacterial activity of iodine compounds (Portenier *et al.* 2001). In another study, Portenier *et al.* (2002) assessed the antibacterial activity of CHX and IKI on *E. faecalis* in the presence of dentine, dentine matrix, dentine pre-treated by ethylenediamine tetraacetic acid (EDTA) and citric acid, collagen and heat-killed cells of *E. faecalis* and *Candida albicans.* Dentine matrix and heat-killed microbial cells were the most effective inhibitors of CHX, whereas dentine pre-treated by citric acid or EDTA showed only slight inhibition. Dentine and skin collagen showed some inhibition at 1 h, but not after 24 h. IKI was effectively inhibited by dentine, dentine matrix and heat-killed microbial cells. Skin collagen and dentine pre-treated by EDTA or by citric acid showed little or no inhibitory effect on IKI. The inhibitory effect of dentine and BSA on the antibacterial activity of CHX and MTAD was assessed in another study (Portenier *et al.* 2006). The presence of dentine or BSA caused a marked delay in the killing of *E. faecalis* by both medicaments. The inhibitory effect of BSA on the antibacterial activity of CHX and NaOCl has been confirmed by Sassone *et al.* (2008). Taken together, it seems that dentine, dentine components (HA and collagen), killed microorganisms and inflammatory exudate in the root canal system all reduce or inhibit the antibacterial activity of medicaments and irrigants. On the whole, it seems that dentine, dentine components (HA and collagen), killed microorganisms and inflammatory exudates in the root canal system reduce the antibacterial activity of CHX.

Tissue-solvent effects of CHX

Several studies have been conducted in the search for an irrigant that meets the four major desirable properties for root canal irrigants – namely: antimicrobial activity, nontoxicity to the periapical tissues, water solubility and the capacity to dissolve organic matter. Therefore, an ideal irrigant should dissolve the organic matter inside the root canal system. Grossman & Meiman (1941) demonstrated the importance of the solvent ability of an endodontic irrigant and emphasized that the elimination of pulp tissue from the root canal was important for the ultimate success of root canal treatment. Moorer & Wesselink (2003) showed that tissue dissolution was dependent on three factors:

the frequency of shaking, the amount of organic matter in relation to the amount of irrigant in the system and the surface area of tissue that was available for contact with the irrigant. Okino *et al.* (2004) evaluated the tissue dissolving ability of 0.5%, 1.0% and 2.5% NaOCl, 2% aqueous solution of CHX-digluconate, 2% CHX digluconate gel and distilled water as the control. Bovine pulp fragments were weighed and placed in contact with 20 mL of each tested substance in a centrifuge at 150 rpm until total dissolution. Dissolution speed was calculated by dividing the pulp weight by the dissolution time. Distilled water and both solutions of CHX did not dissolve the pulp tissue within 6 h. The mean dissolution speeds for 0.5%, 1.0% and 2.5% NaOCl solutions were 0.31, 0.43 and 0.55 mg min^{-1}, respectively. In another study, Naenni *et al.* (2004) assessed the necrotic tissue dissolution capacity of 1% (w/v) NaOCl, 10% CHX, 3% and 30% hydrogen peroxide, 10% peracetic acid, 5% dichloro-isocyanurate (NaDCC) and 10% citric acid. Standardized necrotic tissue samples obtained from pig palates were incubated in these solutions and their weight loss was measured over time. None of the test solutions except NaOCl had any substantial tissue dissolution capacity. It was concluded that this might be important when considering the use of irrigants other than NaOCl. On the whole, one of the major disadvantages of CHX is that it has no tissue solvent activity.

CHX and Ca(OH)$_2$

Chlorhexidine is a cationic biguanide whose optimal antimicrobial activity is achieved within a pH range of 5.5–7.0 (Athanassiadis *et al.* 2007). Therefore, it is likely that alkalinizing the pH by adding Ca(OH)$_2$ to CHX will lead to precipitation of the CHX molecules and thereby decreases its effectiveness. It has been demonstrated that the alkalinity of Ca(OH)$_2$ when mixed with CHX remained unchanged. Therefore, the usefulness of mixing Ca(OH)$_2$ with CHX still remains unclear and controversial (Athanassiadis *et al.* 2007).

When used as an intracanal medicament, CHX was more effective than Ca(OH)$_2$ in eliminating *E. faecalis* from inside dentinal tubules (Athanassiadis *et al.* 2007). In a study by Almyroudi *et al.* (2002), all of the CHX formulations used, including a CHX/ Ca(OH)$_2$ 50:50 mix, were efficient in eliminating *E. faecalis* from the dentinal tubules with a 1% CHX gel working slightly better than the other preparations. These findings were corroborated by Gomes *et al.* (2006) in bovine dentine and Schafer & Bossmann (2005) in

human dentine where 2% CHX gel had greater activity against *E. faecalis*, followed by CHX/ Ca(OH)$_2$ and then Ca(OH)$_2$ used alone.

In a study using agar diffusion, Haenni *et al.* (2003) could not demonstrate any additive antibacterial effect by mixing Ca(OH)$_2$ powder with 0.5% CHX and they showed that the CHX had a reduced antibacterial action. However, Ca(OH)$_2$ did not lose its antibacterial properties in such a mixture. This may be because of the deprotonation of CHX at a pH >10, which reduces its solubility and alters its interaction with bacterial surfaces as a result of the altered charge of the molecule. In an *in vitro* study using human teeth, Ercan *et al.* (2006) showed 2% CHX gel was the most effective agent against *E. faecalis* inside dentinal tubules, followed by a Ca(OH)$_2$/ 2% CHX mix, whilst Ca(OH)$_2$ alone was totally ineffective, even after 30 days. The 2% CHX gel was also significantly more effective than the Ca(OH)$_2$/2% CHX mix against *C. albicans* at 7 days, although there was no significant difference at 15 and 30 days. Ca(OH)$_2$ alone was completely ineffective against *C. albicans*. In another *in vivo* study using primary teeth, a 1% CHX-gluconate gel, both with and without Ca(OH)$_2$, was more effective against *E. faecalis* than CH alone over a 48-h period (Oncag *et al.* 2006).

Schafer & Bossmann (2005) reported that 2% CHX-gluconate was significantly more effective against *E. faecalis* than Ca(OH)$_2$ used alone or a mixture of the two. This was also confirmed by Lin *et al.* (2003b) although in a study by Evans *et al.* (2003) using bovine dentine, 2% CHX with Ca(OH)$_2$ was shown to be more effective than Ca(OH)$_2$ in water. In an animal study, Lindskog *et al.* (1998) reported that teeth dressed with CHX for 4 weeks had reduced inflammatory reactions in the periodontium (both apically and marginally) and less root resorption. Waltimo *et al.* (1999) reported that 0.5% CHX-acetate was more effective at killing *C. albicans* than saturated Ca(OH)$_2$, whilst Ca(OH)$_2$ combined with CHX was more effective than Ca(OH)$_2$ used alone. The high pH of Ca(OH)$_2$ was unaffected when combined with CHX in this study. Taken together, it seems that the usefulness of mixing Ca(OH)$_2$ with CHX remains unclear and controversial.

CHX and coronal penetration of bacteria

Because of its antimicrobial substantivity, it seems that CHX preparations delay entry of bacteria through the coronal portion of the tooth into the root canal system. In a laboratory study, Gomes *et al.* (2003b) investigated the time required for recontamination of the root canal system of teeth with coronal restorations medicated with either calcium hydroxide, 2% CHX gel or with a combination of both. The canals without a coronal restoration, but medicated with CHX, showed recontamination after an average time of 3.7 days; the group with Ca(OH)$_2$ after 1.8 days and the group with CHX+Ca(OH)$_2$ after 2.6 days. The canals medicated with CHX and restored with IRM showed recontamination within 13.5 days; the group with Ca(OH)$_2$+IRM after 17.2 days and the group with CHX+ Ca(OH)$_2$+IRM after 11.9 days. The group with no medication, but restored with IRM, showed recontamination after an average time of 8.7 days. There were statistically significant differences between the groups ($P < 0.05$). All groups without a coronal restoration were recontaminated significantly more quickly than those restored with IRM, except those teeth that had a restoration, but no medicament. The groups with intracanal medication and a coronal restoration were not significantly different from each other.

Vivacqua-Gomes *et al.* (2002) assessed *ex vivo* coronal dye penetration of extracted human teeth after root canal treatment using 1% NaOCl, 1% NaOCl + 17% EDTA, 2% CHX gel, 2% CHX gel + 1% NaOCl and distilled water. After root canal filling, the teeth were incubated at 37 °C for 10 days followed by 10 days immersion in human saliva and an additional 10 days in India ink. The teeth were cleared and the maximum depth of dye penetration was determined digitally in millimetres. Results revealed that the least dye penetration occurred with 1% NaOCl + 17% EDTA and 2% CHX gel. NaOCl, distilled water and 2% CHX gel + 1% NaOCl had more dye penetration with a significant difference compared with NaOCl + 17% EDTA and 2% CHX gel and compared with one another.

Other studies have shown that viscous irrigants, including those containing CHX gluconate, were less soluble substances and they can leave residues on the root canal surfaces, which may affect the root canal filling. Lambrianidis *et al.* (2006) investigated the efficiency of removing Ca(OH)$_2$/CHX gel, Ca(OH)$_2$/ CHX solution and Ca(OH)$_2$/saline pastes with the use of instrumentation and irrigation with NaOCl and EDTA solutions. None of the techniques used in this study removed the inter-appointment root canal medicaments effectively (Lambrianidis *et al.* 2006). Overall, Ca(OH)$_2$/CHX (gel) paste was associated with significantly larger amount of residue, whereas the Ca(OH)$_2$/ CHX mixture was associated with less residue than the other two medicaments. When all these studies are considered it appears as although CHX used as an

intracanal medicament and/or irrigant may delay recontamination of the root canal system via the coronal route because of its substantivity. Overall, because of its substantivity, CHX as an intracanal medicament/irrigant delays recontamination of the root canal system via the coronal route.

CHX and apical fluid penetration

Marley *et al.* (2001) assessed the effect of 0.12% CHX-gluconate as an endodontic irrigating solution on the apical seal of root filled canals using three different cements (Roth's 801, AH26 and Sealapex). At 90 and 180 days after root filling, apical fluid penetration was measured using the fluid filtration method. The results showed no significant differences related to the irrigants at both the 90- and 180-day observation periods. Furthermore, the same group reported that at long-term periods (270 and 360 days), CHX-gluconate irrigant did not adversely affect the apical penetration of fluid with the different root canal cements (Ferguson *et al.* 2003). Wuerch *et al.* (2004) investigated the effect of CHX gel and $Ca(OH)_2$ on the apical seal of root canal fillings. They reported that 2% CHX gel and $Ca(OH)_2$ paste did not adversely affect the apical seal. These findings were also confirmed by Engel *et al.* (2005). Overall, it seems that medication and/or irrigation with CHX does not adversely affect the ability of root fillings to prevent fluid penetration into the root canal system through the apical foramen.

Interaction between CHX and NaOCl

A suggested clinical protocol by Zehnder (2006) for treating the dentine before root canal filling consists of irrigation with NaOCl to dissolve the organic components, irrigation with EDTA to eliminate the smear layer and irrigation with CHX to increase the anti-microbial spectrum of activity and to impart substantivity. Although such a combination of irrigants may enhance the overall antimicrobial effectiveness (Kuruvilla & Kamath 1998), the possible chemical interactions amongst the irrigants have to be considered. Some studies have reported the occurrence of colour change and precipitation when NaOCl and CHX are combined (Vivacqua-Gomes *et al.* 2002, Zehnder 2006, Basrani *et al.* 2007). Furthermore, concern has been raised that the colour change may have some clinical relevance because of staining and that the precipitate might interfere with the seal of the root filling (Vivacqua-

Gomes *et al.* 2002). The formation of a precipitate could be explained by the acid–base reaction that occurs when NaOCl and CHX are mixed together. CHX, a dicationic acid has the ability to donate protons whilst NaOCl is alkaline and can accept protons from the dicationic acid. This proton exchange results in the formation of a neutral and insoluble substance, referred to as the 'precipitate' (Basrani *et al.* 2007). Basrani *et al.* (2007) evaluated the chemical nature of this precipitate and reported that there was an immediate reaction when 2% CHX was combined with NaOCl, even at the low concentration (0.023%). Increasing of the concentration of NaOCl to 0.19% (the sixth dilution in their series) resulted in the formation of a precipitate, which consisted mainly of para-chloroaniline (PCA). This occurred through a substitution of the guanidine group in the CHX molecule. They found that the amount of PCA directly increased with the increasing concentration of NaOCl. PCA has been shown to be toxic with short-term exposure of humans to PCA resulting in cyanosis, which is a manifestation of methemoglobin formation. In another study, Bui *et al.* (2008) evaluated the effect of irrigating root canals with a combination of NaOCl and CHX on root dentine and dentinal tubules by using the environmental scanning electron microscope (FEI Quanta 200, Hillsboro, OR, USA) and a computer program (PHOTOSHOP CS2, Adobe Systems, San Jose, CA, USA). Their findings indicated that there were no significant differences in the amount of debris remaining between the negative control group and the experimental groups although there were significantly fewer patent tubules in the experimental groups when compared with the negative control group. They concluded that the NaOCl/CHX precipitate tends to occlude the dentinal tubules and suggested that until this precipitate is studied further, caution should be exercised when irrigating with both NaOCl and CHX. Taken together, the combination of NaOCl and CHX causes colour changes and formation of a neutral and insoluble precipitate, which may interfere with the seal of the root filling. Copious amounts of CHX irrigant should be administered to prevent discolouring of the tooth by this precipitate. Alternatively, the canal can be dried using paper points before the final CHX rinse (Zehnder 2006).

CHX and dentine bonding (anticollagenolytic activity)

During the last two decades, chemical and technical advances have contributed to increases in resin–dentine

bond strength. However, the premature loss of bond strength is one of the problems that still affects adhesive restorations (Mjör *et al.* 2000) and markedly reduces their durability (Carrilho *et al.* 2005b, De Munck *et al.* 2005, Frankenberger *et al.* 2005). The loss of bond strength has been attributed mainly to the degradation of the hybrid layer at the dentine-adhesive interface. Numerous publications have demonstrated this lack of bond stability (Wang & Spencer 2003, 2005, Yiu *et al.* 2004, Carrilho *et al.* 2005a). The notion that deterioration of dentine collagen fibrils contributes to the mechanism responsible for bond degradation has been reported (Hashimoto *et al.* 2003, Pashley *et al.* 2004). In this context, it has been speculated that a decreasing concentration gradient of resin monomer diffusion within the acid-etched dentine and a subsequent resin elution from hydrolytically unstable polymeric hydrogels within the hybrid layers (Wang & Spencer 2003) leaves the collagen fibrils unprotected and vulnerable to degradation by endogenous metalloproteinases (MMPs). The MMPs are a group of 23 mammalian enzymes capable of degrading all extracellular matrix components. Human dentin contains at least collagenase (MMP-8), gelatinases MMP-2 and -9 and enamelysin MMP-20 (Martin-De Las Heras *et al.* 2000, Sulkala *et al.* 2002, 2007, Mazzoni *et al.* 2006). Dentine collagenolytic and gelatinolytic activities (Pashley *et al.* 2004) can be suppressed by protease inhibitors (Pashley *et al.* 2004), indicating that MMP inhibition could be beneficial in the preservation of hybrid layers. This was demonstrated in an *in vivo* study, in which the application of CHX, known to have a broad-spectrum MMP-inhibitory effect (Gendron *et al.* 1999), significantly improved the integrity of the hybrid layer in a 6-month clinical trial (Hebling *et al.* 2005). Carrilho *et al.* (2007a) evaluated the effect of CHX on the resin–dentine bond stability *ex vivo*. Results showed that with CHX, significantly better preservation of bond strength was observed after 6 months and protease inhibitors in the storage medium had no effect. Failure analysis showed significantly less failure in the hybrid layer with CHX, compared with controls after 6 months. Furthermore, they evaluated the effect of CHX on the preservation of the hybrid layer *in vivo*. Findings showed that bond strength remained stable in the CHX-treated specimens, whilst bond strength decreased significantly in control teeth. Resin-infiltrated dentine in CHX-treated specimens exhibited normal structural integrity of the collagen network. Conversely, progressive disintegration of the fibrillar network was identified in control specimens. They concluded that auto-degradation of collagen matrices can occur in resin-infiltrated dentine, but may be prevented by the application of a synthetic protease inhibitor, such as CHX (Carrilho *et al.* 2007b). On the whole, because of its broad-spectrum MMP-inhibitory effect, CHX can significantly improve the resin–dentine bond stability.

Cytotoxicity of CHX

Results from a study of the cytotoxic effect of chlorhexidine on canine embryonic fibroblasts and *Staphylococcus aureus* showed that bactericidal concentrations of CHX were lethal to canine embryonic fibroblasts whilst noncytotoxic concentrations allowed significant bacterial survival (Sanchez *et al.* 1988). In a study by Tatnall *et al.* (1990), the cytotoxic effects of CHX, hydrogen peroxide and NaOCl were examined on cultured human fibroblasts, basal keratinocytes and a transformed keratinocyte line (SVK 14 cells). At concentrations recommended for wound cleansing, all agents produced 100% killing of all cell types. Comparison of the ED_{50} concentration for each agent on all cell types produced a ranking order of toxicity showing CHX to be the least toxic antiseptic agent.

Results from a laboratory study on the toxicity of CHX to human gingival cells showed that the toxic potency of CHX is dependent on the length of exposure and the composition of the exposure medium (Babich *et al.* 1995). The addition of foetal bovine serum, albumin, lecithin and heat-killed *Escherichia coli* reduced the cytotoxicity of CHX, presumably because of the binding of the cationic CHX to the negatively charged chemical moieties/sites of these components/bacteria (Babich *et al.* 1995). These findings suggest that similar reactions within a root canal may reduce the potential of a cytotoxic reaction in the periapical tissues (Boyce *et al.* 1995). Boyce *et al.* (1995) found CHX (0.05%) uniformly toxic to both cultured human cells and microorganisms. Agarwal *et al.* (1997) found that CHX rapidly disrupts the cell membrane of both crevicular and peripheral blood neutrophils at concentrations above 0.005% within 5 min, indicating that its inhibitory effect on neutrophil function is mostly because of its lytic properties. Yesilsoy *et al.* (1995) assessed the short-term toxic effects of CHX in the subcutaneous tissue of guinea pigs and found moderate inflammation present after 2 days, followed by a foreign-body granuloma formation at 2 weeks. Ribeiro *et al.* (2005) evaluated the genotoxicity

(potential damage to DNA) of formocresol, para-monochlorophenol, calcium hydroxide and CHX against Chinese hamster ovary cells. Results showed that none of the mentioned agents contributed to DNA damage. Taken together, in the clinically used concentrations, the biocompatibility of CHX is acceptable. The potentially toxic interactions between CHX and NaOCl were considered previously.

Allergic reactions to CHX

Although sensitivity to CHX is rare, contact dermatitis is a common adverse reaction to CHX (Krautheim *et al.* 2004). Apart from this, CHX may have a number of rare side effects, such as desquamative gingivitis, discolouration of the teeth and tongue or dysgeusia (distorted taste). Contact with conjunctiva can cause permanent damage and accidental contact with the tympanum can cause ototoxicity (Dukes 1992). Various allergic reactions to CHX have been described. Contact sensitivity to CHX was first reported by Calnan (1962). Today, CHX is known to elicit allergic contact dermatitis, including connubial contact dermatitis, generally after prolonged and repeated application (Krautheim *et al.* 2004). It can also cause contact urticaria, photosensitivity, fixed drug eruption and occupational asthma. People at particular risk of contact allergy (apart from medical and dental staff) are patients with leg ulcers and leg eczema (Krautheim *et al.* 2004). Overall, contact sensitivity to CHX seems to be rare as some large studies have shown a sensitization rate of about 2% (Osmundsen 1982, Bechgaard *et al.* 1985, Nomura *et al.* 1989). Even rarer are reports of immediate anaphylactic reactions because of CHX. Ohtoshi *et al.* (1986) demonstrated IgE antibodies in the sera of patients with anaphylaxis to CHX. Application of CHX to intact skin can cause immediate allergic reactions, such as urticaria, Quincke's edema or dyspnea and very rarely severe anaphylactic reactions (Torricelli & Wüthrich 1996, Snellman & Rantanen 1999). These reports of reactions to CHX indicate that practitioners should always be aware of this potential risk of using CHX. On the whole, although sensitivity to CHX is rare, this complication should be kept in mind during CHX application.

Conclusions

1. CHX has a wide range of activity against both Gram positive and Gram negative bacteria.
2. CHX is an effective antifungal agent especially against *C. albicans*.

3. The effect of CHX on microbial biofilms is significantly less than that of NaOCl.
4. CHX has antibacterial substantivity in dentine for up to 12 weeks.
5. Dentine, dentine components (HA and collagen), killed microorganisms and inflammatory exudate in the root canal system may reduce or inhibit the antibacterial activity of CHX.
6. CHX has little to no ability to dissolve organic tissues.
7. Mixing CHX with $Ca(OH)_2$ may enhance its antimicrobial activity.
8. Medication and/or irrigation with CHX may delay the contamination of root filled teeth by bacteria entering through the coronal restoration/tooth interface.
9. Medication and/or irrigation with CHX will not adversely affect the penetration of fluid through the root filled apical foramen.
10. Combination of NaOCl and CHX causes colour changes and formation a precipitate, which may interfere with the seal of the root filling.
11. CHX can significantly improve the integrity of the hybrid layer andresin–dentine bond stability.
12. The biocompatibility of CHX is acceptable.
13. In rare cases, CHX may cause allergic reactions.

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