

Chlorhexidine gluconate in endodontics: an update review

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The major objective in root canal therapy is to disinfect the entire root canal system. This requires that the pulpal contents be eliminated as sources of infection. This goal may be accomplished using mechanical instrumentation and chemical irrigation, in conjunction with medication of the root canal between treatment sessions. Microorganisms and their by-products are considered to be the major cause of pulpal and periradicular pathosis. In order to reduce or eliminate bacteria, various irrigation solutions have been used during treatment. Chlorhexidine is a cationic molecule which can be used during treatment. It has a wide range antimicrobial activity. Furthermore, because of its cationic structure, chlorhexidine has a unique property named substantivity. The purpose of this paper is to review different aspects of the use of chlorhexidine gluconate in endodontics.

Key words: Antibacterial, antifungal, chlorhexidine gluconate, endodontics, intracanal medication, irrigation, substantivity

The essential role of microorganisms in the development and perpetuation of pulpal and periapical diseases have clearly been demonstrated in animal models and human studies¹⁻³. Elimination of microorganisms from infected root canals is a complicated task. Numerous measures have been described to reduce the numbers of root canal microorganisms, including the use of various instrumentation techniques, irrigation regimens and intra-canal medicaments. There is no evidence in the literature that mechanical instrumentation alone results in a bacteria-free root canal system. Considering the complex anatomy of root canal pulp space, this is not surprising⁴. On the contrary, there is *in vitro* and clinical evidence that mechanical instrumentation leaves a significant portion of the root canal walls untouched⁵ and complete elimination of bacteria from the root canal by cleaning the root canal by instrumentation alone is unlikely⁶. It is assumed, but not demonstrated, that any pulp tissue left in the root canals can serve as bacterial nutrient. Furthermore, tissue remnants also impede the antimicrobial effects of root canal irrigants and medicaments. Therefore some sort of irrigation / disinfection is necessary to remove tissue from the root canals and to kill microorganisms.

Chlorhexidine (CHX) is available as the acetate, gluconate and hydrochloride salts. It is widely used as an endodontic irrigant and medicament. However, there is no adequate review of the literature on the different

aspects of CHX. The purpose of this paper is to review the use of CHX in root canal treatment.

Structure and mechanism of action

CHX is a synthetic cationic bis-guanide that consists of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain⁷. 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 mechanism⁸. Its efficacy is due to the interaction of positive charge of the molecule and negatively charged phosphate groups on the microbial cell walls⁹, thereby altering the cell's 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, chlorhexidine gluconate, is water-soluble and at physiological pH, readily dissociates and releases the positively charged chlorhexidine component⁷. At low concentration (0.2%), low molecular weight substances specifically potassium and phosphorous will leak out. On the other hand, at higher concentration (2%), CHX is bactericidal; precipitation of cytoplasmic contents occurs resulting in cell death⁹.

Antibacterial activity

Delany *et al.*¹⁰ evaluated 0.2% CHX gluconate on infected root canals. Bacteriologic samples were obtained before during, immediately and 24 hours after instrumentation, irrigation, and medication either with CHX gluconate or with sterile saline. There was a highly significant reduction in microorganisms in the CHX-treated specimens after the instrumentation and irrigation procedures. Basson and Tait¹¹ compared the effectiveness of calcium hydroxide, iodine potassium iodide (IKI) and a CHX solution in disinfecting *Actinomyces israelii*-infected root canal walls and dentinal tubules *in vitro*. 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 the samples at all periods while 25% of the specimens treated with iodine potassium iodide and 50% of the specimens treated with calcium hydroxide still had viable *A. israelii* after treatment.

Oncag *et al.*¹² evaluated the antibacterial properties of 5.25% sodium hypochlorite (NaOCl), 2% CHX and 0.2% CHX plus 0.2% cetrимide (Cetrexidin) after 5 min and after 48 h in extracted human teeth, whose canals were infected by *Enterococcus faecalis*. The 2% CHX and Cetrexidin were significantly more effective on *E. faecalis* than the 5.25% NaOCl at both time periods. Gomes *et al.*¹³ investigated *in vitro* the antimicrobial activity of three concentrations (0.2%, 1% and 2%) of two forms of CHX (gel and liquid) against endodontic pathogens and compared the results with those achieved by five concentrations of NaOCl (0.5%, 1%, 2.5%, 4% and 5.25%). Both 2% gel and liquid formulation of CHX eliminated *Staphylococcus aureus* and *Candida albicans* in 15 seconds, whereas the gel formulation killed *E. faecalis* in 1 minute. All tested irrigants eliminated *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, and *Prevotella intermedia* in 15 seconds. The time required to eliminate all microorganisms was the same for 5.25% NaOCl. In another study, Vianna *et al.*¹⁴ also assessed the antimicrobial activity of 0.2%, 1%, and 2% CHX gluconate (gel and liquid), against endodontic pathogens and compared the results with those achieved by 0.5%, 1%, 2.5%, 4%, and 5.25% NaOCl. Both 2.0% gel and liquid formulations eliminated *Staphylococcus aureus* and *Candida albicans* in 15 seconds, whereas the gel formulation killed *E. faecalis* in 1 minute. All tested irrigants eliminated *P. endodontalis*, *P. gingivalis*, and *Prevotella intermedia* in 15 seconds. The timing required for 1.0% and 2.0% CHX liquid to eliminate all microorganisms was the same as required for 5.25% NaOCl. The antimicrobial action is related to type, concentration, and presentation form of the irrigants as well as the microbial susceptibility.

Zamany *et al.*¹⁵ examined the addition of a 2% CHX rinse to the conventional treatment protocol on the successful disinfection of the root canal system. Results showed that cultivable bacteria were retrieved at the conclusion of the first visit in 1 out of 12 CHX

cases, whereas in the control group 7 out of 12 cases showed growth. This difference was significant. Siqueira *et al.*¹⁶ compared the effectiveness of 2.5% sodium hypochlorite and 0.12% CHX as irrigants in reducing the cultivable bacteria in infected root canals of teeth with apical periodontitis. They found that both solutions revealed comparable results as to the bacterial elimination from infected root canals and suggested that both can be used as irrigants. In a randomised clinical trial, Manzur *et al.*¹⁷ assessed the antibacterial efficacy of intracanal medication with calcium hydroxide, 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, and after medication in the second session one week later. They concluded that the antibacterial efficacy of Ca(OH)₂, CHX, and Ca(OH)₂/CHX was comparable. Zerella *et al.*¹⁸ investigated the effect of a slurry of Ca(OH)₂ mixed in aqueous 2% CHX versus aqueous Ca(OH)₂ slurry alone on the disinfection of the pulp space of failed root-filled teeth during endodontic retreatment. Of the total sample population, 12 of 40 (30%) were positive for bacteria before root filling. The control medication disinfected 12 of 20 (60%) teeth including 2 of 4 teeth originally diagnosed with enterococci. The experimental medication resulted in 16 of 20 (80%) teeth being disinfected at the beginning of the third appointment. None of the teeth originally containing enterococci showed remaining growth. They concluded that canal dressing with a mixture of 2% CHX and Ca(OH)₂ slurry is as efficacious as aqueous Ca(OH)₂ on the disinfection of failed root-filled teeth.

Ercan *et al.*¹⁹ evaluated the antibacterial activity of 2% CHX and 5.25% sodium hypochlorite in infected root canals of incisors and premolars. They concluded that both CHX and sodium hypochlorite were significantly effective in reducing the microorganisms in the teeth with necrotic pulps, periapical pathologies, or both, and could be used successfully as an irrigant solution. Tanomaru *et al.*²⁰ evaluated the effect of biomechanical preparation with 5% sodium hypochlorite, 2% CHX and physiological saline irrigating solutions and calcium hydroxide dressing in dog root canals containing bacterial endotoxin. They found that biomechanical preparation with the irrigating solutions did not inactivate the effects of the endotoxin but the calcium hydroxide intracanal dressing did appear to inactivate the effects induced by the endotoxin *in vivo*. Another interesting topic is the additive effect of CHX and hydrogen peroxide. Heling and Chandler²¹ studied the antimicrobial effect of irrigant combinations within dentinal tubules *in vitro* against *E. faecalis* and found that a specific combination of 3% hydrogen peroxide (H₂O₂) and CHX was superior in its antibacterial activity in dentine compared with other regimens such as CHX alone and NaOCl. Steinberg *et al.*²² challenged *E. faecalis* suspensions in trypticase soy

broth (a culture medium rich in peptides) with various combinations of CHX and H₂O₂. The experiments demonstrated that the combination of the two substances killed *E. faecalis* totally in concentrations much lower than each component alone. According to that study, the bactericidal effect of CHX derives from its ability to denature the bacterial cell wall while forming pores in the membrane, while H₂O₂ is effective against intracellular organelles such as DNA. Although the exact synergistic mechanism of CHX and H₂O₂ is not known, it can be postulated that the exposure of bacteria to CHX leads to a more permeable cell wall that H₂O₂ can penetrate easily and hence damage the intracellular organelles²².

Overall, 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 effect *in vitro* (infected dentine) and *in vivo* (in the root canal system) is similar.

Antifungal activity

Fungi constitute a small part of the oral microbiota, the largest proportion being made up of *Candida* species. *Candida albicans* is the fungal species most commonly detected in the oral cavity of both healthy (30-45%) and medically compromised (95%) individuals²³. Fungi have occasionally been found in primary root canal infections, but they seem to be more common in the root canals of obturated teeth in which treatment has failed²³. Overall, the occurrence of yeasts reported in infected root canals varies between 1-17%²⁴.

Because fungi may be involved in cases of persistent and secondary infections associated with recalcitrant periradicular lesions, the spectrum of antimicrobial activity of endodontic medicaments and irrigants should include these microorganisms. Thus, strategies with medicaments that have antifungal effectiveness may assist in the successful management of persistent or secondary endodontic infections caused by fungi^{23,24}. To improve antisepsis in a one-appointment regime, it has been suggested to rinse/soak the canals with CHX or iodine potassium iodide (IPI) solutions following irrigation with sodium hypochlorite. Aqueous CHX solution has a wide-spectrum antimicrobial activity at low concentrations, and is especially effective against *C. albicans*. Furthermore, it binds to surrounding tissues to be released again slowly over extended periods, a phenomenon called substantivity.

Interestingly, it appears that chlorhexidine can efficiently inhibit the initial adherence and perhaps further accumulation and biofilm formation of yeasts and other microorganisms. 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

sodium hypochlorite alone^{23,24}. Sen *et al.*²⁵ evaluated the antifungal properties of 0.12% CHX, 1% NaOCl, and 5% NaOCl against *Candida albicans* using cylindrical dentine tubes. They found that *C. albicans* was more resistant in the presence of smear layer than in the absence of smear layer. When smear layer was absent, NaOCl started to display antifungal activity after 30 minutes. Waltimo *et al.*²⁶ evaluated the susceptibility of seven strains of *C. albicans* to four disinfectants: IKI, CHX acetate, sodium hypochlorite, and calcium hydroxide. In addition, all possible pairs of the disinfectants were tested to compare the effect of the combination and its components. *C. albicans* cells were highly resistant to calcium hydroxide. Sodium hypochlorite (5% and 0.5%) and IKI killed all yeast cells within 30 s, whilst CHX acetate (0.5%) showed complete killing after 5 min. Combinations of disinfectants were equally or less effective than the more effective component. All *C. albicans* strains tested showed similar susceptibility to the medicaments tested.

Siqueira *et al.*²⁷ evaluated the effectiveness of four intracanal medications in disinfecting the root dentine in bovine teeth experimentally infected with *C. albicans*. Infected dentine cylinders were exposed to four different medications: calcium hydroxide/glycerin; calcium hydroxide /0.12% CHX; calcium hydroxide/ camphorated paramonochlorophenol/glycerin; and 0.12% CHX/ zinc oxide. Results showed that the specimens treated with calcium hydroxide/ camphorated paramonochlorophenol/glycerin paste or with CHX/zinc oxide paste were completely disinfected after 1 hour of exposure and calcium hydroxide/ glycerin paste consistently eliminated *C. albicans* infection 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.*²⁸ investigated the antifungal ability of several medicaments against *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. parapsilosis*, and *S. cerevisiae*. Whereas the paste of calcium hydroxide in CPMC/glycerin showed the most pronounced antifungal effects, calcium hydroxide in glycerin or CHX and CHX in detergent also showed antifungal activity that was much lower than the paste of calcium hydroxide in CPMC/glycerin. Ferguson *et al.*²⁹ 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 calcium hydroxide were determined. Their results revealed that NaOCl, hydrogen peroxide, and CHX digluconate were effective against *C. albicans* even when significantly diluted. Aqueous calcium hydroxide had no activity.

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, so-called planktonic microorganisms are a prerequisite for biofilm formation³⁰. Such films may thus become established on any organic or inorganic surface substrate where planktonic 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 for the organisation of this specific biofilm³⁰. However, in endodontics the biofilm concept has so far gained limited attention. It has been discussed mainly within the framework of bacterial appearances on root tips of teeth with non-vital pulps. Such bacterial aggregations have been thought to be the cause of therapy-resistant apical periodontitis³⁰. Although not described in great detail, bacterial condensations on the walls of infected root canals have been observed. Anti-microbial agents have often been developed and optimised for their activity against fast growing, dispersed populations containing a single microorganism. However, microbial communities grown in biofilms are remarkably difficult to eradicate with anti-microbial agents and microorganisms in mature biofilms can be notoriously resistant for reasons that have yet to be adequately explained³⁰. There are reports showing that microorganisms grown in biofilms could be two- to 1,000-fold more resistant than the corresponding planktonic form³¹.

Spratt *et al.*³² evaluated the effectiveness of NaOCl (2.25%), 0.2% CHX, 10% povidone iodine, 5ppm colloidal silver and phosphate buffered solution ((PBS) as control) against monoculture biofilms of five root canal isolates including *P. intermedia*, *Peptostreptococcus* *avios*, *Streptococcus intermedius*, *F. nucleatum*, *E. faecalis*. Results showed that NaOCl was the most effective anti-microbial followed by the iodine solution. Clegg *et al.*³³ evaluated the effectiveness of three concentrations of sodium hypochlorite (6%, 3%, and 1%), 2% CHX and BioPure MTAD on apical dentine biofilms *in vitro*. Results showed that 6% NaOCl and 3% NaOCl were capable of disrupting and removing the biofilm; 1% NaOCl and 1% NaOCl, followed by MTAD were capable of disrupting the biofilm, but not eliminating bacteria; 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.*³⁴ evaluated the efficacy of 6% NaOCl, 1% NaOCl, Smear ClearTM, 2% CHX, REDTA, and BioPureTM MTADTM against *E. faecalis* biofilms using a novel *in vitro* testing system. Biofilms grown in a flow cell system were submerged in test irrigants for either 1 or 5 minutes. There was a significant relationship between test agent and percentage kill of the biofilm bacteria. No significant relationship between time and percentage kill was found. The percentage kill of the biofilms bacte-

ria was: 6% NaOCl (>99.99%), 1% NaOCl (99.78%), Smear ClearTM (78.06%), 2% CHX (60.49%), REDTA (26.99%), and BioPureTM MTADTM (16.08%). There was a significant difference between 1% and 6% NaOCl, 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.*³⁵ assessed the effectiveness of CHX- or antibiotics (clindamycin with metronidazole)-based medications in eliminating *E. faecalis* biofilms. One-day and three-day biofilms of *E. faecalis* were used. Each biofilm-containing membrane was thoroughly covered with 1ml of the test medications and incubated for 1 day at 37°C. Treated biofilms were then aseptically transferred to vials containing a neutralising agent in saline solution and vortexed. Suspensions were 10-fold diluted, seeded onto *Mitis salivarius* agar plates, and the colony-forming units counted after 48h of incubation. There were significant differences between the formulations tested. The association of clindamycin with metronidazole significantly reduced the number of cells in 1-day biofilms. However of all medications tested, only 2% CHX-containing medications were able to thoroughly eliminate most of both 1-day and 3-day *E. faecalis* biofilms.

Substantivity

CHX has a unique feature in that dentine medicated with it acquires antimicrobial substantivity. The positively-charged molecules of CHX can adsorb onto dentine and prevent microbial colonisation on the dentine surface for some time beyond the actual medication period⁸.

Antimicrobial substantivity of CHX has been assessed in several periodontal and endodontic studies. In an *in vivo* periodontal study, Stabholz *et al.*³⁶ evaluated the substantivity of human root surfaces after *in situ* subgingival irrigation with tetracycline HCL and CHX. They found that the substantivity of tetracycline 50mg/ml was significantly greater than CHX for 12 days and greater than saline for 16 days.

In an *in vitro* study, White *et al.*³⁷ evaluated the antimicrobial substantivity of a 2% CHX solution as an endodontic irrigant. Findings showed that substantivity lasted 72h. In an *in vivo* study, Leonardo *et al.*³⁸ 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 found that CHX prevented microbial activity with residual effects in the root canal system for up to 48h. However, some other studies revealed the substantivity of CHX for longer periods. Khademi *et al.*³⁹ found that 5-min treatment with 2% CHX solution induced substantivity for up to 4 weeks. Rosenthal *et al.*⁴⁰ evaluated the substantivity of CHX within the root canal system after 10min treatment with 2% CHX solution.

They found that 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 colonisation. Recently, antibacterial substantivity of three concentrations of CHX solution (4%, 2% and 0.2%) after 5-min has been evaluated. Results revealed a direct relationship between the concentration of CHX and its substantivity⁴¹. On the contrary, Lin *et al.*⁴² attributed the substantivity of CHX to the absorption of the agent to dentine during the first hour and stated that only after the saturation point, after the first hour, did the antimicrobial capability of CHX increase with time. Furthermore, Komorowski *et al.*⁴³ revealed that 5-min CHX treatment did not induce substantivity, and dentine should be treated with CHX for 7 days.

Buffering effect of dentine on CHX

The root canal milieu is a complex mixture of a variety of organic and inorganic compounds. Hydroxylapatite, 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 restrictively⁴⁴. Difficulties in designing experiments that will give reliable and comparable data were one of the great challenges for researchers for many years. Ultimately, Haapasalo *et al.*⁴⁴ introduced a new dentine powder model for studying the inhibitory effect of dentine on various root canal irrigants and medicaments. Haapasalo *et al.*⁴⁴ investigated the buffering effect of dentine on the antibacterial activity of saturated calcium hydroxide solution, 1% sodium hypochlorite, 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 the time the medicament was pre-incubated with 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% sodium hypochlorite 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.*⁴⁵ evaluated the inhibition of the antibacterial effect of saturated calcium hydroxide solution, CHX acetate and IKI by dentine, hydroxylapatite (HA) and bovine serum albumin (BSA). Calcium hydroxide was totally inactivated by the presence of 28mg 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 (28mg), but was practically unaffected by HA or BSA. A stepwise reduction of dentine from 28mg 150 μL^{-1} to 2.8mg 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 less than 28mg. However, the effect of saturated calcium hydroxide solution was totally eliminated by dentine, in all four concentrations. It could be assumed that inhibition by dentine of the antibacterial activity of calcium hydroxide, CHX and IKI occurs by different mechanisms⁴⁵.

Surprisingly, calcium hydroxide was sensitive to the inhibitory effect of all three materials tested. The inhibition of calcium hydroxide by dentine and by the other compounds is, of course, dependent on their quantitative relationships⁴⁵. One major mechanism for resistance of survival of *E. faecalis* in the root canal filled with calcium hydroxide may be the buffering effect of dentine against the pH rise. Inorganic HA had little or no inhibitory activity against CHX as compared to dentine, whereas BSA was the strongest inhibitor of CHX, with more than 10% of *E. faecalis* cells still viable after 24h 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 compounds⁴⁵.

In another study Portenier *et al.*⁴⁶ assessed the antibacterial activity of CHX and IKI on *E. faecalis* in the presence of dentine, dentine matrix, dentine pretreated by 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 1h but not after 24h. 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 iodine potassium iodide. The inhibitory effect of dentine and BSA on the antibacterial activity of CHX and MTAD was assessed in another study⁴⁷. The presence of dentine or BSA caused a marked delay in killing *E. faecalis* by both medicaments. The inhibitory effect of BSA on the antibacterial activity of CHX and NaOCl has been confirmed recently by Sassone *et al.*⁴⁸.

Taken together, it seems that dentine, dentine components (HA and collagen), killed microorganisms and

inflammatory exudates in the root canal system reduce or inhibit the antibacterial activity of medicaments and irrigants.

Tissue solubility of CHX

Several studies have been conducted in the search for an irrigant that meets four major criteria:

- Antimicrobial activity
- Non-toxicity to periapical tissues
- Water solubility
- Capacity to dissolve organic matter.

Therefore, an ideal irrigant should dissolve the organic matter inside the root canal system. Grossman⁴⁹ demonstrated the importance of the solvent ability of an endodontic irrigant and emphasised that the elimination of pulp tissue from the root canal was important for the ultimate success of root canal treatment. Moorer and Wesselink⁵⁰ showed that tissue dissolution was dependent on three factors: frequency of agitation, amount of organic matter in relation to amount of irrigant in the system and surface area of tissue that was available. Okino *et al.*⁵¹ evaluated the tissue dissolving ability of 0.5, 1.0 and 2.5% sodium hypochlorite; 2% aqueous solution of CHX digluconate; 2% chlorhexidine digluconate gel (NatrosolTM); and distilled water as control. Bovine pulp fragments were weighed and placed in contact with 20mL of each tested substance in a centrifuge at 150rpm until total dissolution. Dissolution speed was calculated by dividing pulp weight by dissolution time. Distilled water and both solutions of CHX did not dissolve the pulp tissue within 6h. Mean dissolution speeds for 0.5, 1.0 and 2.5% sodium hypochlorite solutions were 0.31, 0.43 and 0.55 mg/min, respectively. The solvent ability of chlorhexidine solutions was similar to that of distilled water.

In another study, Naenni *et al.*⁵² assessed the necrotic tissue dissolution capacity of 1% (wt/vol) sodium hypochlorite (NaOCl), 10% chlorhexidine, 3% and 30% hydrogen peroxide, 10% peracetic acid, 5% dichloroisocyanurate (NaDCC), and 10% citric acid. Standardised 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 sodium hypochlorite had any substantial tissue dissolution capacity. It was concluded that this might be important when considering the use of irrigants other than NaOCl.

CHX and calcium hydroxide

CHX is a cationic biguanide with an optimal antimicrobial activity which is achieved within a pH range of 5.5 to 7.0⁸. Therefore, it seems that alkalinising the pH by adding calcium hydroxide (CH) to CHX, precipitates

CHX molecules and decreases its effectiveness. However, it has been demonstrated that the alkalinity of calcium hydroxide in the mixture remained unchanged. Therefore, the usefulness of mixing CH with CHX has still remained unclear and is controversial⁸.

When used as an intracanal medicament, CHX was more effective than calcium hydroxide in eliminating *E. faecalis* from inside dentinal tubules⁸. In a study by Almyroudi *et al.*⁵³, all of the chlorhexidine formulations used, including a CHX/CH 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.*⁵⁴ in bovine dentine and Schafer and Bossmann⁵⁵ in human dentine where 2% CHX gel had greater activity against *E. faecalis*, followed by CHX/CH and then CH used alone.

In a study using agar diffusion, Haenni *et al.*⁵⁶ could not demonstrate any additive antibacterial effect by mixing CH powder with 0.5% CHX. In fact, they showed that the CHX had a reduced antibacterial action. However, CH did not lose its antibacterial properties in such a mixture. This may be due to the deprotonation of CHX at a pH greater than 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.*⁵⁷ showed 2% CHX gel was the most effective agent against *E. faecalis* inside dentinal tubules, followed by a CH/2% CHX mix, whilst CH alone was totally ineffective, even after 30 days. The 2% CHX gel was also significantly more effective than the CH/2% CHX mix against *C. albicans* at seven days, although there was no significant difference at 15 and 30 days. CH alone was completely ineffective against *C. albicans*. In another *in vivo* study using primary teeth, a 1% CHX gluconate gel, both with and without CH, was more effective against *E. faecalis* than CH alone within a 48-hour period⁵⁸.

Schafer *et al.*⁵⁵ reported that 2% CHX gluconate was significantly more effective against *E. faecalis* than CH used alone, or a mixture of the two. This was also confirmed by Lin *et al.*⁵⁹ although in a study by Evans *et al.*⁶⁰ using bovine dentine, 2% CHX with CH was shown to be more effective than CH in water. In an animal study, Lindskog *et al.*⁶¹ reported that teeth dressed with CHX for four weeks had reduced inflammatory reactions in the periodontium (both apically and marginally) and less root resorption. Waltimo *et al.*²⁶ reported that 0.5% CHX acetate was more effective at killing *C. albicans* than saturated CH, while CH combined with CHX was more effective than CH used alone. The high pH of CH was unaffected when combined with CHX in this study.

CHX and coronal leakage

Due to its antimicrobial substantivity, it seems that CHX preparations delay microleakage into the root

canal. In an *in vitro* study, Gomes *et al.*⁶² investigated the time required for recontamination of coronally sealed canals medicated with either calcium hydroxide, 2% CHX gel or with a combination of both. The canals without coronal seal, 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 + 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 sealed with IRM, showed recontamination after an average time of 8.7 days.

There were statistically significant differences between the groups. All groups without coronal seal were recontaminated significantly more quickly than those sealed with IRM, except those teeth coronally sealed but without medicament. The groups with intracanal medication and sealed were not significantly different from each other. Vivacqua-Gomes *et al.*⁶³ assessed *in vitro* coronal microleakage in extracted human teeth after root-canal treatment using 1% NaOCl, 1% NaOCl + 17% EDTA, 2% CHX gel, 2% CHX gel + 1% NaOCl, 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 maximum dye penetration was determined digitally in millimetres. Results revealed that least leakage occurred with 1% NaOCl + 17% EDTA and 2% CHX gel. NaOCl, distilled water and 2% CHX gel + 1% NaOCl gave increased leakage with a significant difference compared to NaOCl + 17% EDTA and 2% CHX gel, and compared to one another. On the other hand, some studies showed that viscous irrigants, including those containing chlorhexidine gluconate, were less soluble substances, leaving residues on the root-canal surfaces which impaired final obturation.

Lambrianidis *et al.*⁶⁴ investigated the efficiency of removing calcium hydroxide/CHX gel, Ca(OH)₂/CHX solution and Ca(OH)₂/saline pastes with the use of instrumentation and irrigation with sodium hypochlorite and ethylene diamine tetraacetic acid (EDTA) solutions. None of the techniques used in this study removed the inter-appointment root canal medicaments effectively⁶⁴. Overall, Ca(OH)₂/CHX (gel) paste was associated with a significantly larger amount of residue, whereas Ca(OH)₂/CHX (solution) paste was associated with less residue than the other two medicaments. Taken together, due to its substantivity, CHX as an intracanal medicament/irrigant delays recontamination of the root canal system via the coronal route.

CHX and apical leakage

Marley *et al.*⁶⁵ assessed the effect of 0.12% CHX gluco-

nate as an endodontic irrigating solution on the apical seal of obturated root canals using three different sealers (Roth's 811, AH26, and Sealapex). At 90 and 180 days after obturation apical leakage was measured using the fluid filtration method. The results showed no significant difference in seal related to the irrigant 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 seal of the root canal cements. Wuerch *et al.*⁶⁶ investigated the effect of CHX gel and calcium hydroxide on the apical seal of the root-canal system. Results demonstrated that 2% CHX gel and calcium hydroxide paste did not adversely affect the apical seal of the root-canal system. These findings were confirmed by Engel *et al.*⁶⁷. Overall, it seems that medication and/or irrigation with CHX does not adversely affect the apical seal of the root canal.

Toxicity of CHX

Results from a study on the cytotoxic effect of chlorhexidine on canine embryonic fibroblasts and *Staphylococcus aureus* showed that bactericidal concentrations of chlorhexidine were lethal to canine embryonic fibroblasts whilst noncytotoxic concentrations allowed significant bacterial survival⁶⁸. In a study by Tatnall *et al.*⁶⁹ the cytotoxic effects of CHX, hydrogen peroxide and sodium hypochlorite 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 ED₅₀ 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 an *in vitro* study on the toxicity of CHX to human gingival cells showed that the toxic potency of chlorhexidine is dependent on the length of exposure and the composition of the exposure medium⁷⁰. Addition of foetal bovine serum, albumin, lecithin and heat-killed *Escherichia coli* reduced the cytotoxicity of CHX, presumably due to the binding of the cationic CHX to the negatively charged chemical moieties/sites of these components/bacteria⁷⁰. These findings suggest that similar reactions within a root canal may reduce the potential of a cytotoxic reaction in the periapical tissues⁷¹. Boyce *et al.*⁷¹ found chlorhexidine (0.05%) uniformly toxic to both cultured human cells and microorganisms. Agarwal *et al.*⁷² 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 due to its lytic properties. Yesilsoy *et al.*⁷³ assessed the short-term toxic effects of CHX in the subcutaneous tissue of guinea pigs and found a moderate inflammation present after 2 days, followed

by a foreign-body granuloma formation at 2 weeks. Ribeiro *et al.*⁷⁴ evaluated the genotoxicity (potential damage to DNA) of formocresol, paramonochlorophenol, calcium hydroxide, and CHX against Chinese hamster ovary (CHO) cells. Results showed that none of the mentioned agents contributed to the DNA damage.

Allergic reactions to CHX

Although sensitivity to CHX is rare, contact dermatitis is a common adverse reaction⁷⁵. Apart from that, CHX is liable to a number of rare side effects, such as desquamative gingivitis, discolouration of teeth and tongue or dysgeusia (distorted taste). Contact with conjunctiva can cause permanent damage, and accidental contact with the tympanum can cause ototoxicity⁷⁶. Various allergic reactions due to CHX have been described. Contact sensitivity to CHX was first reported by Calnan in 1962⁷⁷. Today, CHX is known to elicit allergic contact dermatitis, including connubial contact dermatitis, generally after prolonged and repeated application⁷⁵. It can also cause contact urticaria, photosensitivity, fixed drug eruption and occupational asthma. People at particular risk of contact allergy are, apart from medical staff, patients with leg ulcers and leg eczema⁷⁵. Altogether, contact sensitivity to CHX seems to be rare. Some larger studies showed a sensitisation rate of about 2%⁷⁸⁻⁸⁰. Even rarer are reports of immediate anaphylactic reactions due to CHX. Ohtoshi⁸¹ demonstrated IgE antibodies in the sera of patients with anaphylaxis due to CHX. Application of CHX to intact skin can cause immediate allergic reactions such as urticaria, Quincke's oedema or dyspnoea and very rarely severe anaphylactic reactions^{82,83}. Taken together, it is important to keep in mind this potential risk of CHX.

Discussion

CHX is a strong base and is most stable in the form of its salts. It has a wide range of activity against both Gram-positive and Gram-negative bacteria. However, it is less effective on Gram-negative than on Gram-positive bacteria. On the other hand, in primary endodontic infections, which are usually poly-microbial, Gram-negative anaerobes predominate⁸⁴. Furthermore, efficacy of CHX on *E. faecalis* and *C. albicans* has been well documented⁸. This is important because endodontically treated teeth with persistent apical periodontitis are frequently found to be infected with *E. faecalis* and *C. albicans*⁴⁰. In addition, it has been reported that CHX does not inactivate endotoxin (lipopolysaccharide), which is a structural component on the Gram-negative bacterium's outer cell envelope¹⁴. Therefore, it could be concluded that the effectiveness of CHX preparations in primary endodontic infections is less than in post-treatment (secondary) endodontic infections.

A biofilm can be defined as communities of microorganisms attached to a surface, embedded in an extra-cellular matrix of polysaccharides. Within these microcolonies, bacteria have developed into organised communities with functional heterogeneity³¹. 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 killing³¹. Unfortunately, most of the *in vitro* tests use planktonic cultures for testing the antimicrobial efficacy of endodontic irrigants. Depending on the concentration of the substance tested and the susceptibility of the microorganism, the latter can be eliminated in seconds using the planktonic cells and the direct contact method. Such a killing effect may not happen clinically. Therefore, the use of a biofilm model could reproduce more precisely the *in vivo* conditions. It has been shown that susceptibility of microbial biofilm to CHX preparations is less than sodium hypochlorite³²⁻³⁴, which is a shortcoming for CHX.

In cases with necrotic pulps as well as in re-treatment cases, treatment should be performed in two visits, which is more time-consuming than one-visit treatment³⁹. Furthermore, some studies have suggested that calcium hydroxide is ineffective against *E. faecalis*⁴⁰. To overcome the aforementioned problems, an alternative protocol is to use antimicrobial agents that exhibit substantivity, that is, agents that can have a therapeutic effect for a prolonged period³⁹. It has been demonstrated that CHX preparations, due to their cationic nature, exhibit the best substantivity among all endodontic agents^{39,40}. Depending on its concentration, CHX demonstrated substantivity from 48h to 12 weeks in different studies^{37-41,43}. Furthermore, it has been demonstrated that CHX, possibly due to its substantivity, may delay coronal leakage in endodontically treated teeth^{62,63}. In addition, it seems that medication and/or irrigation with CHX does not adversely affect the apical seal of the root canal⁶⁵⁻⁶⁷.

It is clear that the *in vivo* effectiveness of irrigants and medicaments in the root canal against the infecting microflora is somewhat disappointing in light of the more promising *in vitro* results, which show killing of practically all microorganisms in a few seconds, when concentrated solutions are used⁸⁵. One natural explanation is root canal anatomy, in particular the difficulty in reaching the most apical region of the canal with large volumes of fresh irrigant. However, it should not be forgotten that the chemical milieu in the canal is quite different from a simplified test tube environment. It has been shown that dentine, its components, root canal system contents, and dead microorganisms reduce or inhibit the antibacterial activity of CHX⁴⁴⁻⁴⁷.

Antibacterial effect of calcium hydroxide combined with CHX is still controversial. There are two concepts in this regard. Some studies have shown that mixing

calcium hydroxide with CHX enhances the antimicrobial activity of both agents⁸. On the contrary, other investigations have demonstrated that mixing calcium hydroxide with CHX not only does not increase the efficacy of calcium hydroxide, but also may decrease it dramatically⁸. The possible reason for this decrease is that the CHX is a cationic bisbiguanide that is most stable within a pH range of 5-8⁷. Optimal antimicrobial activity is achieved with CHX within a pH range of 5.5-7.0^{7,8}. It precipitates out of solution above pH8. In the case of calcium hydroxide (pH12.0), calcium ion replaces the chlorhexidine molecule in chlorhexidine gluconate solution and chlorhexidine precipitates.

One of the potential weaknesses of CHX in the root canal may be its susceptibility to the presence of organic matter⁸⁵. Furthermore, tissue solubility of CHX is little to none⁴⁹⁻⁵², which is one of the obvious benefits of sodium hypochlorite.

Biocompatibility of CHX is acceptable, which is one of the benefits of CHX over sodium hypochlorite. Finally, it should be noted that in rare cases CHX may cause allergic reactions.

Conclusion

In spite of the fact that CHX possesses a wide range of antimicrobial activity and substantivity, due to the lack of tissue solubility, sensitivity to organic load, and inactivation by dentine, its components, and root canal contents, it should not be used as a routine root canal irrigant. Rather, it should be considered as a final rinse or an intracanal medicament.

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