Abstract: Use of an appropriate root canal irrigant is essential during endodontic treatment, due to the complex and unpredictable anatomy of the root canal system and limitations in the mechanical instrumentation techniques used to obtain a clean, bacteria-free canal. Several irrigants, such as sodium hypochlorite, chlorhexidine, hydrogen peroxide, and normal saline, have been proposed as canal system irrigants in endodontic treatment. The widely used endodontic irrigant chlorhexidine is a positively charged lipophilic/hydrophobic molecule that interacts with phospholipids and lipopolysaccharides on the bacterial cell membrane. In endodontics, its mode of antibacterial activity is determined by its concentration (0.2% or 2%). This article reviews findings from available endodontic studies on the antibacterial, antifungal, and antibiofilm activities of chlorhexidine. (J Oral Sci 56, 99-103, 2014)

Keywords: antibacterial; antifungal; biofilm; chlorhexidine; endodontics.

Introduction

Bacteria have a fundamental role in the pathogenesis of pulpo-periapical diseases (1-3). However, elimination of bacteria from an infected canal is a difficult process that requires the use of various instrumentation techniques, irrigants, and intracanal medications. Because of the complex and unpredictable anatomy of the canal system, mechanical preparation of the canal is not sufficient to ensure that a canal system is free of bacteria (4). Ex vivo and in vivo evidence indicates that mechanical preparation can leave many areas of the canal walls uninstrumented (5) and that instrumentation alone is unlikely to completely remove microorganisms (6).

Remnant pulp can serve as a nutrient source for remaining bacteria. Tissues left in the canal can also limit the antibacterial effects of irrigants. Agents used for chemical debridement of canals are divided into three categories: irrigants, root canal rinses, and interappointment medicaments.

The effectiveness of mechanical instrumentation of the canal can be improved by supplementation with suitable irrigants, ie, medicated fluids used to wash out a cavity inside the body. The goals of this procedure are mechanical and biological. The effectiveness of irrigation, defined as removal of debris and elimination of bacteria, depends on the diameter of the root canal, the penetration depth of the irrigating needle, irrigation pressure, and the properties of the solution. Several irrigants, such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), hydrogen peroxide (H₂O₂), and normal saline, have been proposed as endodontic irrigants of the root canal system. Some of these irrigants, such as NaOCl, have proven beneficial properties in canal irrigation (such as antimicrobial action and removal of the smear layer), whereas others have no antimicrobial effects and thus may not be effective irrigants (7,8). CHX is a widely used endodontic irrigant and medicament that was develop-
oped in the United Kingdom in the late 1940s (9). The present article will review evidence from studies of the antimicrobial efficacy of CHX as an endodontic irrigant.

**Structure**
CHX (C_{22}H_{30}C_{12}N_{10}) is a synthetic material comprising two biguanide groups and two symmetric 4-chloro-phenyl rings, connected by a hexamethylene chain (10).

**Mechanism of action**
CHX is a hydrophobic and lipophilic molecule that interacts with lipopolysaccharides and phospholipids on the bacterial cell membrane (11). The beneficial effects of CHX are due to the interaction of its positive charge with the negatively charged phosphate on bacteria cell walls (12) and its ability to alter the osmotic equilibrium of bacteria cells. This can increase cell wall permeability and allow CHX to penetrate the cell. At high concentrations (≥2%), CHX is a bactericide, as it causes precipitation of cytoplasmic contents; at a lower concentration (0.2%) it causes phosphorous and potassium to leak out of cell structures (12).

**Antibacterial activity**
In an *in vitro* study, Delany et al. (13) studied the effects of 0.2% CHX in infected canals and found that the number of microorganisms was greatly reduced after instrumentation and irrigation. Another *in vitro* study reported that CHX was the only disinfectant that eliminated *Actinomyces israelii* compared to calcium hydroxide (Ca(OH)_2) (14). An *in vitro* study (15) evaluated the antibacterial properties of 2% CHX and 0.2% CHX/0.2% cetrimide at 5 min and 48 h after infecting canals with *Enterococcus faecalis*: 2% CHX was effective against *E. faecalis* at both time points. Both the 2% liquid and 2% gel formulations removed *Candida albicans* and *Staphylococcus aureus* within 15 s, and the gel product eliminated *E. faecalis* within 1 min (16,17). Addition of 2% CHX to an endodontic treatment protocol significantly improved root canal disinfection (18). A clinical study (19) showed that 0.12% CHX reduced bacteria numbers in infected canal systems.

A clinical trial demonstrated that the antibacterial effects of CHX, Ca(OH)_2, and a combination of Ca(OH)_2 and CHX were equivalent (20). Another *in vivo* study showed that the combination of a Ca(OH)_2 slurry and 2% CHX was as effective as aqueous Ca(OH)_2 (21).

Both 2% CHX and NaOCl are effective in reducing the number of bacteria in necrotic teeth and treating periapical pathosis (22).

In an *ex vivo* study, a combination of 3% H_2O_2 and CHX was better than CHX alone and NaOCl in eliminating bacteria (23). A combination of CHX and H_2O_2 killed *E. faecalis* at concentrations lower than those required for each irrigant alone. The reasons for the synergistic effect of CHX and H_2O_2 are unknown; however, exposure of microorganisms to CHX might increase permeability of bacteria cell walls, thereby allowing H_2O_2 to easily penetrate the cell and damage cellular organelles (24).

**Antifungal activity**
Vital fungi are present in 1-17% of infected root canals (25). As compared with canals irrigated with only NaOCl, canals irrigated with a final rinse of 2% CHX were free of bacteria significantly more often (25,26).

Using a cylindrical dentin tube model, Sen et al. (27) studied the antifungal effect of 0.12% CHX against *C. albicans* and found that *C. albicans* was more resistant to CHX when the smear layer was present than when it was removed. Waltimo et al. (28) showed that 0.5% CHX killed all *C. albicans* cells after only 5 min. Another study found that bovine dentin treated with CHX/zinc oxide (ZO) paste was disinfected after 60 min (29). An interesting *in vitro* study (30) found that CHX digluconate was effective against *C. albicans* even when substantially diluted.

**Effect on biofilm**
The biofilm concept in endodontics was first discussed in the context of microorganisms on the tips of roots with infected canals (31,32).

Some recent evidence indicates that the resistance of bacteria grown in biofilms is 2-1,000 times that of the planktonic forms of the same microorganisms (32). Spratt et al. (33) assessed the effectiveness of 0.2% CHX, 10% iodine, 2.25% NaOCl, and phosphate-buffered saline (PBS) against single-species biofilms of some microorganisms and found that NaOCl and iodine were more effective than CHX; NaOCl and iodine resulted in complete eradication after a 1-h incubation period. In another *in vitro* study 2% CHX did not disrupt biofilm (34). Using a novel *in vitro* testing system, Dunavant et al. (35) showed that 2% CHX killed 60% of bacterial cells in *E. faecalis* biofilms. In contrast, an *in vitro* study (36) showed that formulations containing 2% CHX eliminated most *E. faecalis* biofilms.

An *in vitro* study of single-species biofilm (37) showed that mechanical agitation improves the antibacterial properties of 2% CHX. Williamson et al. (38) found that 6% NaOCl was significantly more effective than 2% CHX against a monoculture biofilm of an isolate of *E. faecalis* at 1 and 3 min. Arias-Moliz et al. (39) noted that
CHX eradicated *E. faecalis* biofilm after 5 min. Using confocal microscopy, Chavez de Paz et al. (40) assessed the effect of 2.5% CHX on biofilms of *E. faecalis* and other species isolated from canals with persistent infections and concluded that the treatment removed only half of biofilm cells. Arias-Moliz et al. (39) found that CHX did not eradicate *E. faecalis* biofilms at any concentration. A laboratory study showed that microorganisms in nutrient-limited biofilms and mature biofilms are more resistant to a CHX solution than those in young biofilms (41).

A 2% CHX solution did not improve biofilm dissolution or increase dentin cleaning in comparison with NaOCl (42). An *in vitro* study showed that treatment with 2% CHX for 5 min killed most microorganisms in *E. faecalis* biofilms (43). DNase I and dextranase may decrease bacteria adhesion to dentin and sensitize bacterial biofilms to 2% CHX (44).

**Substantivity**

Dentin medicated with CHX acquires antibacterial substantivity. The absorption of positively charged ions released by CHX prevents bacterial colonization on the dentin surface, and the duration of this effect exceeds the period of medicament application (11).

In an *in vivo* study (45), the substantivity of tetracycline was greater than that of CHX for 12 days and greater than that of normal saline for 16 days. An *in vitro* study showed that the antibacterial substantivity of 2% CHX may persist for 3 days (46). A clinical study found that the substantivity of 2% CHX persisted for up to 2 days (47). However, other studies noted that CHX substantivity may persist for 4 (48) to 12 (49) weeks.

The duration of dentin treatment needed to induce substantivity is unknown. While some studies showed that a 5- to 10-min dentin treatment induced substantivity (36,37), another study (38) found that CHX substantivity was related to the capacity of dentin to absorb CHX. Using infected dentinal tubules from cylindrical bovine specimens, Lin et al. (50) showed that microorganisms in all dentin layers were reduced by CHX and were completely eliminated with a CHX slow-release device. Komorowski et al. (51) showed that 7 days was the optimal duration for CHX treatment of dentin.

**Combining CHX with Ca(OH)₂**

In a study using agar diffusion, the addition of Ca(OH)₂ to 0.5% CHX significantly reduced the antimicrobial effectiveness of CHX. The researchers attributed this effect to deprotonation of CHX, which alters its interaction with microbial surfaces (52). This was confirmed in studies by Almyroudi et al. (53) and Schäfer and Bössmann (54).

A combination of 2% CHX and Ca(OH)₂ reduced the antifungal activity of CHX for 1 week (55). However, there was no significant difference between CHX and the CHX/Ca(OH)₂ combination at 15 days. Using agar diffusion, de Souza-Filho et al. (56) showed that 2% CHX gel was more effective than a combination of 2% CHX and Ca(OH)₂.

In contrast, Al-Nazhan and Al-Obaida (57) studied the effectiveness of 2% CHX combined with Ca(OH)₂ against *C. albicans* and noted additive benefits when combining these agents. Another study revealed that a mixture of CHX and Ca(OH)₂ was more effective than Ca(OH)₂ against *C. albicans* and *E. faecalis* (58). A clinical study (59) found no significant difference between CHX and CHX/Ca(OH)₂ in effectiveness against anaerobic microorganisms.

**Clinical implications and concerns**

Although CHX is useful as a final irrigant, its use as a main endodontic irrigant of the canal is not advised due to its inability to dissolve necrotic remnants (60,61) and to the fact that it is less effective against gram-negative microorganisms than against gram-positive microorganisms (9,62,63). The possibility of anaphylactic reactions due to CHX is another concern (64). Acute allergic reactions such as Quincke edema and urticaria have been reported after skin exposure to CHX (65,66). The potential risks of CHX should be assessed in future research.

In endodontics, CHX is used in two concentrations (0.2% and 2%), and its mode of antibacterial activity is related to concentration. CHX is bacteriostatic at a concentration of 0.2% and bactericidal at a concentration of 2%. Although studies of the antibacterial effectiveness of NaOCl and CHX have revealed some differences between these agents, their antibacterial effects *ex vivo* (in infected dentin) and *in vivo* (in the canal) appear to be similar when they are used at identical concentrations. Furthermore, although CHX has a strong antifungal effect, its effectiveness was considerably less than that of NaOCl. CHX is considered an effective agent against microbial biofilms; however, NaOCl is the only endodontic irrigant that can disrupt biofilm. The principal challenge that prevents the use of CHX as a routine irrigant in endodontics is its lack of tissue solubility during chemomechanical preparation (67). Nevertheless, the substantivity of CHX is a major advantage and makes it an ideal irrigant for final rinsing of the canal.

**References**

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