

Antimicrobial Activity of Calcium Hydroxide in Endodontics: A Review

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The purpose of endodontic therapy is to preserve the patient's natural teeth without compromising the patient's local or systemic health. Calcium hydroxide has been included in several materials and antimicrobial formulations that are used in several treatment modalities in endodontics, such as inter-appointment intracanal medications. The purpose of this article was to review the antimicrobial properties of calcium hydroxide in endodontics. Calcium hydroxide has a high pH (approximately 12.5-12.8) and is classified chemically as a strong base. The lethal effects of calcium hydroxide on bacterial cells are probably due to protein denaturation and damage to DNA and cytoplasmic membranes. Calcium hydroxide has a wide range of antimicrobial activity against common endodontic pathogens but is less effective against *Enterococcus faecalis* and *Candida albicans*. Calcium hydroxide is also a valuable anti-endotoxin agent. However, its effect on microbial biofilms is controversial.

Key Words: *Biofilm; Calcium hydroxide; Candida albicans; Endotoxin*

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INTRODUCTION

Calcium hydroxide was originally introduced to the field of endodontics by Herman in 1920 as a pulp-capping agent.¹ It is a white odorless powder with the formula $\text{Ca}(\text{OH})_2$ (Fig. 1) and has a molecular weight of 74.08. $\text{Ca}(\text{OH})_2$ has low solubility in water (about $1.2 \text{ g} \cdot \text{L}^{-1}$ at 25°C), which decreases as the temperature rises.² The dissociation coefficient of $\text{Ca}(\text{OH})_2$ (0.17) permits a slow, controlled release of both calcium and hydroxyl ions. The low solubility is a good clinical characteristic because a long period is necessary for $\text{Ca}(\text{OH})_2$ to become soluble in tissue fluids when in direct contact with vital tissues.³ $\text{Ca}(\text{OH})_2$ has a high pH (about 12.5-12.8), is insoluble in alcohol, and is chemically classified as a strong base. Its main actions result from the ionic dissociation of the Ca^{2+} and OH^- ions and their effect on vital tissues, such as inducing hard tissue deposition and being antibacterial.² $\text{Ca}(\text{OH})_2$ dissociates into calcium and hydroxyl ions on contact with aqueous fluids.⁴ $\text{Ca}(\text{OH})_2$ in water has a thixotropic behavior, which means that it will be very fluid when agitated.³ When $\text{Ca}(\text{OH})_2$ is exposed to carbon dioxide (CO_2) or carbonate ions (CO_3^{2-}) in bio-

logical tissue, the dissociation of the chemical leads to the formation of calcium carbonate (CaCO_3) and an overall consumption of Ca^{2+} ions. However, one study showed that after 30 days of exposure to carbon dioxide, six preparations of $\text{Ca}(\text{OH})_2$ still maintained a purportedly bactericidal pH within the root canal.²

MECHANISM OF ANTIMICROBIAL ACTIVITY

According to Siqueira,⁵ the antimicrobial activity of $\text{Ca}(\text{OH})_2$ is dependent on the release of hydroxyl ions in an aqueous environment. Furthermore, Siqueira and Lopes⁶ stated that hydroxyl ions are highly oxidant free radicals that show extreme reactivity with several biomolecules. This reactivity is indiscriminate; thus, this free radical rarely diffuses away from sites of generation.⁶ The lethal effects of hydroxyl ions on bacterial cells are probably due to damage to the bacterial cytoplasmic membrane, denaturation of proteins, or damage to the DNA (Fig. 2).

Although these three mechanisms may occur, it is difficult to establish, in a chronological sense, which is the main mechanism involved in the death of bacterial cells after ex-

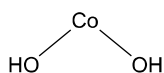


FIG. 1. Structure of the calcium hydroxide molecule.

posure to a strong base.⁶ Hydroxyl ions from $\text{Ca}(\text{OH})_2$ exert their mechanism of action in the cytoplasmic membrane because that is where enzymatic sites are located.⁷ Extracellular enzymes act on nutrients, carbohydrates, proteins, and lipids that, through hydrolysis, favor digestion. Intracellular enzymes located in the cell favor the respiratory activity of the cellular wall structure. The pH gradient of the cytoplasmic membrane is altered by the high concentration of hydroxyl ions from calcium hydroxide acting on the proteins of the membrane (protein denaturation). The high pH of $\text{Ca}(\text{OH})_2$ alters the integrity of the cytoplasmic membrane by means of chemical injury to the organic components and transport of nutrients or by means of the destruction of phospholipids or unsaturated fatty acids of the cytoplasmic membrane during the peroxidation process, which is a saponification reaction.⁸

Adjustment of intracellular pH is influenced by different cellular processes, such as cellular metabolism, alterations in shape, mobility, adjustment of transporters and polymerization of cytoskeleton components, activation of cellular proliferation and growth, conductivity and transport through the membrane, and isosmotic cellular volume. Thus, many cellular functions can be affected by pH, including the enzymes that are essential for cellular metabolism.⁹ Estrela et al.¹⁰ found that bacterial enzymatic inactivation under extreme conditions of pH for a long period of time is irreversible.

ANTIBACTERIAL ACTIVITY

Calcium hydroxide exerts antibacterial effects in the root canal system as long as a high pH is maintained.⁶ An *in vivo* study showed that root canals treated with $\text{Ca}(\text{OH})_2$ had fewer bacteria than did those dressed with camphorated phenol or camphorated monochlorophenol.¹¹ Another study reported $\text{Ca}(\text{OH})_2$ to be effective in preventing the growth of microorganisms but to a limited extent when compared to camphorated chlorophenol, stressing the necessity of direct contact to achieve the optimum antibacterial effect.¹² It was shown that a 7-day application of a $\text{Ca}(\text{OH})_2$ medicament was sufficient to reduce canal bacteria to a level that gave a negative culture.¹³ It has also been shown that an aqueous $\text{Ca}(\text{OH})_2$ paste and a silicone oil-based $\text{Ca}(\text{OH})_2$ paste are effective in the elimination of *Enterococcus faecalis* in dentinal tubules.¹⁴

Estrela et al.¹⁵ demonstrated that both the direct exposure test and the agar diffusion test are useful in establishing the antimicrobial spectrum of $\text{Ca}(\text{OH})_2$ and in developing improved infection control protocols. A complete antimicrobial effect was observed after 48 h with both tests, irrespective of the $\text{Ca}(\text{OH})_2$ paste vehicle.¹⁵ Another study

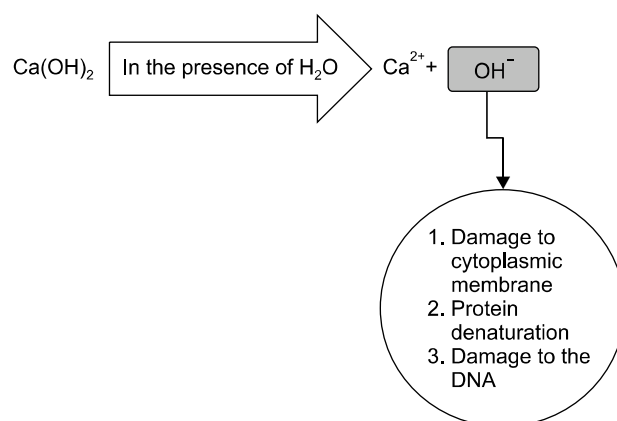


FIG. 2. A schematic view of the mechanisms of the antibacterial activity of calcium hydroxide.

showed that $\text{Ca}(\text{OH})_2$ decreased the numbers of *E. faecalis* at all depths within dentinal tubules up to 24 h and that less viscous preparations of $\text{Ca}(\text{OH})_2$ were more effective in the elimination of *E. faecalis* from dentinal tubules than were viscous preparations.¹⁶

In a study to evaluate the effect of electrophoretically activated $\text{Ca}(\text{OH})_2$ on bacterial viability in dentinal tubules, Lin et al.¹⁷ reported that treatment with electrophoresis was significantly more effective than pure $\text{Ca}(\text{OH})_2$ up to depths of 200 to 500 μm . Specimens treated with electrophoretically activated $\text{Ca}(\text{OH})_2$ revealed no viable bacteria in dentinal tubules to a depth of 500 μm from the root canal space within 7 days.¹⁷

E. faecalis cells in the exponential growth phase have been shown to be the most sensitive to $\text{Ca}(\text{OH})_2$ and are killed within 3 s to 10 min. Cells in a stationary phase were more resistant, with living cells being recovered at 10 min. However, cells in a starvation phase were the most resistant and were not totally eliminated during a 10-minute test period.¹⁸

By contrast, several studies have attested to the ineffectiveness of $\text{Ca}(\text{OH})_2$ in eliminating bacterial cells. Two studies revealed that $\text{Ca}(\text{OH})_2$ had no antibacterial effect as a paste or as the commercial preparation Pulpdent when used against *Streptococcus sanguis*.^{19,20} It was also shown that a $\text{Ca}(\text{OH})_2$ paste (Calasept; Speiko, Darmstadt, Germany) failed to eliminate, even superficially, *E. faecalis* in dentinal tubules.²¹ Safavi et al.²² indicated that *E. faecium* remained viable in dentinal tubules after relatively extended periods of $\text{Ca}(\text{OH})_2$ /saline mixture treatment. Another study demonstrated that $\text{Ca}(\text{OH})_2$ could take up to 10 days to disinfect dentinal tubules infected by facultative bacteria.²³ Siqueira and Uzeda²⁴ demonstrated that, after 1 week of contact, $\text{Ca}(\text{OH})_2$ mixed with saline was ineffective in eliminating *E. faecalis* and *E. faecium* inside dentinal tubules. Estrela et al.⁸ found that $\text{Ca}(\text{OH})_2$ in infected dentinal tubules had no antimicrobial effect on *S. faecalis*, *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, or on the bacterial mixture used throughout the

experiment. It has been revealed that the viability of *E. faecalis* in infected root dentine was not affected by $\text{Ca}(\text{OH})_2$.²⁵ In a systematic review to assess the antibacterial efficacy of $\text{Ca}(\text{OH})_2$, Sathorn et al.²⁶ showed that $\text{Ca}(\text{OH})_2$ had limited effectiveness in eliminating bacteria from human root canal when assessed by culture techniques.

In a polymerase chain reaction (PCR) study to evaluate the effect of root canal filling with or without prior $\text{Ca}(\text{OH})_2$ or 2% chlorhexidine (CHX) on the persistence of bacterial DNA in infected dentinal tubules, Cook et al.²⁷ showed that 2% CHX treatment followed by canal filling was more effective in removing the DNA of *E. faecalis* than placement of $\text{Ca}(\text{OH})_2$ or immediate canal filling. Furthermore, in failed root canal treatments, a 2% CHX gel was a more effective intracanal medicament than $\text{Ca}(\text{OH})_2$ paste against *E. faecalis*.²⁸ Krithikadatta et al.²⁹ indicated that 2% CHX gel alone was more effective against *E. faecalis* than was $\text{Ca}(\text{OH})_2$. Another study found that a polymeric chlorhexidine-controlled release device was significantly more effective in reducing intradentinal bacteria than was $\text{Ca}(\text{OH})_2$ (Table 1).³⁰

EFFECTS ON ENDOTOXIN

Endotoxin, which is present on all Gram-negative bacteria, is composed of polysaccharides, lipids, and proteins and is referred to as lipopolysaccharide (LPS), emphasizing its chemical structure.^{31,32} Lipid A is the region of the

endotoxin molecule responsible for its toxic effects. When free to act, endotoxins do not cause cell or tissue pathosis directly but instead stimulate competent cells to release chemical mediators.³³ Macrophages are the main target of endotoxins.³³ Therefore, endotoxins are not intrinsically toxic.

During root canal treatment, LPS is released during multiplication or bacterial death, thus causing a series of biological effects³⁴ that lead to an inflammatory reaction³² and periapical bone resorption.^{35,36}

Currently, one of the concerns in endodontics is the treatment of teeth with necrotic pulps and periapical pathosis, because treatment failure is higher than in cases without periapical disease.^{37,38} In teeth with chronic periapical lesions, there is a greater prevalence of Gram-negative anaerobic bacteria disseminated throughout the root canal system (dentinal tubules, apical resorptive defects, and cementum lacunae), including apical bacterial biofilm.³⁷⁻⁴⁰ Because these areas are not reached by instrumentation, the use of a root canal medicament is recommended to aid in the elimination of these bacteria and to increase the possibility of clinical success.³⁷⁻³⁹ Teeth with and without radiographically visible periapical disease could be considered as different pathological entities requiring different treatment regimens. Where bone loss has occurred, the use of a root canal medicament between treatment sessions is recommended by some,⁴¹ because the success of treatment in cases with periapical pathosis is directly related to the

TABLE 1. Studies on the antibacterial activity of calcium hydroxide

Researchers	Year of publication	Result
Bystrom et al.	1985	Was more effective than other tested compounds
Stevens & Grossman	1983	Was more effective than CMCP
Sjögren et al.	1991	Was sufficient to reduce canal bacteria to a level that gave a negative culture
Han et al.	2001	Was effective in the elimination of <i>E. faecalis</i> in dentinal tubules
Estrela et al.	2001	Complete antimicrobial effect was observed after 48 h
Behnen et al.	2001	$\text{Ca}(\text{OH})_2$ decreased the numbers of <i>E. faecalis</i> at all depths within dentinal tubules up to 24 h
Lin et al.	2005	Was less effective than electrophoresis up to depths of 200-500 μm
Portenier et al.	2005	<i>E. faecalis</i> cells in the exponential growth phase were the most sensitive to $\text{Ca}(\text{OH})_2$ and were killed within 3 s to 10 min. Cells in a stationary phase were more resistant, with living cells being recovered at 10 min.
DiFiore et al.	1983	$\text{Ca}(\text{OH})_2$ had no antibacterial effect
Siqueira et al.	1998	$\text{Ca}(\text{OH})_2$ had no antibacterial effect
Haapasalo & Ørstavik	1987	$\text{Ca}(\text{OH})_2$ paste failed to eliminate <i>E. faecalis</i> in dentinal tubules
Safavi et al.	1990	<i>E. faecium</i> remained viable in dentinal tubules after relatively extended periods of $\text{Ca}(\text{OH})_2$ /saline mixture treatment
Ørstavik & Haapasalo	1990	$\text{Ca}(\text{OH})_2$ could take up to 10 days to disinfect dentinal tubules
Siqueira et al.	1996	$\text{Ca}(\text{OH})_2$ mixed with saline was ineffective in eliminating <i>E. faecalis</i> and <i>E. faecium</i> inside dentinal tubules
Weiger et al.	2002	Viability of <i>E. faecalis</i> in infected root dentine was not affected by $\text{Ca}(\text{OH})_2$
Sathorn et al.	2007	$\text{Ca}(\text{OH})_2$ had limited effectiveness in eliminating bacteria from human root canal
Cook et al.	2007	Calcium hydroxide was less effective than 2% chlorhexidine
Ballal et al.	2007	Calcium hydroxide was less effective than 2% chlorhexidine gel
Krithikadatta et al.	2007	A polymeric chlorhexidine-controlled release device (PCRD) was significantly more effective in than $\text{Ca}(\text{OH})_2$

elimination of bacteria from the root canal system. The procedures and medicaments used in root canal treatment should lead not only to bacterial death but also to the inactivation of bacterial endotoxin.³³

An *in vitro* study demonstrated that Ca(OH)₂ hydrolyzed the highly toxic lipid A molecule that is responsible for the damaging effects of endotoxin.⁴² Another study showed that Ca(OH)₂ transformed lipid A into fatty acids and amino sugars, which are nontoxic components.⁴³ These findings were confirmed by some other *in vitro* studies.^{34,44}

In two *in vivo* studies, Nelson-Filho et al.³⁹ as well as Silva et al.⁴⁵ revealed that endotoxin caused the formation of periapical lesions and that Ca(OH)₂ inactivated bacterial LPS. In a study on dogs' teeth, Tanomaru et al.⁴⁶ showed that a biomechanical preparation with only irrigating solutions did not inactivate the endotoxin; however, the same treatment associated with the use of the Ca(OH)₂ dressing was effective in inactivation of the toxic effects of this endotoxin. Another study indicated that Ca(OH)₂ significantly reduced osteoclast differentiation.⁴⁷ It has been demonstrated that long-term Ca(OH)₂ treatment did detoxify LPS molecules by hydrolysis of ester bonds in the fatty acid chains of the lipid A moiety (Table 2).⁴⁸

ANTI-FUNGAL ACTIVITY

Fungi have occasionally been found in primary root canal infections,^{49,50} 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 treatment.⁵¹ Overall, the occurrence of fungi in infected root canals varies between 1% and 17%.⁵² A large number of other yeasts have also been isolated from the oral cavity, including *Candida glabrata*, *C. guilliermondii*, *C. parapsilosis*, *C. krusei*, *C. inconspicua*, *C. dubliniensis*, *C. tropicalis*, and *Saccharomyces* species.⁵¹

It has been demonstrated that *C. albicans* cells are highly resistant to Ca(OH)₂ and that all *Candida* species (*C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, and *C. tropi-*

calis) are either equally high or had higher resistance to aqueous calcium hydroxide than did *E. faecalis*.^{53,54} Because *C. albicans* survives at a wide range of pH values, the alkalinity of saturated Ca(OH)₂ solution may not have any effect on *C. albicans*. In addition, Ca(OH)₂ pastes may provide the Ca²⁺ ions necessary for the growth and morphogenesis of *Candida*. These mechanisms may explain why Ca(OH)₂ has been found to be ineffective against *C. albicans*.⁵¹

Siqueira et al.⁵⁵ investigated the antifungal ability of several medicaments against *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. parapsilosis*, and *Saccharomyces cerevisiae*. They reported that whereas the paste of Ca(OH)₂ in camphorated paramonochlorophenol (CPMC)/glycerin had the most pronounced antifungal effects, Ca(OH)₂ in glycerin or chlorhexidine and chlorhexidine in detergent also had antifungal activity, but at a lower level than the paste of Ca(OH)₂ in CPMC/glycerin.⁵⁵ In another study, Ferguson et al.⁵⁶ evaluated the *in vitro* susceptibility of *C. albicans* to various irrigants and medicaments. The minimum inhibitory concentrations of NaOCl, hydrogen peroxide, chlorhexidine digluconate, and aqueous Ca(OH)₂ were determined. The results showed that NaOCl, hydrogen peroxide, and chlorhexidine digluconate were effective against *C. albicans*, even when diluted significantly.⁵⁶ Furthermore, aqueous Ca(OH)₂ had no antifungal activity when maintained in direct contact with *C. albicans* cells, whereas Ca(OH)₂ paste and CPMC were effective antifungal agents.⁵⁶

Valera et al.⁵⁷ showed that, as an intra-canal medicament, CPMC was more effective against *C. albicans* than Ca(OH)₂/CPMC paste. Siqueira et al.⁵⁸ evaluated the effectiveness of four intracanal medicaments in disinfecting the root dentine of bovine teeth experimentally infected with *C. albicans*. Infected dentine cylinders were exposed to 4 different medicaments: Ca(OH)₂/glycerin, Ca(OH)₂/0.12% chlorhexidine digluconate, Ca(OH)₂/CPMC/glycerin, and 0.12% chlorhexidine digluconate/zinc oxide. The specimens were left in contact with the medicaments for 1 hour, 2 days, and 7 days. The specimens treated with Ca(OH)₂/

TABLE 2. Studies on the anti-endotoxin activity of calcium hydroxide

Researchers	Year of publication	Result
Barthel et al.	1997	Ca(OH) ₂ transformed lipid A into fatty acids and amino sugars
Nelson-Filho	2002	1. Endotoxin caused the formation of periapical lesions 2. Ca(OH) ₂ inactivated bacterial LPS
Safavi & Nichols	1993	Ca(OH) ₂ hydrolyzed the highly toxic lipid A molecule that is responsible for the damaging effects of endotoxin
Safavi & Nichols	1994	Ca(OH) ₂ transformed lipid A into fatty acids and amino sugars
Olsen et al.	1999	Ca(OH) ₂ transformed lipid A into fatty acids and amino sugars
Silva et al.	2002	1. Endotoxin caused the formation of periapical lesions 2. Ca(OH) ₂ inactivated bacterial LPS
Tanomaru et al.	2003	Biomechanical preparation associated with the use of the Ca(OH) ₂ dressing was effective in the inactivation of the toxic effects of endotoxin
Jiang et al.	2003	Ca(OH) ₂ significantly reduced osteoclast differentiation
Buck et al.	2001	long-term Ca(OH) ₂ did detoxify LPS molecules by hydrolysis of ester bonds in the fatty acid chains of the lipid A moiety

CPMC/glycerin paste or with chlorhexidine/zinc oxide paste were completely disinfected after 1 hour of exposure. Only $\text{Ca}(\text{OH})_2$ /glycerin paste consistently eliminated *C. albicans* infection after 7 days of exposure. $\text{Ca}(\text{OH})_2$ mixed with chlorhexidine was ineffective in disinfecting dentine even after 1 week of exposure. Of the medicaments tested, the $\text{Ca}(\text{OH})_2$ /CPMC/glycerin paste and chlorhexidine digluconate mixed with zinc oxide were the most effective in eliminating *C. albicans* cells from dentine specimens.⁵⁸

EFFECTS ON BIOFILMS

The term *biofilm* was introduced to designate the thin-layered (sessile) condensations of microbes that may occur on various surface structures in nature.⁵⁹ 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.^{60,61} Such bacterial aggregations have been thought to be the cause of therapy-resistant apical periodontitis.^{61,62} Using transmission electron microscopy (TEM), Nair⁶⁰ examined the root canal contents of 31 teeth that had gross coronal caries and to which the periapical inflammatory lesion was attached upon extraction. In addition to his observations of the microstructure of the inflammatory tissue, he noted that the major bulk of the organisms existed as loose collections of cocci, rods, filaments, and spirochetes.⁶⁰ Whereas most of these organisms appeared to be suspended in what was described as a moist canal space, dense aggregates were also observed sticking to the canal walls and forming layers of bacterial condensations. Amorphous material filled the interbacterial spaces and was interpreted as an extracellular matrix of bacterial origin. When they occurred, the bacterial condensations had a palisade structure similar to the one for dental plaque on external tooth surfaces, suggesting similar mechanisms for bacterial attachment as those for dental plaque.⁶⁰ Sen et al.⁶³ examined untreated extracted teeth with apical periodontitis by scanning electron microscopy (SEM) and found that the root canals were heavily infected, with microorganisms being observed in all areas of the canal. Cocci and rods predominated and formed colonies on the root canal walls and also, to a varying degree, penetrated the dentinal tubules.⁶³ Nair et al.⁶¹ found that even after instrumentation, irrigation, and canal filling in a one-visit treatment, microbes existed as biofilms in untouched locations in the main canal, isthmuses, and accessory canals in most of the specimens.

Antimicrobial agents have often been developed and optimized for their activity against fast-growing, dispersed populations containing a single microorganism.^{59,64} 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.^{60,65} Some reports have shown that microorganisms grown in biofilms could be 2-fold to 1000-fold more re-

sistant than the corresponding planktonic form of the same organisms.⁵⁹ Using scanning electron microscopy and scanning confocal laser microscopy, Distel et al.⁶⁶ reported that despite intracanal dressing with $\text{Ca}(\text{OH})_2$, *E. faecalis* formed biofilms in root canals. Another study showed that $\text{Ca}(\text{OH})_2$ was 100% effective in eliminating *E. faecalis* biofilm.⁶⁷ Brändle et al.⁶⁸ evaluated the effects of growth condition (planktonic, mono- and multi-species biofilms) on the susceptibility of *E. faecalis*, *Streptococcus sobrinus*, *C. albicans*, *Actinomyces naeslundii*, and *Fusobacterium nucleatum* to alkaline stress. The findings showed that planktonic microorganisms were most susceptible; only *E. faecalis* and *C. albicans* survived in saturated solution for 10 minutes, and the latter also survived for 100 minutes.⁶⁸ Dentine adhesion was the major factor in improving the resistance of *E. faecalis* and *A. naeslundii* to calcium hydroxide, whereas the multispecies context in a biofilm was the major factor in promoting resistance of *S. sobrinus* to the disinfectant. In contrast, the *C. albicans* response to calcium hydroxide was not influenced by growth conditions.⁶⁸

COMBINATION OF $\text{Ca}(\text{OH})_2$ AND CHLORHEXIDINE

Chlorhexidine (CHX) is a cationic biguanide whose optimal antimicrobial activity is achieved within a pH range of 5.5 to 7.0.⁶⁹ Therefore, it is likely that alkalinizing the pH by adding $\text{Ca}(\text{OH})_2$ to CHX will lead to precipitation of CHX molecules, thereby decreasing its effectiveness.⁷⁰ It has been demonstrated that the alkalinity of $\text{Ca}(\text{OH})_2$ when mixed with CHX remains unchanged.⁷¹ Therefore, the usefulness of mixing $\text{Ca}(\text{OH})_2$ with CHX remains unclear and controversial.⁶⁹

When used as an intracanal medicament, CHX was more effective than $\text{Ca}(\text{OH})_2$ in eliminating *E. faecalis* from inside dentinal tubules.⁶⁹ One report revealed that all of the chlorhexidine formulations studied, including a CHX/ $\text{Ca}(\text{OH})_2$ 50:50 mix, were effective in eliminating *E. faecalis* from dentinal tubules, with a 1% CHX gel working better than the other preparations.⁷² These findings were corroborated by two other studies in bovine dentine and⁷³ and human dentine.⁷⁴

Haenni et al.⁷¹ found no additive antibacterial effect by mixing $\text{Ca}(\text{OH})_2$ powder with 0.5% CHX. They indicated that CHX had a reduced antibacterial action. However, $\text{Ca}(\text{OH})_2$ did not lose its antibacterial properties in such a mixture.⁷¹ An *in vitro* study showed that 2% CHX gel was the most effective agent against *E. faecalis* inside dentinal tubules, followed by a $\text{Ca}(\text{OH})_2$ /2% CHX mixture, whereas $\text{Ca}(\text{OH})_2$ alone was totally ineffective, even after 30 days.⁷⁵ The 2% CHX gel was also significantly more effective than the $\text{Ca}(\text{OH})_2$ /2% CHX mixture against *C. albicans* at 7 days, although there was no significant difference at 15 and 30 days. $\text{Ca}(\text{OH})_2$ alone was completely ineffective against *C. albicans*.⁷⁵ In an *in vivo* study using primary teeth, Onçag et al.⁷⁶ showed that a 1% CHX-gluconate gel, both with and without $\text{Ca}(\text{OH})_2$, was more effective against *E. faecalis* than $\text{Ca}(\text{OH})_2$ alone over a 48-hour period.

Another study showed that that 2% CHX-gluconate was significantly more effective against *E. faecalis* than $\text{Ca}(\text{OH})_2$ used alone or a mixture of the two.⁷⁴ Although this was also confirmed by Lin et al.,⁷⁷ a study by Evans et al.⁷⁸ using bovine dentine concluded that 2% CHX with $\text{Ca}(\text{OH})_2$ was more effective than $\text{Ca}(\text{OH})_2$ in water. An animal study demonstrated that teeth dressed with CHX for 4 weeks had reduced inflammatory reactions in the periodontium (both apically and marginally) and less root resorption.⁷⁹ Waltimo et al.⁵³ reported that 0.5% CHX-acetate was more effective at killing *C. albicans* than was saturated $\text{Ca}(\text{OH})_2$, whereas $\text{Ca}(\text{OH})_2$ combined with CHX was more effective than $\text{Ca}(\text{OH})_2$ used alone.

BUFFERING EFFECT OF DENTINE ON THE ANTIBACTERIAL ACTIVITY OF $\text{Ca}(\text{OH})_2$

The root canal milieu is a complex mixture of a variety of organic and inorganic components. Hydroxyapatite is the major representative of the inorganic components, whereas pulp tissue, microorganisms, and inflammatory exudate rich in proteins such as albumin are the major organic components.⁸⁰ The relative importance of the various organic and inorganic compounds in the inactivation of root canal disinfectants has been studied to a limited extent only.⁸¹ Difficulties in designing experiments that will give reliable and comparable data have been some of the greatest challenges. Haapasalo et al.⁸¹ introduced a new dentine powder model for studying the inhibitory effect of dentine on various root canal irrigants and medicaments. They concluded that dentine powder effectively abolished the killing of *E. faecalis* by $\text{Ca}(\text{OH})_2$.⁸¹ Hydroxyapatite had an effect similar to dentine on $\text{Ca}(\text{OH})_2$, preventing the killing of *E. faecalis*.⁸²

The substantial effect of dentine on the antibacterial activity of $\text{Ca}(\text{OH})_2$ can be attributed to the buffering action of dentine against alkali.⁸³ $\text{Ca}(\text{OH})_2$ is used as a thick paste *in vivo*; however, its solubility is low and saturation is achieved at a relatively low concentration of hydroxyl ions. Both laboratory and *in vivo* studies have shown that buffering by dentine, particularly in the subsurface layers of the root canal walls, might be the main factor behind the reduced antibacterial effect of $\text{Ca}(\text{OH})_2$. It is possible that deeper in dentine (outside the main root canal), $\text{Ca}(\text{OH})_2$ is present as a saturated solution or at concentrations even below that level.⁸¹ Besides dentine, remnants of necrotic pulp tissue as well as inflammatory exudate might affect the antibacterial potential of endodontic disinfectants.⁸⁰

In conclusion, (1) The antimicrobial activity of $\text{Ca}(\text{OH})_2$ is related to the release of highly reactive hydroxyl ions in an aqueous environment, which mainly affects cytoplasmic membranes, proteins, and DNA.

(2) Although some clinical studies have supported the efficacy of $\text{Ca}(\text{OH})_2$ as an intracanal medicament, others studies have questioned its efficacy and have indicated CHX instead of $\text{Ca}(\text{OH})_2$.

(3) Endotoxin, a component of the cell wall of Gram-neg-

ative bacteria, plays a fundamental role in the genesis and maintenance of periapical lesions due to the induction of inflammation and bone resorption. $\text{Ca}(\text{OH})_2$ inactivates endotoxin, *in vitro* and *in vivo*, and appears currently to be the only clinically effective medicament for inactivation of endotoxin.

(4) Fungi have occasionally been found in primary root canal infections, but they appear to occur more often in filled root canals of teeth in which treatment has failed. *C. albicans* is by far the fungal species most commonly isolated from infected root canals. It seems that the combinations of $\text{Ca}(\text{OH})_2$ with camphorated paramonochlorophenol or chlorhexidine have the potential to be used as effective intracanal medicaments for cases in which fungal infection is suspected.

(5) The few studies conducted on the antimicrobial potential of $\text{Ca}(\text{OH})_2$ on biofilms have demonstrated inconsistent results. Further studies are required to elucidate the anti-biofilm efficacy of $\text{Ca}(\text{OH})_2$.

(6) Although the usefulness of mixing $\text{Ca}(\text{OH})_2$ with CHX remains unclear and controversial, it seems that by mixing $\text{Ca}(\text{OH})_2$ with CHX, the antimicrobial activity of $\text{Ca}(\text{OH})_2$ is increased. In other words, the descending order of the antimicrobial activity of $\text{Ca}(\text{OH})_2$, CHX, and their combination is as follows: CHX, $\text{Ca}(\text{OH})_2$ /CHX, and $\text{Ca}(\text{OH})_2$.

(7) It seems that dentine, hydroxyapatite, and remnants of necrotic pulp tissue as well as inflammatory exudate decrease the antibacterial potential of $\text{Ca}(\text{OH})_2$. In other words, $\text{Ca}(\text{OH})_2$ is likely to be effective under laboratory conditions but relatively ineffective as a medicament *in vivo*.

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