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Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part I. *In vitro* studies

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The goal of endodontic treatment is the prevention and control of pulpal and periradicular infections. Calcium hydroxide ($Ca(OH)_2$) has been widely used in endodontics as an intracanal medicament to eliminate the remaining microorganisms after chemomechanical preparation. The purpose of this article is to review the antimicrobial properties of $Ca(OH)_2$ as an intracanal medicament in root canal treatment. The first part of this review details the characteristics of $Ca(OH)_2$ and summarizes the results of *in vitro* studies related to its antimicrobial effect. The antimicrobial effect of $Ca(OH)_2$ results from the release of hydroxyl ions when it comes into contact with aqueous fluids. $Ca(OH)_2$ has a wide range of antimicrobial effects against common endodontic pathogens, but is less effective against *Enterococcus faecalis* and *Candida albicans*. The addition of vehicles or other agents might contribute to the antimicrobial effect of $Ca(OH)_2$. (*Restor Dent Endod* 2014;39(4):241-252)

Key words: Antimicrobial effect; Calcium hydroxide; Endodontics; Intracanal medicament; Microorganism

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Introduction

Microorganisms are the cause of apical inflammatory lesions, and the goal of endodontic treatment is the prevention and control of pulpal and periradicular infections.[1] Numerous measures have been introduced to reduce the number of microorganisms from the root canal system, including various mechanical instrumentation techniques, irrigation regimes, and intracanal medicaments.[2-6] It is difficult to eliminate all microorganisms from an infected root canal system by mechanical instrumentation alone.[7-9] Therefore, chemical irrigation and disinfection are necessary to remove microorganisms, their byproducts, pulp tissue remnants, and other debris from the root canal. Intracanal medicaments may perform these roles by remaining in the root canal between treatment appointments.[10]

Since Hermann introduced it in 1920 as a pulp-capping agent, calcium hydroxide ($Ca(OH)_2$) has been widely used in endodontics.[11] Various biological properties of $Ca(OH)_2$, such as antimicrobial activity, tissue-dissolving ability, inhibition of tooth resorption, and hard tissue formation, have been investigated, and its wide use in root canal treatment has been associated with periradicular healing and few adverse reactions.[3,12-16]

Currently, $Ca(OH)_2$ is considered the first choice of root canal dressing materials. The

purpose of this article is to review the antimicrobial effect of $Ca(OH)_2$ as an intracanal medicament in root canal treatment. A PubMed search was performed to identify laboratory and clinical studies that investigated the antimicrobial effect of $Ca(OH)_2$ from 1970 to 2013 and was limited to English-language papers. Studies that included $Ca(OH)_2$ as one of the comparative groups as well as the main subject were all reviewed. The articles were classified and analyzed according to their experimental methods. The first part of this review will detail the characteristics of $Ca(OH)_2$ and summarize the results of *in vitro* studies related to its antimicrobial effect.

Review

Chemical characteristics of calcium hydroxide and mechanisms of antimicrobial effect

Calcium hydroxide is a white odorless powder with the formula $Ca(OH)_2$. It has low solubility in water and releases calcium (Ca^{2+}) and hydroxyl (OH^-) ions slowly. The low solubility is a good clinical characteristic because a long period is necessary for $Ca(OH)_2$ to become soluble in tissue fluids when in direct contact with vital tissues.[17] $Ca(OH)_2$ has a high pH (12.5 - 12.8) and is chemically classified as a strong base. It dissociates into calcium and hydroxyl ions on contact with an aqueous solution, and the main actions of $Ca(OH)_2$ are attributed to the effect of these ions on vital tissues, such as inducing hard tissue deposition and being antibacterial.[17] Hydroxyl ions are responsible for the highly alkaline nature of $Ca(OH)_2$. Most of the pathogens are unable to survive in the highly alkaline environment provided by $Ca(OH)_2$.[18] Since the pH of $Ca(OH)_2$ is about 12.5, bacteria in the infected root canal are eliminated when in direct contact with this substance.[3]

The antimicrobial activity of $Ca(OH)_2$ is related to the release of hydroxyl ions in contact with aqueous fluids. Hydroxyl ions are highly oxidant free radicals that show extreme reactivity with biomolecules.[19] The lethal effect on microorganisms has been attributed to the following mechanisms - damage to the bacterial cytoplasmic membrane, protein denaturation, and/or damage to the DNA - yet, it is difficult to establish the main mechanism involved in the death of bacteria.[20-23] Kontakiotis *et al.* suggested that the ability of $Ca(OH)_2$ to absorb carbon dioxide may contribute to its antibacterial activity.[24]

Laboratory studies

1. Antimicrobial susceptibility tests

A series of studies demonstrated the antimicrobial effect of $Ca(OH)_2$ (Table 1). Several researchers investigated the effect of root canal medicaments by a direct exposure

Table 1. Studies reporting antimicrobial effect of $Ca(OH)_2$

Year	Researcher	Test method	Microbial strains	Major ingredients	Period
1991	Pavelić *et al.*[36]	ADT	2 (+)	CH	1, 2 day
1992	Gencoglu & Kulekci[25]	DET	4	CH, CMCP, IKI, cresophene	10, 15 min
1993	Alaçam *et al.*[26]	DET	6	CH, NaOCl, metronidazole	0 - 3 day
1993	Georgopoulou *et al.*[27]	DET	30	CH, CMCP	3 - 60 min
1995	Kontakiotis *et al.*[24]	DET	40	CH	3 day
1996	Barnard *et al.*[28]	DET	1	CH, NaOCl	1 - 30 min, 7 day
1998	Estrela *et al.*[29]	DET	6	CH	0 - 7 day
2000	Leonardo *et al.*[31]	ADT	7 (+)	CH, ZnO	2 hr
2003	Morrier *et al.*[32]	ADT	3 (+)	CH, 5 commercial pastes	
2003	Podbielski *et al.*[37]	DET	5 (+)	CH, ZnO	0 - 14 day
2006	Amorim Lde *et al.*[33]	DET/ADT	4 (+)	CH, Vitapex, ZOE, TC	0 - 3 day
2007	Ferreira *et al.*[38]	BDT	5 (+)	CH, 6 antibiotics	1 hr
2007	Tanomaru *et al.*[30]	ADT	5	CH, CH + CMCP	2 day
2008	Blanscet *et al.*[35]	ADT	6 (+)	40, 50, 60%-CH, UltraCal, Vitapex	2, 4 day
2011	Mehrvarzfar *et al.*[34]	DET	1 (+)	CH, BAG	0 - 3 day

(+), *E. faecalis* was included as a subject of the experiment.
ADT, Agar diffusion test; DET, Direct exposure test; BDT, Broth dilution test, CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; IKI, Iodine potassium iodide; NaOCl, Sodium hypochlorite; ZnO, Zinc oxide; ZOE, Zinc oxide eugenol; TC, Tetracycline; BAG, Bioactive glass.
Vitapex, Neo-Dental Int., Federal Way, WA, USA; UltraCal, Ultradent Products Inc., South Jordan, UT, USA.

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test, and they found that $Ca(OH)_2$ was effective in killing bacteria.[24-30] However, it should be noted that they did not evaluate the susceptibility of *Enterococcus faecalis* (*E. faecalis*). Leonardo *et al.* evaluated the antimicrobial activity of two $Ca(OH)_2$ pastes against seven bacterial strains, and both of them were effective for all strains.[31] Some studies have compared various $Ca(OH)_2$ pastes.[32,33] Mehrvarzfar *et al.* compared bioactive glass with $Ca(OH)_2$ and found that both exhibited antimicrobial effects against *E. faecalis* and that $Ca(OH)_2$ showed a superior disinfecting effect.[34] Blanscet *et al.* found that the higher the concentration of the $Ca(OH)_2$ paste was, the larger were the zones of inhibition observed.[35]

Some authors have insisted that *E. faecalis* is less susceptible to $Ca(OH)_2$ than other bacteria. Pavelic *et al.* evaluated the antimicrobial effect of $Ca(OH)_2$ by using an agar diffusion method.[36] They found that $Ca(OH)_2$ effectively inhibited the growth of all three microorganisms after 24 hours, but there was a difference in the sensitivity of each microorganism, such that *Streptococcus mutans* was the most sensitive and *E. faecalis* was the least. In the studies of Podbielski *et al.* and Ferreira *et al.*, $Ca(OH)_2$ was effective against all experimental strains and *E. faecalis* was the least susceptible.[37,38]

On the other hand, some articles have presented results that bring into question the antimicrobial effect of $Ca(OH)_2$ (Table 2). Ohara *et al.* evaluated the antibacterial effect of several irrigants and found that $Ca(OH)_2$ was totally ineffective as saline.[39] Studies that compared the effect of various agents showed that $Ca(OH)_2$ had only a weak effect.[40-43] More recent studies that compared $Ca(OH)_2$ with other agents reported that $Ca(OH)_2$ was less effective than others.[44-46] Adl *et al.* compared a triple antibiotic paste with $Ca(OH)_2$ and found that $Ca(OH)_2$ exhibited the smallest inhibition zones against *E. faecalis*.[47]

Several researchers have assessed the influence of vehicles or agents mixed with $Ca(OH)_2$ (Table 3). Estrela *et al.* examined the antimicrobial efficacy of $Ca(OH)_2$ with certain vehicles and concluded that the vehicles did not influence the antimicrobial activity of $Ca(OH)_2$.[48,49] However, different results were observed by Gomes *et al.*[50,51] They reported that $Ca(OH)_2$ mixed with water or glycerin showed little or no effect, whereas $Ca(OH)_2$ mixed with camphorated paramonochlorophenol (CMCP) was significantly more effective. The pastes with oily vehicles showed larger zones of inhibition than those with aqueous or viscous vehicles. In Siqueira and Uzeda's study, $Ca(OH)_2$ mixed with distilled water or glycerin was ineffective against all bacterial strains even after 7 days of incubation.[52] However, in another study using a broth dilution test, $Ca(OH)_2$ with saline or glycerin showed antibacterial activity after 1 - 3 days.[53] Vianna *et al.* stated that all tested $Ca(OH)_2$ pastes were able to kill bacteria, but the paste prepared with CMCP was more effective in eliminating *E. faecalis* and *Candida albicans* (*C. albicans*).[54] This coincided with the result reported by Gangwar.[55] Turk *et al.* and Pacios *et al.* reported that *E. faecalis* and *C. albicans* were not inhibited by $Ca(OH)_2$ mixed with distilled water.[56,57]

Some have studied $Ca(OH)_2$, chlorhexidine (CHX), and their mixtures. Basrani *et al.*, Lin *et al.* and Ballal *et al.* found that the CHX gel was more effective than the $Ca(OH)_2$ paste against *E. faecalis* and *C. albicans*.[58-60] Several other

Table 2. Studies reporting $Ca(OH)_2$ to be ineffective

Year	Researcher	Test method	Microbial strains	Major ingredients	Period
1993	Ohara *et al.*[39]	DET	6	CH, CMCP, IKI, cresophene	5 - 60 min
1994	Barbosa *et al.*[40]	DET	11 (+)	CH, CHX, NaOCl, H_2O_2, EDTA	1 - 60 min, 1 wk
2002	Ferreira *et al.*[41]	BDT	4	CH, CHX, CMCP	2, 4 day
2002	Rosa *et al.*[42]	BDT	3	CH, CHX, CMCP, FC	2, 4 day
2007	Reddy & Ramakrishna[43]	ADT	26 (+)	CH, ZOE, CP, Metapex	
2011	Badr *et al.*[44]	ADT/BDT	1 (+)	CH, Liquorice	2 day
2012	Adl *et al.*[47]	ADT	1 (+)	CH, antibiotics	7 day
2012	Hegde *et al.*[45]	ADT	7 (+)	ApexCal, Metapex, Endoflas, ZOE	1 - 2 day
2012	Mattigatti *et al.*[46]	ADT	2 (+)	CH, CHX, NaOCl, EDTA, MTAD, propolis	2 day

(+), *E. faecalis* was included as a subject of the experiment.
DET, Direct exposure test; BDT, Broth dilution test; ADT, Agar diffusion test, CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; IKI, Iodine potassium iodide; CHX, Chlorhexidine; NaOCl, sodium hypochlorite; H_2O_2, Hydrogen peroxide; EDTA, Ethylenediaminetetraacetic acid; FC, Formocresol; ZOE, Zinc oxide euginol; CP, Camphorated phenol.
Metapex, Meta Biomed Co., Ltd., Cheongju, Korea; ApexCal, Ivoclar Vivadent, Schaan, Liechtenstein; Endoflas, Sanlor Laboratory, Miami, FL, USA; MTAD, Dentsply Tulsa Dental Specialties, Tulsa, OK, USA.

Table 3. Studies on the effect of $Ca(OH)_2$ mixed with vehicles or other agents

Year	Researcher	Test method	Microbial strains	Major ingredients (Mixed)	Period	Effect (+/–)
1997	Siqueira & Uzeda[52]	ADT	12 (+)	CH, CHX, CMCP	7 day	+/–
1998	Siqueira & Uzeda[53]	BDT	4 (+)	CH, CMCP	0 - 3 day	+
2001	Estrela *et al.*[48]	DET	5 (+)	CH, CHX, CMCP	0 - 7 day	+
2001	Estrela *et al.*[49]	DET/ADT	4 (+)	CH, PEG, CMCP	0 - 3 day	+
2002	Gomes *et al.*[50]	ADT	11 (+)	CH + 7 vehicles	0 - 7 day	+/–
2002	Gomes *et al.*[51]	ADT	11 (+)	CH + 7 vehicles	1 - 7 day	+/–
2003	Basrani *et al.*[58]	ADT	1 (+)	CH, CHX	7 day	–
2003	Lin *et al.*[59]	ADT	1 (+)	CH, CHX	1, 3 day	–
2005	Vianna *et al.*[54]	BDT	5 (+)	CH + 6 vehicles	0 - 7 day	+
2006	Gomes *et al.*[61]	DET/ADT	5 (+)	CH, CHX	1 - 2 day	–
2007	Ballal *et al.*[60]	ADT	1 (+)	CH, CHX	2, 3 day	+/–
2007	Neelakantan *et al.*[62]	ADT	3 (+)	CH, CHX	0 - 3 day	–
2008	de Souza-Filho *et al.*[63]	ADT	6 (+)	CH, CHX	1 - 2 day	+/–
2009	Turk *et al.*[56]	ADT	1 (+)	CH + 7 vehicles	1 day	+/–
2010	Jhamb *et al.*[64]	ADT	1 (+)	CH, CHX	3 day	–
2011	Gangwar[55]	ADT	6 (+)	CH + 4 vehicles	1,4,7 day	+/–
2012	Pacios *et al.*[57]	ADT	6 (+)	CH + 9 vehicles	2 day	+/–

(+), *E. faecalis* was included as a subject of the experiment; +/–, The result showed a limited effect.
ADT, Agar diffusion test; BDT, Broth dilution test; DET, Direct exposure test, CH, Calcium hydroxide; CHX, Chlorhexidine; CMCP, Camphorated paramonochlorophenol; PEG, Polyethylene glycol.

authors reported that the antimicrobial effect of $Ca(OH)_2$ alone was significantly lower than that of $Ca(OH)_2$ mixed with CHX.[61-64]

Some studies have reported other factors that could affect the antimicrobial effect of $Ca(OH)_2$. Portenier *et al.* found that the susceptibility of *E. faecalis* differed with its various phases such as the starvation, stationary, or exponential growth phases.[65] Oliveira *et al.* reported that the existence of serum or necrotic tissue slowed down the antimicrobial activity of intracanal medicaments.[66] Irrespective of the results, we have to take care in using phenolic compounds such as CMCP, because they may have toxic and/or antigenic effects.[67]

2. Infected dentin models - bovine teeth

In 1987, Haapasalo and Orstavik introduced an *in vitro* model for a dentinal tubule infection of the root canal.[68] Cylindrical dentin specimens made from extracted bovine incisors were infected with *E. faecalis*, and medicaments were applied to the lumen of the dentin blocks. After a certain period, bacterial samples were taken with sterile round burs, and the dentin chips obtained with each bur were immediately collected in separate test tubes. These tubes were then incubated and inspected. In this study, liquid CMCP rapidly and completely disinfected the dentinal tubules, whereas $Ca(OH)_2$ failed to eliminate them even superficially. Since then, a number of experiments have been performed using this method (Table 4). This model enabled an evaluation of the infection status at different depths of the dentinal tubules. Even though some researchers have modified the details of the model, the main objective of these studies, to evaluate the effect of antimicrobial agents in the root canal system with its own structures and components, was the same.

The results have been controversial. Siqueira & Uzeda reported that the $Ca(OH)_2$/saline paste was ineffective against *E. faecalis* and *Fusobacterium nucleatum* (*F. nucleatum*) even after 1 week of exposure.[69] The results of other experiments supported that $Ca(OH)_2$ had little or no antimicrobial effect.[70-74] A recent study used an infected dentin biofilm model that showed only a minimal inhibition of bacterial growth by $Ca(OH)_2$ for 1 week.[75] Behnen *et al.* found that a thin preparation of $Ca(OH)_2$ was more effective than thick preparations.[76] Fuss *et al.* reported that $Ca(OH)_2$ significantly reduced bacterial viability in dentinal tubules up to a depth of 300 μm.[77] Lynne *et al.* found that a 10% $Ca(OH)_2$ paste effectively eliminated *E. faecalis*

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Table 4. Studies on the antimicrobial effect of $Ca(OH)_2$ using infected dentin models with bovine teeth

Year	Researcher	Microbial strains	Major ingredients	Period	Effect (+/–)
1987	Haapasalo & Orstavik[68]	*E. faecalis*	CH, CMCP	10 day	–
1996	Siqueira & Uzeda[69]	*E. faecalis* + 2	CH, CMCP	1 wk	–
2003	Evans *et al.*[70]	*E. faecalis*	CH, CHX	1 wk	–
2003	Gomes *et al.*[71]	*E. faecalis*	CH, CHX	1 - 30 day	–
2004	Baker *et al.*[72]	*E. faecalis*	CH, Betadine, IKI	15 min, 1 day	–
2004	Sirén *et al.*[73]	*E. faecalis*	CH, CHX, IKI	1, 7 day	–
2009	Lin *et al.*[74]	*E. faecalis*	CH, IKI	2, 7 day	–
2013	Ordinola-Zapata *et al.*[75]	*E. faecalis* biofilm	CH, CHX, TAB	7 day	–
2001	Behnen *et al.*[76]	*E. faecalis*	CH	1 day	+/–
2002	Fuss *et al.*[77]	*E. faecalis*	CH, IKI, Cu	1 wk	+
2003	Lynne *et al.*[78]	*E. faecalis*	CH, CHX	1 day	+
2003	Siqueira *et al.*[79]	*C. albicans*	CH, CHX, CMCP	1 - 7 day	+
2003	Zehnder *et al.*[80]	*E. faecalis*	CH, CHX, NaOCl	1, 5 day	+
2004	Evanov *et al.*[81]	*E. faecalis*	CH, CHX	35 min	+

+/–, The result showed limited effect.
CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; CHX, Chlorhexidine; IKI, Iodine potassium iodide; TAB, Triple antibiotic paste; Cu, Copper; NaOCl, Sodium hypochlorite.

(89 - 94%).[78] Siqueira *et al.* and Zehnder *et al.* reported that $Ca(OH)_2$ eliminated *C. albicans* and *E. faecalis* after 7 days.[79,80]

Evanov *et al.* investigated whether the antimicrobial action of $Ca(OH)_2$ or CHX was enhanced by heat.[81] Both agents at 46℃ produced significantly less growth than either group at 37℃.

To be effective against the bacteria located inside the dentinal tubules, hydroxyl ions from $Ca(OH)_2$ must diffuse through the dentin and reach sufficient levels to be lethal. Dentin has the buffering property of alkaline substances because of the proton donors in the hydrated layer of the hydroxyapatite.[82] It has been demonstrated that $Ca(OH)_2$ alkalinizes dentin, but the pH values may be insufficient to kill some bacterial strains, particularly *E. faecalis*, which can survive at a pH value of 11.5.[3] Some authors supposed that excessively large numbers of bacteria might be used, which may have mechanically blocked the dentinal tubules, preventing $Ca(OH)_2$ from entering.

3. Infected dentin models - human teeth

Considerably more studies have been performed with extracted human teeth than with bovine teeth.

Safavi *et al.* used the roots of extracted human teeth.[83] The specimens were infected with *Streptococcus faecium* and exposed to $Ca(OH)_2$ or iodine potassium iodide (IKI) for a duration ranging from 1 minute to 24 hours and the viability of the microorganisms was determined by the incubation of the entire roots in a culture medium. IKI disinfected dentin effectively, but bacteria remained viable in the dentin after extended periods of $Ca(OH)_2$ treatment. This study was followed by several researchers who supported the ineffectiveness of $Ca(OH)_2$ as an intracanal medicament.[84-86] Some authors have reported that $Ca(OH)_2$ showed almost no antimicrobial effect at all (Table 5).[58,87-90]

Nevertheless, the opposite results have also been exhibited (Table 6). The study of Stuart *et al.* that compared the antimicrobial effectiveness of three medicaments in the root canals of extracted human teeth showed a 99.9% reduction against four species of bacteria in the teeth treated with $Ca(OH)_2$.[91] Han *et al.* found that $Ca(OH)_2$ with an aqueous vehicle could successfully reduce the bacteria in the dentinal tubules.[92] The results of other experiments showed that the bacteria were almost eliminated by $Ca(OH)_2$.[93-98] Noetzel *et al.* and Valera *et al.* found that $Ca(OH)_2$ effectively reduced microorganisms that remained even after cleaning and shaping procedures.[99,100]

Lastly, there were authors who reported the limited antimicrobial effect of $Ca(OH)_2$ that could partially reduce the number of bacteria (Table 7). Tanriverdi *et al.* found that the effectiveness of $Ca(OH)_2$ was inferior to that of the other agents.[101] However, a significant reduction in the bacterial number was observed. Similar results were exhibited in other studies.[102-105] Lana *et al.* said that a $Ca(OH)_2$ paste induced a 70% elimination of *E. faecalis* for

Table 5. Studies reporting $Ca(OH)_2$ to be ineffective using infected dentin models with human teeth

Year	Researcher	Microbial strains	Major ingredients	Period
1990	Safavi *et al.*[83]	*S. faecium*	CH, IKI	
1999	Estrela *et al.*[84]	4 types of bacteria	CH	0 - 7 day
2001	Basson & Tait[85]	*A. israelii*	CH, IKI, CHX	3, 7, 60 day
2001	Valera *et al.*[86]	*C. albicans*	CH, CMCP, IKI, FC	14 day
2003	Basrani *et al.*[58]	*E. faecalis*	CH, CHX	7 day
2005	Schäfer *et al.*[87]	*E. faecalis*	CH, CHX	3 day
2006	Ercan *et al.*[88]	*E. faecalis, C. albicans*	CH, CHX	7 - 30 day
2009	Gomes *et al.*[89]	3 types of bacteria + *C. albicans*	CH, CHX, ZnO	
2013	Lee *et al.*[90]	*E. faecalis*	CH, HBD3, CHX	7 day

CH, Calcium hydroxide; IKI, Iodine potassium iodide; CHX, Chlorhexidine; CMCP, Camphorated paramonochlorophenol; FC, Formocresol; ZnO, Zinc oxide; HBD3, Human β-defensin 3.

Table 6. Studies reporting antimicrobial effect of $Ca(OH)_2$ using infected dentin models with human teeth

Year	Researcher	Microbial strains	Major ingredients	Period
1991	Stuart *et al.*[91]	4 bacteria	CH, Pulpdent, CMCP, FC	1 hr
2001	Han *et al.*[92]	*E. faecalis*	CH (aqueous), CH (oil-based)	1 wk
2002	Barthel *et al.*[93]	Bacteria from oral cavity (*in situ*)	CH, CHX	1 wk
2004	Menezes *et al.*[94]	*E. faecalis, C. albicans*	CH, CMCP, FC, FPMC	15 day
2006	Zehnder *et al.*[95]	*E. faecalis*	CH, BAG	10 day
2013	Atila-Pektaş *et al.*[96]	*E. faecalis, S. mutans*	CH, CHX, BAG	1 wk
2013	Maekawa *et al.*[97]	*E. faecalis, C. albicans*	CH, CHX, propolis, ginger extract	2 wk
2009	Noetzel *et al.*[99]	*E. faecalis*	CH, ozone, Er:YAG laser	1 wk
2012	Valera *et al.*[98]	*E. coli*	CH, Polymyxin B, CHX, castor oil extract	2 wk

CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; FC, Formocresol; CHX, Chlorhexidine; FPMC, Furacin paramonochlorophenol; BAG, Bioactive glass.
Pulpdent, Pulpdent Corp., Watertown, MA, USA.

14 days.[106] Delgado *et al.*, Harrison *et al.*, Kandaswamy *et al.*, and Madhubala *et al.* reported 75%, 83%, 55%, and 59% of bacterial reduction, respectively.[107-110] Almyroudi *et al.*, Oncag *et al.* and Pavaskar *et al.* said that the antimicrobial efficacy of $Ca(OH)_2$ might decrease over time.[111-113]

Some have compared the effect of $Ca(OH)_2$ with different vehicles (Table 8). Sukawat and Srisuwan found that $Ca(OH)_2$ mixed with CMCP killed all of the *E. faecalis* inside the tubules, whereas $Ca(OH)_2$ mixed with distilled water or CHX was ineffective against these bacteria.[114] Cwikla *et al.* said that there were significant differences in the disinfecting ability among the three $Ca(OH)_2$ formulations.[115] Lima *et al.* reported that all $Ca(OH)_2$-based medicaments used in the study were able to significantly reduce the colony-forming units (CFU) of *E. faecalis* in the extracted teeth.[116] Prabhakar *et al.* said that a combination of IKI or CHX with $Ca(OH)_2$ may be beneficial.[117]

One study applied molecular techniques to the infected dentin model. Cook *et al.* evaluated the effect of $Ca(OH)_2$ or CHX prior to root canal obturation on the survival of *E. faecalis* by using culture and polymerase chain reaction (PCR) techniques.[118] A significant finding was exhibited such that no bacterial growth was seen on any of the cultures. However, PCR results showed a positive result on most of the experimental groups. These molecular techniques have been actively used for *in vivo* studies, which we shall discuss in the next part of this article.

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Table 7. Studies reporting limited effect of $Ca(OH)_2$ using infected dentin models with human teeth

Year	Researcher	Microbial strains	Major ingredients	Period	Note
1997	Tanriverdi *et al.*[101]	*E. faecalis*	CH, cresophene, phenic acid	1, 3 day	CH < CPCP
2002	Almyroudi *et al.*[111]	*E. faecalis*	CH, CHX	3, 8, 14 day	3, 8 day: Effective 14 day: Reduced effect
2006	Oncag *et al.*[112]	*E. faecalis*	CH, CHX, propolis	2, 10 day	2 day: Effective 10 day: Reduced effect
2007	Krithikadatta *et al.*[102]	*E. faecalis*	CH, CHX, BAG	1, 3, 5 day	CH < BAG < CHX
2009	Lana *et al.*[106]	*E. faecalis*	CH, CMCP	7, 14 day	CH (70%) < CH + CMCP (100%)
2010	Delgado *et al.*[107]	*E. faecalis*	CH, CHX	14 day	CH (75%) < CHX
2010	Harrison *et al.*[108]	*E. faecalis*	CH	7 day	83% reduction
2010	Kandaswamy *et al.*[109]	*E. faecalis*	CH, CHX, MC juice	1, 3, 5 day	CH (55%) < CHX (100%)
2011	Kayaoglu *et al.*[103]	*E. faecalis*	CH, CHX, propolis	1, 7 day	CH < propolis < CHX
2011	Madhubala *et al.*[110]	*E. faecalis*	CH, TAB, propolis	1, 2, 7 day	CH (59%) < TAB < propolis
2012	Pavaskar *et al.*[113]	*E. faecalis*	CH, Vitapex, linezolid	3, 8, 14 day	3 day: Effective 8 day: Reduced effect
2013	Delgado *et al.*[104]	*C. albicans*	CH, CHX	14 day	CH < CHX
2013	Pan *et al.*[105]	*E. faecalis*	CH, cold plasma therapy	7 day	Reduced but not eliminated

CH, Calcium hydroxide; CPCP, Camphorated parachlorophenol; CHX, Chlorhexidine; BAG, Bioactive glass; CMCP, Camphorated paramonochlorophenol; MC juice, Morinda citrifolia juice; TAB, Triple antibiotic paste.
Vitapex, Neo-Dental Int., Federal Way, WA, USA.

Table 8. Studies on the effect of $Ca(OH)_2$ mixed with vehicles or other agents using infected dentin models with human teeth

Year	Researcher	Microbial strains	Major ingredients (Mixed)	Period	Effect (+/−)	Note
2002	Sukawat & Srisuwan[114]	*E. faecalis*	CH, CHX, CMCP	7 day	+/−	CH, CHX: Ineffective CH + CMCP: Killed all
2005	Cwikla *et al.*[115]	*E. faecalis*	CH, IKI, Metapex	7 day	+/−	CH < CH + IKI < Metapex
2011	Lima *et al.*[116]	*E. faecalis*	CH, CMCP, CHX	7 day	+	
2012	Prabhakar *et al.*[117]	*E. faecalis*	CH, CHX, IKI	1, 7 day	+/−	CH < CH + CHX < CH + IKI

+/-, The result showed that $Ca(OH)_2$ alone was less effective than when mixed with others.
CH, Calcium hydroxide; CHX, Chlorhexidine; CMCP, Camphorated paramonochlorophenol; IKI, Iodine potassium iodide.
Metapex, Meta Biomed Co., Ltd., Cheongju, Korea.

Conclusions

Studies on the antimicrobial effect of $Ca(OH)_2$ have differed with regard to methodology, inoculum size and age, culture medium, and bacterial strains used. Furthermore, experimental conditions completely equivalent to the root canal environment could not be ensured. Therefore, the studies showed varied, even conflicting, results. Although some studies have supported the antimicrobial effect of $Ca(OH)_2$, others have questioned its efficacy.

In summary of the first part of this review, the antimicrobial effect of $Ca(OH)_2$ is related to the hydroxyl ions released in an aqueous environment, which affects cytoplasmic membranes, proteins, and the DNA of microorganisms. $Ca(OH)_2$ has a wide range of antimicrobial effects against common endodontic pathogens, but it is less effective against specific species such as *E. faecalis* or *C. albicans*. The addition of vehicles or other agents might contribute to the antimicrobial effect of $Ca(OH)_2$. Although it remains controversial, it seems that by mixing $Ca(OH)_2$ with CHX, the antimicrobial activity of $Ca(OH)_2$ can be increased.

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