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

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.³⁻⁰⁻¹⁶

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 (Ca²⁺) 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.²⁷ 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)₂.²⁸ 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

| Year | Researcher            | Test method | Microbial strains | Major ingredients | Period |
|------|-----------------------|-------------|-------------------|-------------------|-------|
| 1991 | Pavelić et al.³⁶      | ADT         | 2 (+)             | CH                | 1, 2 day |
| 1992 | Gencoglu & Kulekci²⁵ | 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.²¹     | 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         | 6 (+)             | 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.
test, and they found that Ca(OH)₂ was effective in killing bacteria. 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. Some studies have compared various Ca(OH)₂ pastes. 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. Blanschet et al. found that the higher the concentration of the Ca(OH)₂ paste was, the larger were the zones of inhibition observed.

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. 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.

On the other hand, some articles have presented results that bring into question the antimicrobial effect of Ca(OH)₂. Ohara et al. evaluated the antibacterial effect of several irrigants and found that Ca(OH)₂ was totally ineffective as saline. Studies that compared the effect of various agents showed that Ca(OH)₂ had only a weak effect. More recent studies that compared Ca(OH)₂ with other agents reported that Ca(OH)₂ was less effective than others.

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. However, different results were observed by Gomes et al. They reported that Ca(OH)₂ mixed with water or glycerin showed little or no effect, whereas Ca(OH)₂ mixed with camphorated monochlorophenol (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. However, in another study using a broth dilution test, Ca(OH)₂ with saline or glycerin showed antibacterial activity after 1 - 3 days. 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). This coincided with the result reported by Gangwar. 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.

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. 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. | DET | 6 | CH, CMCP, IKI, cresophene | 5 - 60 min |
| 1994 | Barbosa et al. | DET | 11 (+) | CH, CHX, NaOCl, H₂O₂, EDTA | 1 - 60 min, 1 wk |
| 2002 | Ferreira et al. | BDT | 4 | CH, CHX, CMCP | 2, 4 day |
| 2002 | Rosa et al. | BDT | 3 | CH, CHX, CMCP, FC | 2, 4 day |
| 2007 | Reddy & Ramakrishna | ADT | 26 (+) | CH, ZOE, CP, Metapex | |
| 2011 | Badr et al. | ADT/BDT | 1 (+) | CH, Liquorice | 2 day |
| 2012 | Adl et al. | ADT | 1 (+) | CH, antibiotics | 7 day |
| 2012 | Hegde et al. | ADT | 7 (+) | ApexCal, Metapex, Endoflas, ZOE | 1 - 2 day |
| 2012 | Mattigatti et al. | 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 monochlorophenol; IKI, Iodine potassium iodide; CHX, Chlorhexidine; NaOCl, sodium hypochlorite; H₂O₂, Hydrogen peroxide; EDTA, Ethylenediaminetetraacetic acid; FC, Formocresol; ZOE, Zinc oxide eugenol; CP, Camphorated phenol. Metapex, Meta Biomed Co., Ltd., Cheongju, Korea; ApexCal, Ivoclar Vivadent, Schaen, Liechtenstein; Endoflas, Sanlor Laboratory, Miami, FL, USA; MTAD, Dentsply Tulsa Dental Specialties, Tulsa, OK, USA.
authors reported that the antimicrobial effect of Ca(OH)$_2$ alone was significantly lower than that of Ca(OH)$_2$ mixed with CHX. 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. Oliveira et al. reported that the existence of serum or necrotic tissue slowed down the antimicrobial activity of intracanal medicaments. 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.

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. 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. The results of other experiments supported that Ca(OH)$_2$ had little or no antimicrobial effect. 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. Behnen et al. found that a thin preparation of Ca(OH)$_2$ was more effective than thick preparations. Fuss et al. reported that Ca(OH)$_2$ significantly reduced bacterial viability in dentinal tubules up to a depth of 300 µm. Lynne et al. found that a 10% Ca(OH)$_2$ paste effectively eliminated E. faecalis

| Year | Researcher       | Test method | Microbial strains | Major ingredients (Mixed) | Period | Effect (+/-) |
|------|------------------|-------------|-------------------|---------------------------|--------|--------------|
| 1997 | Siqueira & Uzeda | ADT         | 12 (+)            | CH, CHX, CMCP             | 7 day  | +/-          |
| 1998 | Siqueira & Uzeda | BDT         | 4 (+)             | CH, CMCP                  | 0 - 3 day | +       |
| 2001 | Estrela et al.   | DET         | 5 (+)             | CH, CHX, CMCP             | 0 - 7 day | +       |
| 2001 | Estrela et al.   | DET/ADT     | 4 (+)             | CH, PEG, CMCP             | 0 - 3 day | +       |
| 2002 | Gomes et al.     | ADT         | 11 (+)            | CH + 7 vehicles           | 0 - 7 day | +/-    |
| 2002 | Gomes et al.     | ADT         | 11 (+)            | CH + 7 vehicles           | 1 - 7 day | +/-    |
| 2003 | Lin et al.       | ADT         | 1 (+)             | CH, CHX                   | 7 day   | -        |
| 2005 | Vianna et al.    | BDT         | 5 (+)             | CH + 6 vehicles           | 0 - 7 day | +       |
| 2006 | Gomes et al.     | DET/ADT     | 5 (+)             | CH, CHX                   | 1 - 2 day | -       |
| 2007 | Ballal et al.    | ADT         | 1 (+)             | CH, CHX                   | 2, 3 day | +/-    |
| 2007 | Neelakantan et al.| ADT      | 3 (+)             | CH, CHX                   | 0 - 3 day | -       |
| 2008 | de Souza-Filho et al. | ADT | 6 (+)             | CH, CHX                   | 1 - 2 day | +/-    |
| 2009 | Turk et al.      | ADT         | 1 (+)             | CH + 7 vehicles           | 1 day   | +/-    |
| 2010 | Jhamb et al.     | ADT         | 1 (+)             | CH, CHX                   | 3 day   | -       |
| 2011 | Gangwar          | ADT         | 6 (+)             | CH + 4 vehicles           | 1,4,7 day | +/-    |
| 2012 | Pacios et al.    | 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.
dentinal tubules, hydroxyl ions from Ca(OH)2 must diffuse either group at 37°C. The result showed limited effect. Considerably more studies have been performed with human teeth than with bovine teeth.

The specimens were infected with Streptococcus faecalis 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. Some authors have reported that Ca(OH)2 showed almost no antimicrobial effect at all (Table 5). 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 eliminated by Ca(OH)2. Han et al. found that Ca(OH)2 with an aqueous vehicle could successfully reduce the bacteria in the dentinal tubules. The results of other experiments showed that the bacteria were almost eliminated by Ca(OH)2. Noetzl et al. and Valera et al. found that Ca(OH)2 effectively reduced microorganisms that remained even after cleaning and shaping procedures.

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. However, a significant reduction in the bacterial number was observed. Similar results were exhibited in other studies. Lana et al. said that a Ca(OH)2 paste induced a 70% elimination of E. faecalis for

### 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 | E. faecalis | CH, CMCP | 10 day | - |
| 1996 | Siqueira & Uzeda | E. faecalis + 2 | CH, CMCP | 1 wk | - |
| 2003 | Evans et al. | E. faecalis | CH, CHX | 1 wk | - |
| 2003 | Gomes et al. | E. faecalis | CH, CHX | 1 - 30 day | - |
| 2004 | Baker et al. | E. faecalis | CH, Betadine, IKI | 15 min, 1 day | - |
| 2004 | Sirén et al. | E. faecalis | CH, CHX, IKI | 1, 7 day | - |
| 2009 | Lin et al. | E. faecalis | CH, IKI | 2, 7 day | - |
| 2013 | Ordinola-Zapata et al. | E. faecalis biofilm | CH, CHX, TAB | 7 day | - |
| 2001 | Behnen et al. | E. faecalis | CH | 1 day | +/- |
| 2002 | Fuss et al. | E. faecalis | CH, IKI, Cu | 1 wk | + |
| 2003 | Lynne et al. | E. faecalis | CH, CHX | 1 day | + |
| 2003 | Siqueira et al. | C. albicans | CH, CHX, CMCP | 1 - 7 day | + |
| 2003 | Zehnder et al. | E. faecalis | CH, CHX, NaOCl | 1, 5 day | + |
| 2004 | Evanov et al. | 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%). Siqueira et al. and Zehnder et al. reported that Ca(OH)2 eliminated C. albicans and E. faecalis after 7 days. The results of this study were followed by several researchers who supported the ineffectiveness of Ca(OH)2 as an intracanal medicament. Some authors reported that Ca(OH)2 showed almost no antimicrobial effect at all (Table 5). 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 treated with Ca(OH)2. Han et al. found that Ca(OH)2 with an aqueous vehicle could successfully reduce the bacteria in the dentinal tubules. The results of other experiments showed that the bacteria were almost eliminated by Ca(OH)2. Noetzl et al. and Valera et al. found that Ca(OH)2 effectively reduced microorganisms that remained even after cleaning and shaping procedures. 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. However, a significant reduction in the bacterial number was observed. Similar results were exhibited in other studies. Lana et al. said that a Ca(OH)2 paste induced a 70% elimination of E. faecalis for
Delgado et al., Harrison et al., Kandaswamy et al., and Madhubala et al. reported 75%, 83%, 55%, and 59% of bacterial reduction, respectively.

Almyroudi et al., Oncag et al. and Pavaskar et al. said that the antimicrobial efficacy of Ca(OH)₂ might decrease over time.

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.

Cwikla et al. said that there were significant differences in the disinfecting ability among the three Ca(OH)₂ formulations. Lima et al. reported that all Ca(OH)₂-based medications used in the study were able to significantly reduce the colony-forming units (CFU) of E. faecalis in the extracted teeth.

Prabhakar et al. said that a combination of IKI or CHX with Ca(OH)₂ may be beneficial.

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. 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.
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.

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

| Year | Researcher           | Microbial strains | Major ingredients                  | Period   | Effect (+/-)                   | Note                        |
|------|----------------------|-------------------|------------------------------------|----------|-------------------------------|-----------------------------|
| 1997 | Tanriverdi et al.    | E. faecalis       | CH, cresophene, phenic acid        | 1, 3 day | CH < CPCP                      |                             |
| 2002 | Almyroudi et al.     | E. faecalis       | CH, CHX                            | 3, 8, 14 day | 3, 8 day: Effective 14 day: Reduced effect |                             |
| 2006 | Oncag et al.         | E. faecalis       | CH, CHX, propolis                 | 2, 10 day | CH < CHX                       |                             |
| 2007 | Krithikadatta et al. | E. faecalis       | CH, CHX, BAG                       | 1, 3, 5 day | CH < BAG < CHX                 |                             |
| 2009 | Lana et al.          | E. faecalis       | CH, CMCP                          | 7, 14 day | CH (70%) < CH + CMCP (100%)   |                             |
| 2010 | Delgado et al.       | E. faecalis       | CH, CHX                           | 14 day    | CH (75%) < CHX                 |                             |
| 2010 | Harrison et al.      | E. faecalis       | CH                                | 7 day     | 83% reduction                  |                             |
| 2010 | Kandaswamy et al.    | E. faecalis       | CH, CHX, MC juice                 | 1, 3, 5 day | CH (55%) < CHX (100%)         |                             |
| 2011 | Kayaoglu et al.      | E. faecalis       | CH, CHX, propolis                 | 1, 7 day  | CH < propolis < CHX            |                             |
| 2011 | Madhubala et al.     | E. faecalis       | CH, TAB, propolis                 | 1, 2, 7 day | CH (59%) < TAB < propolis     |                             |
| 2012 | Pavaskar et al.      | E. faecalis       | CH, Vitapex, linezolid            | 3, 8, 14 day | 3 day: Effective 8 day: Reduced effect |                             |
| 2013 | Delgado et al.       | C. albicans       | CH, CHX                           | 14 day    | CH < CHX                       |                             |
| 2013 | Pan et al.           | 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 | E. faecalis     | CH, CHX, CMCP            | 7 day  | +/-          | CH, CHX: Ineffective         |
|      |            |                   |                           |        |              | CH + CMCP: Killed all        |
| 2005 | Cwikla et al. | E. faecalis     | CH, IKI, Metapex          | 7 day  | +/-          | CH < CH + IKI < Metapex       |
| 2011 | Lima et al.  | E. faecalis     | CH, CMCP, CHX             | 7 day  | +            |                               |
| 2012 | Prabhakar et al.| 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.
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