REVIEW ARTICLE

Zahed Mohammadi[1,2]✉ DDS, MS, **Sousan Shalavi**[3] DMD

Is Chlorhexidine an Ideal Vehicle for Calcium Hydroxide? A Microbiologic Review

1. *Department of Endodontics, Hamedan University of Medical Sciences, Hamedan*
2. *Iranian Center for Endodontic Research, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
3. *Hamedan University of Medical Sciences, Hamedan, Iran*

Abstract: Microorganisms play a major role in the initiation and perpetuation of pulpal and periapical disease. In order to predictably achieve a bacteria-free root canal system, it is necessary to use intracanal medicaments. Calcium hydroxide [$Ca(OH)_2$] is the most common intracanal medicaments. It is effective against primary infections. However, its effectiveness against *Enterococcus (E.) faecalis* and *Candida (C.) albicans* is controversial. On the other hand, chlorhexidine (CHX) is a potent agent against *E. faecalis* and *C. albicans*. For this reason, the combination of $Ca(OH)_2$ and CHX has been suggested as an intracanal medicament. The purpose of this article was to review antimicrobial efficacy of $Ca(OH)_2$, CHX as well as their combination.

Keywords: Calcium Hydroxide, *Candida Albicans*, Chlorhexidine, *Enterococcus Faecalis*

✉ ***Zahed Mohammadi,*** *Department of Endodontics, Hamedan University of Medical Sciences, Hamedan, Iran.* ***Tel:*** *+98-9188729690,* ***Email:*** *zahed_mohammadi@yahoo.com*

Introduction

Micro-organisms play an essential role in the development and perpetuation of pulp and periapical diseases [1-3]. Elimination of micro-organisms from infected root canal systems (RCS) is a complicated task. Numerous measures have been described to reduce the number of micro-organisms from the root canal system, including the use of various mechanical instrumentation techniques, irrigation regimes and intra-canal medicaments. There is no definitive evidence in literature to show that mechanical instrumentation alone will predictably result in bacteria-free RCS's, which is not surprising given the complex anatomy of the RCS [4]. On the contrary, there is both *in vitro* and clinical evidence that mechanical instrumentation leaves significant portions of the root canal walls untouched [5]. Hence complete elimination of bacteria from the RCS by instrumentation alone is unlikely to be achieved [6]. It is assumed, but not demonstrated, that any pulp tissue left in the RCS can serve as a source of nutrient for bacteria. This however is likely to be for a very short time as any remnant pulp tissue is likely to necrose and be digested by the bacteria within 1-2 months, depending on whether the canal is open to the oral environment or not [7]. Furthermore, tissue remnants may impede the antimicrobial effects of root canal irrigants and medicaments. Therefore, some form of chemical irrigation and disinfection is necessary to remove tissue and other debris from the RCS and to kill remaining micro-organisms. Chemical treatment of the RCS can be arbitrarily divided into several phases, namely irrigants, rinses, and inter-appointment medicaments.

Definition of a medicament

Medicament is an effective antimicrobial agent which is placed inside the root canal between treatment appointments in order to destroy remaining micro-organisms and prevent the growth of any new arrivals [8].

Calcium hydroxide

Chemical composition and activity

Calcium hydroxide [$Ca(OH)_2$] was originally introduced to the field of endodontics by Herman as a direct pulp-capping agent [9]. It is a white odorless powder with the formula $Ca(OH)_2$, and a molecular weight of 74.08. It has low solubility in water (about 1.2 gL^{-1} at 25°C), which decreases

as the temperature rises [10]. It has been shown that it's the dissociation coefficient of $Ca(OH)_2$ of 0.17 that permits a slow, controlled release of both calcium and hydroxyl ions. The low solubility is a good clinical characteristic as a long period is necessary before it becomes soluble in tissue fluids when in direct contact with vital tissues [11]. It has a high pH (about 12.5-12.8) and is insoluble in alcohol. The material is chemically classified as a strong base, it main actions come from the ionic dissociation of Ca^{2+} and OH^- ions and their effect on vital tissues, generating the induction of hard tissue deposition and being antibacterial [9]. Estrela and Pesce [12] chemically analyzed the liberation of calcium and hydroxyl ions from $Ca(OH)_2$ pastes with vehicles of different acid-base and hydrosolubility characteristics by means of conductometer analysis of their solutions in connective tissue of a dog. The liberation of hydroxyl ions from the pastes can be demonstrated by the liberation of calcium ions and hydroxyl ions and the molecular weight of $Ca(OH)_2$. In $Ca(OH)_2$, the proportion of hydroxyl ions to calcium ions is 45.89% to 54.11%. $Ca(OH)_2$ in water has a thixotropic behavior. This means it will be very fluid when agitated [11]. When $Ca(OH)_2$ is exposed to carbon dioxide (CO_2) or carbonate ions (CO_3^-) in biological tissue, the dissociation of the chemical leads to formation of calcium carbonate ($CaCO_3$) and an overall consumption of Ca^{2+} ions. However, it has been shown that after 30 days of exposure to carbon dioxide, six preparations of $Ca(OH)_2$ still maintained a purportedly bactericidal pH within the root canal [10]. Estrela and Pesce [13] also showed that when saline vehicles were used with $Ca(OH)_2$ paste, the rate of formation of calcium carbonate was practically unaltered after 30 days and up to 60 days.

Mechanism of antimicrobial action

Antimicrobial activity of $Ca(OH)_2$ is related to the release of hydroxyl ions in an aqueous environment. Their lethal effects on bacterial cells are probably due to the following mechanisms: damage to the bacterial cytoplasmic membrane; protein denaturation; and damage to the DNA.

Although scientific evidence suggests that the 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 exposure to a strong base.

Adjustment of intracellular pH is influenced by different cellular processes such as: ***a)*** cellular metabolism, ***b)*** alterations in shape, mobility, adjustment of transporters and polymerization of cytoskeleton components, ***c)*** activation of cellular proliferation and growth, ***d)*** conductivity and transport through the membrane, and ***e)*** isosmotic cellular volume. Thus, many cellular functions can be affected by pH, including the enzymes that are essential to cellular metabolism [14].

Antimicrobial activity

Calcium hydroxide exerts antibacterial effects in the root canal system as long as the high pH is retained. In their *in vivo* study, Byström *et al.* found that root canals treated with $Ca(OH)_2$ had fewer bacteria than those treated with camphorated phenol or camphorated monochlorophenol [15]. They attributed this to the fact that $Ca(OH)_2$ can be packed into the root canal system allowing hydroxyl ions to be released over a long period of time. Stevens and Grossman [16] also showed $Ca(OH)_2$ to be effective in preventing the growth of microorganisms but to a limited extent when compared to CMCP, stressing the necessity of direct contact to achieve antibacterial effect. Sjogren *et al.* demonstrated that a 7-day usage of $Ca(OH)_2$ medicament was sufficient to reduce canal bacteria to a level that gave a negative culture [17]. In a study to evaluate the effect of electrophoretically activated $Ca(OH)_2$ on bacterial viability in dentinal tubules, Lin *et al.* found that treatment with electrophoresis was significantly more effective than pure $Ca(OH)_2$ in depths of 200-500 micrometres [18]. Specimens treated with electrophoretically activated $Ca(OH)_2$ showed no viable bacteria in dentinal tubules to a depth of 500 micrometres from the root canal space within 7 days. Portenier *et al.* showed that *E. faecalis* cells in their exponential growth phase were the most sensitive to $Ca(OH)_2$ paste and were killed between 3 sec and 10 min [19]. Cells in stationary phase were more resistant and living cells could be recovered in 10 min. However, cells in starvation phase were the most resistant and were not totally eliminated during the 10-min test period.

By contrast, several studies have attested to

the inefficacy of $Ca(OH)_2$ in eliminating bacterial cells. DiFiore *et al.* found that $Ca(OH)_2$ had no antibacterial effect as a paste, or as the commercial preparation Pulpdent when used against *S. Sanguis* [20]. These findings were confirmed by a further study [21].

Haapasalo and Ørstavik reported that a $Ca(OH)_2$ paste (Calasept) failed to eliminate, even superficially, *E. faecalis* in dentinal tubules [22]. Safavi *et al*. demonstrated that *Enterococcus (E.) faecium* remained viable in dentinal tubules after relatively extended periods of $Ca(OH)_2$/saline mixture treatment [23]. Ørstavik and Haapasalo observed that $Ca(OH)_2$ can take up to 10 days to disinfect dentinal tubules infected by facultative bacteria [24]. Siqueira and Uzeda demonstrated that $Ca(OH)_2$ mixed with saline was ineffective in eliminating *E. faecalis* and *E. faecium* inside dentinal tubules even after 1 week of contact [25]. Weiger *et al.* showed that the viability of *E. faecalis* in infected root dentine was not affected by $Ca(OH)_2$ [26]. In a polymerase chain reaction (PCR) study to evaluate the effect of root canal obturation with or without prior $Ca(OH)_2$ or 2% chlorhexidine (CHX) on the persistence of bacterial DNA in infected dentinal tubules, Cook *et al.* found that 2% CHX treatment followed by obturation was more effective in removing *E. faecalis* DNA than placement of $Ca(OH)_2$ or immediate obturation [27]. Ballal *et al*. found that in failed root canal treatments, 2% CHX gel may be a more effective intracanal medicament than $Ca(OH)_2$ paste against *E. faecalis* [28]. Krithikadatta *et al.* showed that as an intracanal medicament, %2 CHX gel alone was more effective against *E. faecalis* when compared to $Ca(OH)_2$ [29].

Waltimo *et al*. found that *C. albicans* cells were highly resistant to $Ca(OH)_2$ [30]. Siqueira *et al.* investigated the antifungal ability of several medicaments against *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. parapsilosis*, and *S. cerevisiae* [31]. Whereas the paste of $Ca(OH)_2$ in CPMC/glycerin showed the most pronounced antifungal effects, $Ca(OH)_2$ in glycerin or CHX and CHX in detergent also showed antifungal activity that was much lower than the paste of $Ca(OH)_2$ in CPMC/glycerin. In another study, the *in vitro* susceptibility of *C. albicans* to various irrigants and medicaments showed that NaOCl, hydrogen peroxide, and CHX digluconate were effective against *C. albicans* even when significantly diluted [32]. Aqueous $Ca(OH)_2$ had no activity. When maintained in direct contact with *C. albicans* cells, $Ca(OH)_2$ paste and CPMC were effective in killing this microorganism. The antifungal effectiveness of CPMC was also shown by a study that investigated the effectiveness of several intracanal medications on *C. albicans* harvested inside root canals, observing that CPMC was the most effective, followed by $Ca(OH)_2$/CPMC paste.

A further study evaluated the effectiveness of 4 intracanal medicaments in disinfecting the root dentin of bovine teeth experimentally infected with *C. albicans*. Infected dentin cylinders were exposed to 5 different medications: $Ca(OH)_2$/glycerin, $Ca(OH)_2$/0.12% CHX digluconate, $Ca(OH)_2$/CPMC/glycerin, and 0.12% CHX digluconate/zinc oxide. Specimens were left in contact with the medicaments for 1 hour, 2 days, and 7 days. The specimens treated with $Ca(OH)_2$/CPMC/glycerin paste or with CHX/zinc oxide paste were completely disinfected after 1 hour of exposure. $Ca(OH)_2$/glycerin paste only consistently eliminated *C. albicans* infection after 7 days of exposure. $Ca(OH)_2$ mixed with CHX was ineffective in disinfecting dentin even after 1 week of medicament exposure. Of the medicaments tested, the $Ca(OH)_2$/CPMC/glycerin paste and CHX digluconate mixed with zinc oxide were the most effective in eliminating *C. albicans* cells from dentinal specimens.

Chlorhexidine gluconate

Structure and mechanism of action

Chlorhexidine consists of two symmetric 4-cholorophenyl rings and two biguanide groups connected by a central hexamethylene chain [33]. CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism [34]. Its efficacy is due to the interaction of the positive charge of the molecule and the negatively charged phosphate groups on the microbial cell walls [34], thereby altering the cells' osmotic equilibrium. This increases the permeability of the cell wall, which allows the CHX molecule to penetrate into the bacterial cell. CHX is a base and is stable as a salt. The most common oral preparation, CHX gluconate, is water-soluble and, at physiologic

pH, it readily dissociates and releases the positively charged CHX component [34]. At low concentration (such as 0.2%), low molecular weight substances-specifically potassium and phosphorous-will leak out. A higher concentrations (*e.g.* 2%), CHX is bactericidal and precipitation of cytoplasmic contents occurs which results in cell death [34].

Antimicrobial activity

Delany *et al.* evaluated 0.2% CHX-gluconate in infected root canals [35]. Bacteriologic samples were obtained before, during, immediately after and 24 hours after instrumentation, irrigation and medication either with CHX-gluconate or with sterile saline. There was a highly significant reduction in the number of microorganisms in the CHX-treated specimens after instrumentation and irrigation. Oncag *et al.* evaluated the antibacterial properties of 5.25% sodium hypochlorite (NaOCl), 2% CHX and 0.2% CHX plus 0.2% cetrimide [Cetrexidin (GABA Vebas, San Giuliano Milanese, Italy)] after 5 minutes and 48 hours in extracted human teeth after the canals had been infected by *E. faecalis* [36]. The 2% CHX and Cetrexidin were significantly more effective on *E. faecalis* than the 5.25% NaOCl at both time periods.

Zamany *et al.* examined the effects of adding a 2% CHX rinse to the conventional treatment protocol [37]. Their results showed that cultivable bacteria were retrieved at the conclusion of the first visit in one of the CHX cases whereas seven of the 12 control cases without CHX showed growth; this difference was statistically significant. Siqueira *et al.* compared the effectiveness of 2.5% sodium hypochlorite and 0.12% CHX as irrigants in reducing the cultivable bacteria in infected root canal systems with apical periodontitis [38]. They found that the two solutions had comparable effects in eliminating bacteria and they suggested that both could be used as irrigants. This result is supported by other studies [39]

In a randomized clinical trial, Manzur *et al.* assessed the antibacterial efficacy of intracanal medication with $Ca(OH)_2$, 2% CHX gel, and a combination of both ($Ca(OH)_2$/CHX) in teeth with chronic apical periodontitis [40]. Findings revealed that the antibacterial efficacies of $Ca(OH)_2$, CHX, and a mixture of $Ca(OH)_2$/CHX were comparable.

Zerella *et al.* investigated the effect of a slurry of $Ca(OH)_2$ mixed in aqueous 2% CHX versus aqueous $Ca(OH)_2$ alone on the disinfection of the root canal system of root filled teeth that required root canal re-treatment because the canals had become infected again [41]. Their results indicated that a mixture of 2% CHX and a $Ca(OH)_2$ slurry is as efficacious as aqueous $Ca(OH)_2$ on the disinfection of infected root filled teeth.

Tanomaru *et al.* evaluated the effect of biomechanical preparation with 5% NaOCl, 2% CHX and physiological saline irrigating solutions and $Ca(OH)_2$ dressing in the root canals of dogs' teeth that contained bacterial endotoxin [42]. They found that biomechanical preparation with the irrigating solutions did not inactivate the endotoxin but the $Ca(OH)_2$ intracanal dressing did inactivate the effects induced by the endotoxin *in vivo*.

Waltimo *et al.* evaluated the susceptibility of seven strains of *C. albicans* to four disinfectants, namely IKI, CHX-acetate (0.5%), NaOCl (5% and 0.5%), and $Ca(OH)_2$ [30]. All *C. albicans* strains tested showed similar susceptibility to these medicaments. They were highly resistant to $Ca(OH)_2$ but the NaOCl and IKI killed all cells within 30 seconds and the CHX-acetate showed complete killing after 5 minutes. Combinations of disinfectants were either equally or less effective than the more effective component of the pair tested.

Siqueira *et al.* also investigated the antifungal activity of several medicaments against *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. parapsilosis*, and *S. cerevisiae* [34]. $Ca(OH)_2$ mixed with CPMC/glycerin as a paste showed the most pronounced antifungal effects. $Ca(OH)_2$ in glycerin, Ca(OH)2 with CHX, and CHX in detergent had less antifungal activity. Ferguson *et al.* sought to determine the *in vitro* susceptibility of *C. albicans* to various irrigants and medicaments [32]. The minimum inhibitory concentrations of NaOCl, hydrogen peroxide, CHX-digluconate, and aqueous $Ca(OH)_2$ were determined. Their results revealed that NaOCl, hydrogen peroxide, and CHX-digluconate were effective against *C. albicans* even when significantly diluted. However, aqueous $Ca(OH)_2$ had no anti-fungal activity.

On the whole, it seems that when used in identical concentrations, the antibacterial effects of CHX and NaOCl are similar. CHX is an

effective antifungal agent and its efficacy is significantly less than NaOCl.

CHX and Calcium hydroxide

Chemical viewpoints

Combined use of CHX and $Ca(OH)_2$ in the root canal may generate excessive reactive oxygen species, which may potentially kill various root canal pathogens [43]. Furthermore, it has been demonstrated that the alkalinity of $Ca(OH)_2$ when mixed with CHX remained unchanged [44].

Antimicrobial activity

In a study by Almyroudi *et al.*, all of the CHX formulations used, including a CHX/CH 50:50 mix, were efficient in eliminating *E. faecalis* from the dentinal tubules with a 1% CHX gel working slightly better than the other preparations [45]. These findings were corroborated by Gomes *et al.* [46] in bovine dentine and Schafer and Bossmann [47] in human dentine where 2% CHX gel had greater activity against *E. faecalis*, followed by CHX/CH and then CH used alone.

In a study using agar diffusion, researchers could not demonstrate any additive antibacterial effect by mixing CH powder with 0.5% CHX and they showed that the CHX had a reduced antibacterial action [44]. However, CH did not lose its antibacterial properties in such a mixture. This may be due to the deprotonation of CHX at a pH greater than 10, which reduces its solubility and alters its interaction with bacterial surfaces as a result of the altered charge of the molecule. In an *in vitro* study using human teeth, Ercan *et al.* [48] showed 2% CHX gel was the most effective agent against *E. faecalis* inside dentinal tubules, followed by a CH/2% CHX mix, whilst CH alone was totally ineffective, even after 30 days. The 2% CHX gel was also significantly more effective than the CH/2% CHX mix against *C. albicans* at seven days, although there was no significant difference at 15 and 30 days. CH alone was completely ineffective against *C. albicans*. These results were further validated by another *in vivo* study using primary teeth. A 1% CHX-gluconate gel, both with and without CH, was more effective against *E. faecalis* than CH alone over a 48-hour period [49].

Schafer and Bossnamm reported that 2% CHX-gluconate was significantly more effective against *E. faecalis* than CH used alone, or a mixture of the two [47]. This was also confirmed by Lin *et al.* [50] although in a study by Evans *et al.* [51] using bovine dentine, 2% CHX with CH was shown to be more effective than CH in water. In an animal study, Lindskog *et al.* reported that teeth dressed with CHX for four weeks had reduced inflammatory reactions in the periodontium (both apically and marginally) and less root resorption [52]. Waltimo *et al.* [30] reported that 0.5% CHX-acetate was more effective at killing *C. albicans* than saturated CH, while CH combined with CHX was more effective than CH used alone. The high pH of CH was unaffected when combined with CHX in this study. Another study evaluated the effectiveness of 2% CHX solution mixed with CH against *C. albicans* and found that combining these agents was beneficial [53].

Conclusion

1. Antimicrobial activity of $Ca(OH)_2$ is related to the release of hydroxyl ions in an aqueous environment.
2. $Ca(OH)_2$ seems to be ineffective against *E. faecalis* and *C. albicans*, and therefore has no efficacy in retreatment cases.
3. CHX has a wide range of activity against both Gram positive and Gram negative bacteria.
4. CHX is an effective antifungal agent especially against *C. albicans*.
5. Mixing CHX with $Ca(OH)_2$ may enhance its antimicrobial activity.

Conflict of Interest: 'none declared'.

References

[1] Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. Oral Surg Oral Med Oral Pathol. 1965;20:340-9.

[2] Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res. 1981;89(6):475-84.

[3] Sundqvist G. Ecology of the root canal flora. J Endod. 1992;18(9):427-30.

[4] Hess W. Anatomy of root canals in the teeth of the permanent dentition. New York: William Wood and Co; 1925. pp.36-9.

[5] Peters OA, Laib A, Gohring TN, Barbakow F. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. J Endod. 2001;27(1):1-6.

[6] Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res. 1981;89(4):321-8.

[7] Jansson L, Ehnevid H, Lindskog S, Blomlof L. Development of periapical lesions. Swed Dent J. 1993;17(3):85-93.

[8] Weine F. Endodontic Therapy. 6th Edition: St. Louis: CV Mosby; 2004. pp. 226-228

[9] Hermann B. Calcium hydroxid als Mittelzurn, Behandeln und Fullen von Wurzelkanalen [Thesis]. Wurzburg; 1920.

[10] Farhad A, Mohammadi Z. Calcium hydroxide: a review. Int Dent J. 2005;55(5):293-301.

[11] Spangberg LSW, Haapasalo M. Rationale and efficacy of root canal medicaments and root filling materials with emphasis on treatment outcome. Endodontic Topics. 2002;2(1):35-58.

[12] Estrela C, Pesce HF. Chemical analysis of the liberation of calcium and hydroxyl ions from calcium hydroxide pastes in connective tissue in the dog. Part I. Braz Dent J. 1996;7(1):41-6.

[13] Estrela C, PESCE HF. Chemical Analysis of the Formation of Calcium Carbonate and its Influence on Calcium Hydroxide Pastes in Connective Tissue of the Dog-Part II. Braz Dent J. 1997;8(1):49-53.

[14] Siqueira JF, Jr., Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J. 1999;32(5):361-9.

[15] Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol. 1985;1(5):170-5.

[16] Stevens RH, Grossman LI. Evaluation of the antimicrobial potential of calcium hydroxide as an intracanal medicament. J Endod. 1983;9(9):372-4.

[17] Sjögren U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short term intracanal dressing. Int Endod J. 1991;24(3):119-25.

[18] Lin S, Tsesis I, Zukerman O, Weiss EI, Fuss Z. Effect of electrophoretically activated calcium hydroxide on bacterial viability in dentinal tubules–in vitro. Dental Traumatol. 2005;21(1):42-5.

[19] Portenier I, Waltimo T, Orstavik D, Haapasalo M. The susceptibility of starved, stationary phase, and growing cells of Enterococcus faecalis to endodontic medicaments. J Endod. 2005;31(5):380-6.

[20] DiFiore PM, Peters DD, Setterstrom JA, Lorton L. The antibacterial effects of calcium hydroxide apexification pastes on Streptococcus sanguis. Oral Surg Oral Med Oral Pathol. 1983;55(1):91-4.

[21] Siqueira JF, Jr., Lopes HP, de Uzeda M. Recontamination of coronally unsealed root canals medicated with camphorated paramonochlorophenol or calcium hydroxide pastes after saliva challenge. J Endod. 1998;24(1):11-4.

[22] Haapasalo M, Ørstavik D. In vitro Infection and of Dentinal Tubules. J Dent Res. 1987;66(8):1375-9.

[23] Safavi KE, Spangberg LS, Langeland K. Root canal dentinal tubule disinfection. J Endod. 1990;16(5):207-10.

[24] Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol. 1990;6(4):142-9.

[25] Siqueira JF, Jr., de Uzeda M. Disinfection by calcium hydroxide pastes of dentinal tubules infected with two obligate and one facultative anaerobic bacteria. J Endod. 1996;22(12):674-6.

[26] Weiger R, de Lucena J, Decker HE, Lost C. Vitality status of microorganisms in infected human root dentine. Int Endod J. 2002;35(2):166-71.

[27] Cook J, Nandakumar R, Fouad AF. Molecular- and culture-based comparison of the effects of antimicrobial agents on bacterial survival in infected dentinal tubules. J Endod. 2007;33(6):690-2.

[28] Ballal V, Kundabala M, Acharya S, Ballal M. Antimicrobial action of calcium hydroxide, chlorhexidine and their combination on endodontic pathogens. Aust Den J. 2007;52(2):118-21.

[29] Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. J Endodo. 2007;33(12):1473-6.

[30] Waltimo T, Ørstavik D, Siren E, Haapasalo M. In vitro susceptibility of Candida albicans to

four disinfectants and their combinations. Int Endod J. 1999;32(6):421-9.

[31] Siqueira JF, Jr., Rocas IN, Magalhaes FA, de Uzeda M. Antifungal effects of endodontic medicaments. Aust Endod J. 2001;27(3):112-4.

[32] Ferguson JW, Hatton JF, Gillespie MJ. Effectiveness of intracanal irrigants and medications against the yeast Candida albicans. J Endod. 2002;28(2):68-71.

[33] Greenstein G, Berman C, Jaffin R. Chlorhexidine. An adjunct to periodontal therapy. J Periodontol. 1986;57(6):370-7.

[34] Athanassiadis B, Abbott P, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. Aust Endod J. 2007;52:S64-S82.

[35] Delany GM, Patterson SS, Miller CH, Newton CW. The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. Oral Surg Oral Med Oral Pathol. 1982;53(5):518-23.

[36] Önçağ Ö, Hoşgör M, Hilmioğlu S, Zekioğlu O, Eronat C, Burhanoğlu D. Comparison of antibacterial and toxic effects of various root canal irrigants. Int Endod J. 2003;36(6):423-32.

[37] Zamany A, Safavi K, Spångberg LSW. The effect of chlorhexidine as an endodontic disinfectant. Oral Surg, Oral Med, Oral Pathol, Oral Radio and Endodontology. 2003;96(5):578-81.

[38] Siqueira JF, Jr., Rocas IN, Paiva SS, Guimaraes-Pinto T, Magalhaes KM, Lima KC. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104(1):122-30.

[39] Ercan E, Ozekinci T, Atakul F, Gul K. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: in vivo study. J Endod. 2004;30(2):84-7.

[40] Manzur A, González AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod. 2007;33(2):114-8.

[41] Zerella JA, Fouad AF, Spångberg LSW. Effectiveness of a calcium hydroxide and chlorhexidine digluconate mixture as disinfectant during retreatment of failed endodontic cases. Oral Surg, Oral Med, Oral Pathol, Oral Radio and Endodo. 2005;100(6):756-61.

[42] Tanomaru JM, Leonardo MR, Tanomaru Filho M, Bonetti Filho I, Silva LA. Effect of different irrigation solutions and calcium hydroxide on bacterial LPS. Int Endod J. 2003;36(11):733-9.

[43] Yeung SY, Huang CS, Chan CP, Lin CP, Lin HN, Lee PH, et al. Antioxidant and pro-oxidant properties of chlorhexidine and its interaction with calcium hydroxide solutions. Int Endod J. 2007;40(11):837-44.

[44] Haenni S, Schmidlin PR, Mueller B, Sener B, Zehnder M. Chemical and antimicrobial properties of calcium hydroxide mixed with irrigating solutions. Int Endod J. 2003;36(2):100-5.

[45] Almyroudi A, Mackenzie D, McHugh S, Saunders W. The effectiveness of various disinfectants used as endodontic intracanal medications: an in vitro study. J Endod. 2002;28(3):163-7.

[46] Gomes B, Souza S, Ferraz C, Teixeira F, Zaia A, Valdrighi L. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. Int Endod J. 2003;36(4):267-75.

[47] Schafer E, Bossmann K. Antimicrobial efficacy of chlorhexidine and two calcium hydroxide formulations against Enterococcus faecalis. J Endod. 2005;31(1):53-6.

[48] Ercan E, Dalli M, Dulgergil CT. In vitro assessment of the effectiveness of chlorhexidine gel and calcium hydroxide paste with chlorhexidine against Enterococcus faecalis and Candida albicans. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;102(2):e27-31.

[49] Önçag Ö, Gogulu D, Uzel A. Efficacy of various intracanal medicaments against Enterococcus faecalis in primary teeth: an in vivo study. JClin Pediatr Dent. 2006;30(3):233-7.

[50] Lin YH, Mickel AK, Chogle S. Effectiveness of selected materials against Enterococcus faecalis: part 3. The antibacterial effect of calcium hydroxide and chlorhexidine on Enterococcus faecalis. J Endod. 2003;29(9):565-6.

[51] Evans MD, Baumgartner JC, Xia T. Efficacy of calcium hydroxide: chlorhexidine paste as an intracanal medication in bovine dentin. J Endod. 2003;29(5):338-9.

[52] Lindskog S, Piercs A, Blomiöf L. Ghlorhexidine as a root canal medicament for treating inflammatory lesions in the periodontal space. Dent Traumatol. 1998;14(4):186-90.

[53] Al-Nazhan S, Al-Obaida M. Effectiveness of a 2% chlorhexidine solution mixed with calcium hydroxide against Candida albicans. Aust Endod J. 2008;34(3):133-5.