Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part II. in vivo studies

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The first part of this study reviewed the characteristics of calcium hydroxide (Ca(OH)_2) and summarized the results of in vitro studies related to its antimicrobial effects. The second part of this review covers in vivo studies including human clinical studies and animal studies. The use of Ca(OH)_2 as an intracanal medicament represented better histological results in animal studies. However, human clinical studies showed limited antimicrobial effects that microorganisms were reduced but not eliminated through the treatment, and that some species had resistance to Ca(OH)_2. Most of clinical outcome studies supported that there is no improvement in healing of periapical lesions when Ca(OH)_2 was applied between appointments. Further studies are required for the antimicrobial effects of Ca(OH)_2, and search for the ideal material and technique to completely clean infected root canals should be continued. (Restor Dent Endod 2015;40(2):97-103)

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

Introduction

The first part of this study reviewed the characteristics of calcium hydroxide (Ca(OH)_2) and selected literatures dealing with in vitro antimicrobial effect of Ca(OH)_2 using antimicrobial susceptibility tests and infected dentin models. It was concluded that the antimicrobial effect of Ca(OH)_2 is related to the release of hydroxyl ions, and that Ca(OH)_2 had antimicrobial effect against common endodontic pathogens while it had limited effect against specific species such as Enterococcus faecalis (E. faecalis) or Candida albicans (C. albicans). The addition of other agents seems to be able to improve the antimicrobial effect of Ca(OH)_2.

The purpose of this article is to review the antimicrobial effect of Ca(OH)_2 as an intracanal medicament in root canal treatment. The second part of this review will cover in vivo studies including human clinical studies and animal studies. A PubMed search was performed to identify 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.
**Review**

**In vivo studies**

1. Animal studies (Table 1)

Stevens and Grossman used adult cats to determine the effectiveness of Ca(OH)_2 as an intracanal medicament.\(^1\) Three of four canines were treated with Ca(OH)_2 solution or camphorated paramonochlorophenol (CMCP), and the remaining was used as control with no treatment. *E. faecalis* persisted in Ca(OH)_2 solution-treated canals for 3 weeks, whereas CMCP eliminated the infection after a single treatment.

Katebzadeh *et al.* histologically and radiographically compared the periapical healing of the infected roots obturated in one-step or with prior Ca(OH)_2 disinfection.\(^2,3\) The result showed that 1 week Ca(OH)_2 disinfection before obturation results in significantly less periapical inflammation. Leonardo *et al.* performed histopathological evaluation of the repair of periapical tissues of dog’s teeth with induced chronic periapical lesions after dressing with Ca(OH)_2 at different time periods.\(^4\) The animals were sacrificed at 7, 15 and 30 days and the teeth specimens were examined with a light microscope. They concluded that more advanced repair was seen at 30 days compared to the other two periods. Tanomaru Filho *et al.* compared the periapical lesions of the dog teeth received immediate canal filling or 15 days Ca(OH)_2 dressing.\(^5\) There was better histological repair in the groups with the Ca(OH)_2 dressing than the groups with immediate obturation. This small number of animal studies were the only ones that gave histopathological evidences.

De Rossi *et al.* evaluated healing of experimentally induced chronic apical lesions in dogs at 35, 75 and 120 days after instrumentation with or without Ca(OH)_2/chlorhexidine (CHX) paste intracanal dressing by standard radiographs.\(^6\) Radiographs taken at 120 days showed that the treatment with intracanal medication resulted in a significant reduction in mean size of the apical lesions in comparison to single-session treatment.

2. Human clinical studies - bacterial culture method (Table 2)

Most clinical studies performed bacterial sampling from the canals for bacterial culture at three stages: S1, after initial access, to confirm that the canal is infected at the time of treatment; S2, after the cleaning and shaping procedure is complete, immediately before canal medication; S3, when the canal is re-accessed later and the medication has been removed. Some researchers additionally culture bacteria at certain days after cleaning the canal from medicaments (S4). S1 is essentially positive in 100% of the cases in teeth with periradicular lesion since they routinely have infected canals. Cleaning and shaping results in an extensive reduction in bacterial count

| Year | Researcher | Test method (animal) | Major ingredient | Period | Result |
|------|------------|----------------------|------------------|--------|--------|
| 1983 | Stevens & Grossman\(^1\) | Culture (cat) | CH solution, slurry, Pulpdent, CMCP | 21 day | CH solution: ineffective CH slurry, Pulpdent: limited effect |
| 1999 | Katebzadeh *et al.*\(^2\) | Histopathology (dog) | CH | 1 wk (sacrificed 6 mon) | CH: less inflammation |
| 2000 | Katebzadeh *et al.*\(^3\) | Radiograph (dog) | CH | 1 wk (x-ray 6 mon) | CH: fewer failed cases more improved cases |
| 2002 | Leonardo *et al.*\(^4\) | Histopathology (dog) | CH + CMCP | 30 day: Better results |
| 2002 | Tanomaru Filho *et al.*\(^5\) | Histopathology (dog) | CH + CMCP | 15 day (sacrificed 210 day) | Better results than immediate obturation |
| 2005 | De Rossi *et al.*\(^5\) | Radiograph (dog) | CH + CHX | 15 day (x-ray 30, 75 and 120 day) | 120 day: Reduction of lesion size |

CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; CHX, Chlorhexidine. Pulpdent (Pulpdent Corp., Watertown, MA, USA).

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In 1985, Byström et al. evaluated the bactericidal efficacy of Ca(OH)$_2$, camphorated phenol (CP), and CMCP as root canal dressings. The mechanical cleansing was completed at the first appointment and a sample was taken with paper points (S2). Then the intracanal medicament was placed into the canal. At the second appointment, 1 month later for canals filled with Ca(OH)$_2$ paste, the intracanal dressing was removed and a sample was collected from the root canal (S3). After 2 to 4 days, the root canal was filled with saline and another bacteriological sample was taken (S4). Bacteria could not be recovered from any of these canals after they had been dressed with Ca(OH)$_2$ paste for 1 month. In samples taken 2 to 4 days after the dressing had been removed, bacteria were recovered from 1 of 35 root canals. Similar studies were performed with other researchers, and they also showed the antibacterial effect of Ca(OH)$_2$ paste used as a long-term intracanal dressing material.\textsuperscript{8-11}

Several studies indicated that the treatment with intracanal medicaments could markedly affect the diversity and quantity of cultivable microorganisms in infected canals, with some groups of microorganisms being more resistant to treatment than others. Peters et al. evaluated microorganism in root canals with or without Ca(OH)$_2$ medication.\textsuperscript{12} They said that although Ca(OH)$_2$ was placed in the prepared canals, the number of positive canals had increased in the period between visits. Ca(OH)$_2$ could not totally prevent regrowth of bacteria. Zerella et al. said that complete disinfection was not achieved but all cases that initially harbored Enterococcus species were successfully disinfected.\textsuperscript{13} Chu et al. found that Gram-positive facultative anaerobic cocci tend to predominate than Gram-negative obligate anaerobic rods after treatment.\textsuperscript{14} Oncag et al. said that CHX was more effective than Ca(OH)$_2$ alone against E. faecalis. In the study of Sinha et al., Ca(OH)$_2$ showed limited efficacy against facultative anaerobes and Candida species, but was effective against obligate anaerobes.\textsuperscript{15,16} Molander et al. treated teeth with Ca(OH)$_2$ for 2 months and found that there was no increased antimicrobial effect of Ca(OH)$_2$ even if it was left for longer periods in the root canal.\textsuperscript{17}

Some authors state that Ca(OH)$_2$ has little effect in reducing microorganisms. Manzur et al. found that bacterial growth and CFU counts decreased significantly from S1 to S2, but differences between S2 to S3 were not statistically significant for Ca(OH)$_2$, CHX, or combination of both.\textsuperscript{18} Vianna et al. reported that after chemomechanical preparation, no improvement was achieved by 7 days of intracanal dressing.\textsuperscript{19}

### 3. Human clinical studies - molecular method (Table 3)

In the middle of 2000s, a major shift had occurred in microbiology from studies based on culturing to ones that utilize molecular techniques. The reasons for this

### Table 2. Human clinical studies on the antimicrobial effect of Ca(OH)$_2$ by bacterial culture

| Year   | Researcher         | Major ingredient          | Period   | Protocol       | Effect   |
|--------|--------------------|---------------------------|----------|----------------|----------|
| 1985   | Byström et al.     | CH, CP, CMCP              | 1 mon    | S2-S3-S4       | +        |
| 1985   | Safavi et al.      | CH, IKI                    |          | S2-S3          | +        |
| 1991   | Sjögren et al.     | CH                        | 1 wk     | S1-S2-S3-S4    | +        |
| 1997   | Barbosa et al.     | CH, CMCP, CHX             | 1 wk     | S2-S3-S4       | +        |
| 1999   | Molander et al.    | CH                        | 2 mon    | S1-S2-S4       | +/-      |
| 2000   | Shuping et al.     | CH                        | 7 - 200 day | S1-S2-S3     | +        |
| 2002   | Peters et al.      | CH                        | 1 mon    | S1-S2-S3       | +/-      |
| 2005   | Zerella et al.     | CH, CHX, CH+CHX           | 7 - 10 day | S1-S2-S3-S2-S3 | +/-      |
| 2006   | Chu et al.         | CH, Ledermix, Septomixine | 1 wk     | S1-S3          | +/-      |
| 2006   | Oncag et al.       | CH, CHX, CH+CHX           | 2 day    | S1-S2-S3       | +/-      |
| 2007   | Manzur et al.      | CH, CHX, CH+CHX           | 1 wk     | S1-S2-S3       | -        |
| 2007   | Vianna et al.      | CH, CHX, CH+CHX           | 1 wk     | S1-S2-S3       | -        |
| 2013   | Sinha et al.       | CH, CHX, CH+CHX           | 1 wk     | S1-S2-S3       | +/-      |

+/-. The result showed a limited effect.

CH, Calcium hydroxide; CP, Camphorated phenol; CMCP, Camphorated paramonochlorophenol; IKI, Iodine potassium iodide; CHX, Chlorhexidine.
shift included the increase in sensitivity, accuracy, and efficiency of molecular techniques, as well as the realization that many microorganisms that populate the oral cavity may not have been cultivated. The most popular techniques were those based on polymerase chain reaction (PCR) amplification of the 16S or other ribosomal DNA sequences. Because of the low sensitivity of culture methods, a negative result does not imply that the canal has been rendered sterile. It could mean that bacterial numbers have reached a threshold that is undetectable by culture and that may be compatible with periradicular tissue healing. Molecular methods can detect far fewer cells than culture and have the potential to demonstrate the actual effectiveness of a given antibacterial protocol and accurately establish the number of bacterial cells that characterize the threshold below which a satisfactory outcome can still be achieved. However, as with any other method, molecular methods have limitations. Most assays are qualitative, and detect only a few target species and cannot detect unexpected species. Enzymes in the sample could suppress the amplifying reaction. Molecular methods cost higher than conventional culture methods. Lastly, they might detect dead microorganisms that we could not distinguish from living ones.\(^20\)

Tang et al. evaluated the efficacy of Ca(OH)\(_2\) or Septomixine (Septodont, St. Maur Des Fosses, France) in eliminating residual intracanal bacteria during interappointment using molecular methods.\(^21\) The PCR results showed that 25 of 31 examined canals were positively detected with residual microorganisms after instrumentation, irrigation with saline, and 1 week medication with either Ca(OH)\(_2\) or Septomixine. They suggested that the conventional 1 week medication might not effectively inhibit residual bacterial growth in all root canals during interappointment intervals. In the studies of Sakamoto et al. and Siqueira et al., significant reductions in bacterial counts were observed between S1 and S2, and S1 and S3.\(^22,23\) However, no statistically significant difference was observed between S2 and S3 samples with regard to the number of cases yielding negative cultures or quantitative bacterial reduction.

De Souza et al. used checkboard DNA-DNA hybridization technique to examine the effects of Ca(OH)\(_2\) therapy on the microorganisms.\(^24\) Significant differences in the microbiota from baseline to post-therapy were found, and the results indicated that conventional therapy with Ca(OH)\(_2\) results in the reduction of pathogenic species associated with pulp necrosis. However, it did not eliminate the whole spectrum of microorganisms. The studies of Siqueira et al. reported that 1 week use of Ca(OH)\(_2\), mixed with CHX or CMCP significantly reduced the bacterial counts.\(^25,26\) Rocas and Siqueira said that bacterial diversity was clearly reduced after chemomechanical preparation and after the supplemental effects of the intracanal medication.\(^27,28\) Most taxa was completely eradicated, or at least reduced in levels. However, detectable levels of rRNA, which is highly likely to represent viable cells, were still observed in S2 and S3 samples. Ito et al. investigated the root canal microbiota of primary teeth with apical periodontitis and the \textit{in vivo} antimicrobial effects of Ca(OH)\(_2\)/CHX paste using both bacterial culture and checkerboard DNA-DNA hybridization technique.\(^29\) The results of both methods exhibited that the prevalence of samples that were positive for the presence of microorganisms did not change, although the overall number of bacteria was dramatically diminished compared with initial contamination. The recent study showed that the number of positive cases decreased between S2 and S3 samples.\(^20\)

### Table 3. Human clinical studies on the antimicrobial effect of Ca(OH)\(_2\) by molecular methods

| Year | Researcher | Major ingredient | Period | Protocol | Effect |
|------|------------|-----------------|--------|----------|--------|
| 2004 | Tang et al.\(^21\) | CH, Septomixine | 1 wk | S1-S3 | - |
| 2005 | de Souza et al.\(^24\) | CH | 2 wk | S1-S3 | +/- |
| 2007 | Sakamoto et al.\(^22\) | CH + CMCP | 1 wk | S1-S2-S3 | - |
| 2007 | Siqueira et al.\(^23\) | CH | 1 wk | S1-S2-S3 | - |
| 2007 | Siqueira et al.\(^25\) | CH + CMCP | 1 wk | S1-S2-S3 | + |
| 2007 | Siqueira et al.\(^26\) | CH + CHX | 1 wk | S1-S2-S3 | + |
| 2010 | Rocas et al.\(^27\) | CH + CMCP | 1 wk | S1-S2-S3 | +/- |
| 2011 | Ito et al.\(^29\) | CH + CHX | 2 wk | S1-S4 | +/- |
| 2011 | Rocas et al.\(^28\) | CH, CH + CMCP | 1 wk | S1-S2-S3 | +/- |
| 2013 | Paiva et al.\(^30\) | CH + CHX | 1 wk | S1-S2-S3 | +/- |

+/-, The result showed a limited effect.  
CH, Calcium hydroxide; CMCP, Camphorated paramonochlorophenol; CHX, Chlorhexidine.
4. Clinical outcome studies (Table 4)

One-visit root canal treatment has been considered ideal for the teeth with vital pulp. On the other hand, root canal treatment for teeth with necrotic pulps associated with a periapical lesion remains controversial. Several studies have shown that it is impossible to eliminate whole microorganisms even after cleaning, shaping and irrigation with disinfectants. Furthermore, a number of above clinical studies emphasizes that Ca(OH)$_2$ cannot completely reduce the remaining bacteria.

Trope et al. evaluated radiographic healing of teeth with apical periodontitis treated in one-visit or two-visit with or without Ca(OH)$_2$ as an intracanal medicament. The Periapical Index (PAI) Scoring Method was used to compare differences in periapical status during one-year follow-up evaluation. It was shown that the use of Ca(OH)$_2$ before obturation resulted in 10% increase in healing rates.

On the other hand, several studies have concluded that one-visit treatment is equally effective or even more effective than multiple-visit treatment. Weiger et al. performed a prospective study to explore the influence of Ca(OH)$_2$ as an inter-appointment dressing on the healing of periapical lesions associated with pulpless teeth. In both treatment groups, the success rate within an observation time of 5 years exceeded 90%. A statistically significant difference between the two groups was not detected. Peters and Wesselink found no significant differences in healing of periapical radiolucency between the teeth that were treated in one-visit and two-visits with Ca(OH)$_2$ for 4 weeks. Waltimo et al. stated that Ca(OH)$_2$ dressing between the appointments did not show significant effect in treatment outcome. In a randomized clinical trial, Molander et al. assessed 2 year clinical and radiographic outcomes of one- and two-visit root canal treatments and found similar healing results.

In summary, there is still considerable controversy about the effect of the use of Ca(OH)$_2$ on the clinical outcome, while the majority of the studies supported that there was no significant difference in healing of periapical lesions between the treatment modalities.

Conclusions

To summarize the results of these in vivo studies, the use of Ca(OH)$_2$ as an intracanal medicament represented better histological results in animal studies. However, human clinical studies showed limited antimicrobial effects which microorganisms were reduced but not completely eliminated after treatments, and some species had resistance to Ca(OH)$_2$. The majority of clinical outcome studies supported that there was no improvement in healing of periapical lesions when Ca(OH)$_2$ was applied between appointments. It was concluded that one-visit treatment is as effective as two-visit treatment with interappointment intracanal medicaments. These may imply that there may be no correlation between the healing of endodontic lesions and the presence and absence of positive bacterial culture.

This series of articles reviewed currently available laboratory and clinical evidences addressing the antimicrobial effects of Ca(OH)$_2$ as an intracanal medicament. Although some studies have supported the effectiveness of Ca(OH)$_2$ as an intracanal medicament, others have questioned its efficacy or indicated that other agents should be mixed to improve its antimicrobial activity. It seems that the limitations of antimicrobial effect of Ca(OH)$_2$ has been suggested following the development of experimental methods. Further scientific investigations are required for better antibacterial protocols and sampling techniques to ensure that bacteria have been reliably eradicated prior to obturation, and it is necessary to assess the clinical outcomes related to intracanal medicaments. Also, further studies are required to assess the antimicrobial effect of Ca(OH)$_2$, and search for an ideal material and/or technique to completely clean infected root canals should be continued.

| Year | Researcher | Test method | Interappointment period | Follow-up period | Result |
|------|------------|-------------|-------------------------|-----------------|--------|
| 1999 | Trope et al.$^{34}$ | Radiograph | 1 wk | 1 yr | + |
| 2000 | Weiger et al.$^{35}$ | Radiograph | 7 - 47 day | 5 yr | - |
| 2002 | Peters and Wessellink$^{36}$ | Radiograph | 4 wk | 4.5 yr | - |
| 2005 | Waltimo et al.$^{37}$ | Radiograph | 1 wk | 1 yr | - |
| 2007 | Molander et al.$^{38}$ | Radiograph | 1 wk | 2 yr | - |

+, The use of Ca(OH)$_2$ resulted better outcome; -, The results did not show significant difference.
Conflict of Interest: No potential conflict of interest relevant to this article was reported.

References

1. Stevens RH, Grossman LI. Evaluation of the antimicrobial potential of calcium hydroxide as an intracanal medicament. J Endod 1983;9:372-374.

2. Katebzadeh N, Hupp J, Trope M. Histological periapical repair after obturation of infected root canals in dogs. J Endod 1999;25:364-368.

3. Katebzadeh N, Sigurdsson A, Trope M. Radiographic evaluation of periapical healing after obturation of infected root canals: an in vivo study. Int Endod J 2000;33:60-66.

4. Leonardo MR, Silveira FF, Silva LA, Tanomaru Filho M, Utrilla LS. Calcium hydroxide root canal dressing. Histopathological evaluation of periapical repair at different time periods. Braz Dent J 2002;13:17-22.

5. Tanomaru Filho M, Leonardo MR, da Silva LA. Effect of irrigating solution and calcium hydroxide root canal dressing on the repair of apical and periapical tissues of teeth with periapical lesion. J Endod 2002;28:295-299.

6. De Rossi A, Silva LA, Leonardo MR, Rocha LB, Rossi MA. Effect of rotary or manual instrumentation, with or without a calcium hydroxide/1% chlorhexidine intracanal dressing, on the healing of experimentally induced chronic periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:628-636.

7. Byström 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;1:170-175.

8. Safavi KE, Dowden WE, Introcaso JH, Langeland K. A comparison of antimicrobial effects of calcium hydroxide and iodine-potassium iodide. J Endod 1985;11:454-456.

9. Sjögren U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991;24:119-125.

10. Barbosa CA, Gonçalves RB, Siqueira JF Jr, De Uzeda M. Evaluation of the antibacterial activities of calcium hydroxide, chlorhexidine, and camphorated paramonochlorophenol as intracanal medicament. A clinical and laboratory study. J Endod 1997;23:297-300.

11. Shuping GB, Ørstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000;26:751-755.

12. Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. Int Endod J 2002;35:13-21.

13. Zerella JA, Fouad AF, Spångberg LS. Effectiveness of a calcium hydroxide and chlorhexidine digluconate mixture as disinfectant during retreatment of failed endodontic cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:756-761.

14. Chu FC, Leung WK, Tsang PC, Chow TW, Samaranayake LP. Identification of cultivable microorganisms from root canals with apical periodontitis following two-visit endodontic treatment with antibiotics/steroid or calcium hydroxide dressings. J Endod 2006;32:17-23.

15. Onçag O,Cogulu D, Uzel A. Efficacy of various intracanal medicaments against Enterococcus faecalis in primary teeth: an in vivo study. J Clin Pediatr Dent 2006;30:233-237.

16. Sinha N, Patil S, Dodwad PK, Patil AC, Singh B. Evaluation of antimicrobial efficacy of calcium hydroxide paste, chlorhexidine gel, and a combination of both as intracanal medicament: an in vivo comparative study. J Conserv Dent 2013;16:65-70.

17. Molander A, Reit C, Dahlén G. The antimicrobial effect of calcium hydroxide in root canals pretreated with 5% iodine potassium iodide. Endod Dent Traumatol 1999;15:205-209.

18. Manzur A, González AM, Pozos A, Silva-Hertzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod 2007;33:114-118.

19. Vianna ME, Horz HP, Conrads G, Zaia AA, Souza-Filho FJ, Gomes BP. Effect of root canal procedures on endotoxins and endodontic pathogens. Oral Microbiol Immunol 2007;22:411-418.

20. Hargreaves KM, Cohen S, Berman LH. Pathways of the pulp. 10th ed. St. Louis: Elsevier Mosby; 2011. p 568-571.

21. Tang G, Samaranayake LP, Yip HK. Molecular evaluation of residual endodontic microorganisms after instrumentation, irrigation and medication with either calcium hydroxide or Septomixine. Oral Dis 2004;10:389-397.

22. Sakamoto M, Siqueira JF Jr, Roçâs IN, Benyo Y. Efficacy of various intracanal medicaments against Endoçoccus faecalis in primary teeth: an in vivo study. J Endod Dent Traumatol 2005;22:411-418.

23. Sakamoto M, Siqueira JF Jr, Roçâs IN, Benyo Y. Efficacy of various intracanal medicaments against Endoçoccus faecalis in primary teeth: an in vivo study. J Endod Dent Traumatol 2007;9:19-23.

24. de Souza CA, Teles RP, Souto R, Chaves MA, Colombo AP. Endodontic therapy associated with calcium hydroxide
as an intracanal dressing: microbiologic evaluation by the checkerboard DNA-DNA hybridization technique. J Endod 2005;31:79-83.

25. Siqueira JF Jr, Magalhães KM, Roçâs IN. Bacterial reduction in infected root canals treated with 2.5% NaOCl as an irrigant and calcium hydroxide/camphorated paraamonochlorophenol paste as an intracanal dressing. J Endod 2007;33:667-672.

26. Siqueira JF Jr, Paiva SS, Roçâs IN. Reduction in the cultivable bacterial populations in infected root canals by a chlorhexidine-based antimicrobial protocol. J Endod 2007;33:541-547.

27. Roçâs IN, Siqueira JF Jr. Identification of bacteria enduring endodontic treatment procedures by a combined reverse transcriptase-polymerase chain reaction and reverse-capture checkerboard approach. J Endod 2010;36:45-52.

28. Roçâs IN, Siqueira JF Jr. In vivo antimicrobial effects of endodontic treatment procedures as assessed by molecular microbiologic techniques. J Endod 2011;37:304-310.

29. Ito IY, Junior FM, Paula-Silva FW, Da Silva LA, Leonardo MR, Nelson-Filho P. Microbial culture and checkerboard DNA-DNA hybridization assessment of bacteria in root canals of primary teeth pre- and post-endodontic therapy with a calcium hydroxide/chlorhexidine paste. Int J Paediatr Dent 2011;21:353-360.

30. Paiva SS, Siqueira JF Jr, Roçâs IN, Carmo FL, Leite DC, Ferreira DC, Rachid CT, Rosado AS. Clinical antimicrobial efficacy of NiTi rotary instrumentation with NaOCl irrigation, final rinse with chlorhexidine and interappointment medication: a molecular study. Int Endod J 2013;46:225-233.

31. Ashkenaz PJ. One-visit endodontics. Dent Clin North Am 1984;28:853-863.

32. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-328.

33. Ørstavik D, Kerekes K, Molven O. Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. Int Endod J 1991;24:1-7.

34. Trope M, Delano EO, Ørstavik D. Endodontic treatment of teeth with apical periodontitis: single vs. multivisit treatment. J Endod 1999;25:345-350.

35. Weiger R, Rosendahl R, Löst C. Influence of calcium hydroxide intracanal dressings on the prognosis of teeth with endodontically induced periapical lesions. Int Endod J 2000;33:219-226.

36. Peters LB, Wesselinck PR. Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms. Int Endod J 2002;35:660-667.

37. Waltimo T, Trope M, Haapasalo M, Ørstavik D. Clinical efficacy of treatment procedures in endodontic infection control and one year follow-up of periapical healing. J Endod 2005;31:863-866.

38. Molander A, Warfvinge J, Reit C, Kvist T. Clinical and radiographic evaluation of one- and two-visit endodontic treatment of asymptomatic necrotic teeth with apical periodontitis: a randomized clinical trial. J Endod 2007;33:1145-1148.