International Journal of Dental and Health Sciences
Volume 01,Issue 03

Original Article

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF THREE CALCIUM HYDROXIDE PASTES WITH ZINC OXIDE EUGENOL USED AS ROOT CANAL FILLING MATERIALS IN PRIMARY TEETH :AN IN VITRO STUDY

Nimitt Baldota[1],Vikas Bendgude[2]
1.Post Gradutate,Department Of Pediatric Dentistry,Dr.D.Y.Patil Dental College & Hospital,India
2.Professor & Head,Department Of Pediatric Dentistry,Dr.D.Y.Patil Dental College & Hospital,India

ABSTRACT:

Objective: The present study aimed to evaluate and compare three different formulation of calcium hydroxide materials commonly used for filling the root canals of primary teeth for antimicrobial efficacy against some of the microorganisms commonly found in infected root canals. Study design: In this experimental in vitro study four root canal filling materials were tested for antimicrobial efficacy against five microbial strains using the agar diffusion method.

Results: Zinc oxide eugenol paste exhibited the strongest antimicrobial potential followed by metapex, calasept plus, vitapex

Conclusions: All the test filling materials demonstrated varying antimicrobial activity against the microorganisms tested. Zinc oxide eugenol paste and materials containing zinc oxide were found to be more effective against the microorganisms compared to materials with calcium hydroxide .

Key words: antimicrobial efficacy, root filling materials, primary teeth

INTRODUCTION:

A successful endodontic therapy depends on elimination of microorganisms from the root canal system and prevention of relapse, achieved by biomechanical cleaning and shaping, and followed by three-dimensional filling of the root canal space. The root canal system, especially of primary teeth owing to their complex anatomy conventional canal debridement procedures may not be effective completely to eliminate the bacteria from root canal system.(1) The literature have shown that mechanical instrumentation with antibacterial irrigation will only render 50-70% of infected canals free of microorganisms, depending on which irrigants are used. Viable bacteria can be recovered from the root canals after being

*Corresponding Author Address: Dr Nimitt Baldota,Post Gradutate,Department Of Pediatric Dentistry,Dr.D.Y.Patil Dental College & Hospital,India Email: nimittbaldota@gmail.com

treated by an effective disinfection process. Therefore, especially in pediatric endodontics, difficulties in antimicrobial control require the use of root canal filling pastes with broad antimicrobial activity. [2]

An ideal root canal filling material should be bactericidal or at least not encourage microbial growth. The antimicrobial activity of filling materials is considered crucial in primary teeth to inhibit the growth of microorganisms and prevent the failure of root canal therapy. [3]

The search for the ideal filling material for primary teeth, over the years is still going on a number of materials have been trialed with varying degrees of success.

Zinc oxide-eugenol cement (ZOE) has long been used as a root canal filling material for deciduous teeth, and in a survey conducted in 1997 it was cited as the preferred root canal filling material by 94% of the chairpersons of predoctoral pediatric dental programs in the Unites States.[1]

Nevertheless, ZOE cannot be considered the ideal root canal filling material because it tends to resorb at a slower rate than the roots of the deciduous teeth . Concerns about these shortcomings of ZOE led to a search for alternative root canal filling materials for deciduous teeth. [1]

Calcium hydroxide is considered to have some antimicrobial action and is easily resorbed when inadvertently forced beyond the root apex. The antimicrobial action of calcium hydroxide is associated with its ionic dissociation into Ca^{++} and OH^{-} ions. The vehicle used in the formulation of the root canal filling paste plays a fundamental role in this process because it influences the speed of ionic dissociation. [1]

Hence the trend is shifting towards the use of calcium hydroxide based pastes as a root canal filling material.

The current study is an attempt to evaluate and compare three calcium hydroxide pastes with zinc oxide eugenol for antimicrobial efficacy against microrganisms commonly isolated from infected root canals using agar diffusion assay technique which has been extensively studied for checking antimicrobial activity of root canal filling materials.

MATERIAL AND METHODS:

In this in-vitro study four root canal fillng materials mentioned below were tested against the were tested for antimicrobial efficacy against five microbial strains. These materials were trialed, as they have been successfully used for filling the primary tooth canals, are easy to manipulate and readily available commercial product. The following four root canal materials were tested in the present study.

1. Zinc oxide mixed with eugenol (Vishal Dento care Pvt.Ltd.Sarkhej District, Ahmedabad, Gujarat- India)
2. Calasept Plus (Nordia Dental Products Co. Ltd, Greece)

3. Vitapex (Neo Dental Chemical Products Co. Ltd, Tokyo, Japan)

4. Metapex (Meta Biomed Co.Ltd Korea.)

The powder liquid ratio of zinc oxide eugenol for the test were standardized according to the formula given by Tchaou et.al[7] An electronic balance and micro-pipette were used to measure the exact amount of powder and liquid to be dispensed. The zinc oxide used was 1 scoop equivalent to 0.2 g and eugenol liquid 7 drops equivalent to 0.07 cc it was mixed on a dry sterile glass slab using a cement spatula at room temperature, just before they were assayed for Agar diffusion assay.

The mixture was back loaded into a Eppendorf pipette and kept ready to be dispensed into the prescribed area on the Agar diffusion plate. The three calcium hydroxide based materials were available in premixed syringes.

The following microbial strains (ATCC) were used are listed below with their numbers

Enterococcus faecalis (ATCC 10541; gram positive coccus), Streptococcus mutans (ATCC 25175; gram positive coccus), Escherichia coli (ATCC 10538; gram negative bacillus), Staphylococcus aureus (ATCC 6538; gram positive coccus), Candida albicans (ATCC 10231; fungi).

Agar Diffusion Assay:

Sensitivity testing of the root canal filling materials against the isolated obtained was performed in Muller-Hinton agar by cup-plate method of Kirby - Bauer.

The Muller- Hinton agar plates were dried and 4 wells of 4mm diameter and depth of 3mm were made in the agar plates using sterile agar puncher.

Using a sterile swab, the entire surface of the agar plate was swabbed 3 times to ensure even distribution of the inoculum and to obtain lawn culture.

The four root canal fillings materials Zinc oxide eugenol, Calasept Plus, Vitapex and Metapex were tested in each plate. Each of the four fillings materials was filled in each well in the agar plate, these plates were incubated at 37° C overnight.

The diameter zones of inhibition in millimeter around the filling materials were measured after 24 hours for aerobic isolates and after 48 hours for anaerobic isolates.

The diameters of the zones of microbial inhibition around each test material were measured in millimeters (HiAntibiotic Zone Scale, HIMEDIA). The experiment was repeated trice for each strain and one observer measured the zones. The mean zone of inhibition for each material-microbial strain combination was then calculated.

Statistical analysis:

Statistical analysis was carried out by one-way ANOVA using SPSS (Statistical package for social sciences) version 13.0 with post-hoc tests to compare the statistical difference of antimicrobial

effects between the materials tested with each of the isolates of bacteria.

RESULTS:

The zones of inhibition produced by the test materials against the selected microorganisms were ranked arbitrarily into four categories as strong, medium, weak and noninhibitory according to the proportional distribution of the data ZOE was most inhibitory, followed by Metapex ,Vitapex and then Calasept Plus against E.faecalis . There was a significant difference in the inhibition assay between ZOE and Calasept Plus that is highly significant (p= 0.008). between Metapex and Calasept Plus that is significant (p= 0.006) between Vitapex and Calasept Plus that is highly significant (p= 0.005).

ZOE was most inhibitory, followed by Metapex which also showed strong inhibition then Calasept Plus followed by Vitapex which showed medium inhibition against S.aureus There was difference in the inhibition assay between ZOE and Calasept Plus that is significant (p= 0.010).between Metapex and Calasept Plus that is significant (p= 0.009).There was a significant difference in the inhibition assay between ZOE and Vitapex, that is highly significant (p= 0.001).

There was a significant difference in the inhibition assay between Metapex and Vitapex, that is highly significant (p= 0.005).

ZOE was most inhibitory, followed by Metapex which also showed strong inhibition then Calasept Plus followed by Vitapex which showed medium inhibition against E.coli . There was a significant difference in the inhibition assay between ZOE and Calasept Plus that is(p= 0.008) between Metapex and Calasept Plus that is significant (p= 0.006).between ZOE and Vitapex that is highly significant (p= 0.001).

ZOE was most inhibitory, followed by Vitapex which also showed strong inhibition then Metapex followed by Calasept plus which showed medium inhibition against S.mutans. There was a significant difference in the inhibition assay between ZOE and Calasept Plus which is significant (p= 0.008) between Vitapex and Calasept Plus which is significant (p= 0.007).

ZOE was showed medium inhibitory action, rest all the three Metapex ,Vitapex and Calasept plus showed weak inhibition against C.albicans. There were no significant differences in the inhibition activities between the four materials tested on C.albicans

It was observed that ZOE showed strong inhibitory activity against Enterococcus faecalis ,Streptococcus mutans , Escherichia coli ,Staphylococcus aureus , and medium inhibition against Candida albicans .Calasept Plus the pure calcium hydroxide formulation showed strong inhibition against E.faecalis and E.coli , medium inhibition against S.aureus and S.mutans and least inhibition against Candida albicans . Vitapex showed strong

antibacterial activity in S.mutans, and E.faecalis and medium inhibition in E,coli and S.aureus weak inhibition against Candida Albicans Metapex showed strong inhibitory activity against Enterococcus faecalis ,Streptococcus mutans , Escherichia coli ,Staphylococcus aureus , and weak inhibition against Candida albicans.

DISCUSSION:

The incidence of dental caries in primary teeth is still high in spite of many preventive measures employed to prevent the decay, still exfoliation of pulpally involved primary teeth remains renders a problem and a challenge for the pediatric dentist.[9]

The tooth itself is a good space maintener, so to maintain it as a functional unit pulpectomy is one of the treatment modalities employed to retain the primary tooth in the dental arch**.** [1] The success of pulpectomy, depends on good instrumentation, irrigation, intracanal medication, and on antibacterial activity of the filling material. [9]

The understanding of the anatomy of primary roots before endodontic therapy is a primary requisite as root morphology of the primary teeth changes continuously because of primary tooth root resorption which causes the transportation of the apical foramen continuously, secondary dentin is deposited within the pulp canal system this deposition produces variations and alterations in the number and size of the root canals and it tortious nature complicates the endodontic therapy.[10]

The primary objective of the endodontic treatment of teeth with necrotic teeth and peri-apical lesions.[33,34] is elimination of infection and their end products particularly in primary teeth because the ample medullary bones spaces favor dissemination of infection and also the developing permanent tooth germ is very close to the roots of primary teeth .

Five microorganisms which are commonly isolated from infected root canals of primary teeth-Enterococcus faecalis Streptococcus mutans, Escherichia coli, Staphylococcus aureus , Candida albicans strains were used for testing four root canal filling materials in the current study.

Streptococci were present in 86.65 % cases, which is the consistent with the findings of Silva et al who reported 85% prevalence of streptococci in the necrotic pulp of human primary teeth and also comparable with the findings of Marsh and Largent who reported 82% prevalence. Enterococci were isolated in 26.66% of the cases in the study by Priya et.al and Rocas et.al found 33% prevalence in teeth with chronic peri-radicular lesions.[4] [20]

Staphyloccocus aureus was isolated in 5% cases by Cohen et.al and 13% by Priya et.al from the root canals of the primary teeth. [4][36]

The in-vitro method requires the filling materials to diffuse into the agar, the net inhibitory effect was a combination of

diffusion potential and antibacterial activity, and it allows direct comparsion of the materials against the tested microorganisms in the environment similar to the root canal system.[7]

The final outcome of the pulpectomy procedure depends on the quality of the root canal fillings materials, which can neutralize any remaining pulp tissuse and microorganisms.

The filling material most commonly used for primary teeth has been ZOE either alone or with a fixative and the success rate ranges from 65-86%.[11]

In this study, ZOE exhibited strong inhibitory action against four groups of bacteria except candida albicans, against which ZOE showed medium inhibitory activity it is similar to that of Bonow et.al which is contradictory to the results obtained in the study by Priya et.al [9]

Broisman et al, [55] Cox et al,[9] Grossman,[11] Pupo et al[12], Rahmat,[13] Candala & Pumarola and Pumarola et also observed that the sealers with Zinc oxide eugenol bases are those that have greater inhibitory effect against the microorganisms found in the root canals.

The antimicrobial activity of ZOE is attributed to the eugenol content of the material, Cox et al demonstrated that zinc oxide had no inhibitory effect and the addition of eugenol to zinc oxide retarted the growth of only the Gram Positive micro-organisms. The inclusion of the zinc acetate as a setting accelerator inhibited both Gram positive and Negative organisms. products.

Calasept Plus the pure calcium hydroxide formulation showed strong inhibition against E.faecalis and E.coli , medium inhibition against S.aureus and S.mutans and least inhibition against Candida albicans in our study.

In case of S.mutans Calasept plus has better result than Metapex . Calasept plus is better than Vitapex in the case of E.coli and S.aureus. This is in agreement with the results of Tchaou et al [7] who found that calcium hydroxide produced medium or medium strong inhibition.

The result of our study contradicts with that of the previous studies [5][28] in which they have mentioned calcium hydroxide exhibited weak antimicrobial activity against facultative/aerobic Gram positive, facultative aerobic Gram-negative organisms.

The result of our study also contradicts that calcium hydroxide failed to inhibit anaerobic bacteria, infact all the three calcium hydroxide based pastes have shown medium to strong antimicrobial activity respectively.

The results also differs from other studies by Difore et al, [24] Abdulkader et al, Siqueira and Gonclaves,[40] they demonstrated that calcium hydroxide was ineffective against several obligatory and facultative anaerobic bacteria. The weak inhibitory effect of calcium hydroxide in agar diffusion assay can be explained by the fact that blood or buffer present in

the agar media might have neutralized calcium hydroxide, a phenomenon that may also occur in vivo where blood and buffering systems are present.

Vitapex showed strong antibacterial activity in S.mutans, and E.faecalis and medium inhibition in E,coli and S.aureus weak inhibition against Candida Albicans, these finding differed from the results of Pabla[21] et al, Amorim[22], Tchaou et.al[7] according to those studies, Vitapex showed the least or no antibacterial activity.

Vitapex showed the lowest antimicrobial activity when compared to the other three root canal filling materials tested in this study.

Nurko and Garcia- Goday [33] studied the effectiveness of Vitapex in the root canal treatment of primary teeth the clinical procedure was deemed successful, on the basis of results obtained so the author rendered the use of vitapex as a root canal filling material.

Metapex showed the strongest antimicrobial activity when compared to the other two calcium hydroxide formulations in this study i.e Calasept plus and Vitapex .It showed strong inhibitory against facultative /aerobic Gram -negative bacteria, anaerobic Gram positive bacteria, and anaerobic Gram negative bacteria but showed weak inhibitory activity against facultative /aerobic Gram positive bacteria in comparsion to Calasept Plus which showed strong inhibitory activity against facultative /aerobic Gram positive bacteria in comparsion of all three calcium hydroxide based paste.[37]

Metapex showed better antimicrobial activity in this study as compared to other studies carried on it by various authors using Agar diffusion assay, the study also contradicts the results of S Reddy [2] study of antimicrobial efficacy of various root canal fillings material in primary teeth in which metapex was found ineffective against most microorganisms.

In this study the results obtained of Zinc oxide eugenol are comparable to that of Metapex which also has strong inhibition activity except against Candida Albicans this contradicts all the other studies carried out on Zinc oxide eugenol and Metapex in which metapex always showed weak inhibition or no inhibition.

The results of the studies should be further evaluated by carrying out in vivo conditions because very few studies are carried out in the literature of pediatric dentistry exclusively on bacterial strains isolated from infected primary teeth.

Most of the studies related to anti microbial activity of root canal filling materials have done using standardized bacterial strains (ATCC- American Type Culture Collection).

It is also possible that different results could be have been derived if other methods of the antimicrobial activity would have been considered for testing the root canal filling materials.

The mean zone of inhibition of the materials in this study cannot be

compared with previous studies because of the variability's bacterial strains, culture media, culture conditions,pH of substrates in the plate and incubation time in our study we incubated aerobic bacteria for 24 hours and anaerobic bacteria for 48 hours, then formulation of ZOE can be different from the other studies carried out, Calasept plus which consists of 41% pure calcium hydroxide was the commercial product in the paste form where else every other studies or investigations have used pure form of calcium hydroxide in the powder and liquid form .

Based on the results of this study, Zinc oxide eugenol showed superior antimicrobial activity against most of the organisms used in this study followed by Metapex, Calasept Plus, Vitapex in descending order.

Metapex showed very comparable results to Zinc Oxide Eugenol in the present study so we can infer that it can be an alternative to Zinc Oxide Eugenol as the root canal filling material in primary teeth.

CONCLUSION:

The following conclusions were inferred from the present study:

1) All the test filling materials showed varied antimicrobial activity against the microorganisms tested.

2) ZOE showed superior inhibitory activity against most of the organisms used in the study followed by Metapex, Calasept Plus and Vitapex in descending order.

3) Metapex can be used as an alternative to the Zinc oxide eugenol as the root canal filling material in the primary teeth.

It is difficult to draw conclusions based on in vitro evaluation of antimicrobial activity with the ATCC isolated organisms. It is well know that endodontic infection is polymicrobial in nature with complex flora. The effect of the test filling materials against a singe strain may not be effective against a mixed variety of infection .The use of artificial media also plays an important role in determining the study results .It is also possible that different findings might have been obtained if other methods of testing antimicrobial activity would have been employed.

REFERENCES:

1. Barja Ferenda. A systemic Review of root canal filing materials for deciduous teeth : Is their any alternative for Zinc Oxide- Eugenol ? ISRN Dentistry Volume 2011.

2. Reddy S. and Ramakrishna Y. Evaluation of Antimicrobial Efficacy of various root canal filling materials used in primary teeth : A Microbiological Study J Clin Pediatr Dent 31;195-199,2007.

3. Estrela Carlos , Toledo Airton. Antimicrobial Analysis of Different Root Canal Filling Pastes used in pediatric

dentistry by two experimental methods.Braz Dent J 17: 317-322, 2006.

4. Priya Harini M, Bhatt Sham S, Hedge Sundeep K. Comparative evaluation of bacteria potential of four root canal filling materials against microflora of infected non-vital primary teeth. J Clin Pediatr Dent 35:23-30, 2010.

5. Gangawar Anshul. Antimicrobial effectiveness of different preparations of calcium hydroxide Indian Journal of Dental Research,22;66-70, 2011.

6. Nelson Paulo, Assed Sada. Antibacterial activity of root canal filling materials for primary teeth: Zinc oxide Eugenol, Calen paste thickened with Zinc oxide ,Sealpex and EndoREZ"Braz Dent J 20;290-296,2009.

7. Tchaou Coll James A. In vitro inhibition of bacteria from root canals of primary teeth by various dental materials American Academy of Pediatric Dentistry – 17; 351-355, 1995.

8. Sapna Hegde, Priti Lala. In vitro evaluation of antimicrobial efficacy of primary root canal filling materials J Clin Pediatr Dent ; 37(1): 59-64 2012.

9. Cox ST, Hembree JH, Mcknight JP et al. The bactericidal potential of various endodontic materials for primary teeth .Oral Surg June 1978; 45(6): 947-54.

10. Fuks AB, Eidelman E, Pauker N. Root fillings with Endoflas in primary teeth: retrospective study.J Clin Pediatr Dent 2002; 27(1): 41-46.

11. Hibbard ED, Ireland HL. Morphology of the root canals of the primary molar teeth. J Dent Child 1957; 24: 250-57.

12. Payne RG, Kenny OJ, Johnston DH et al. Two-year outcome study of zinc oxide eugenol root canal treatment for vital primary teeth. J Can Dent Assoc 1993; 59:528-536.

13. American Academy of Pediatric Dentistry: Guidelines for pulp therapy for primary and young permanent teeth.Reference manual 2000-2001.

14. Ranly OM. Pulp therapy in primary teeth. A review and prospectus. Acta Odont Pediatr 1992; 3: 63-68.

15. Marsh SJ, Largent MD. A bacteriological study of the pulp canals of infected primary molars. J Dent Child 1967; 34: 460-470.

16. Leonardo MR, Rossi MA, Silva LAB, Ito IY, BonifacioKC EM. Evaluation of bacterial biofilm and microorganisms on the apical external root surface of human teeth. J Endod 2002; 28: 815-818.

17. Silva LAB, Nelson-Filho P, Faria G et al. Bacterial profile in primary teeth with necrotic pulp and periapical lesions. Braz Dent J 2006; 17(2): 144-48.

18. Tronstad L. Recent development in endodontic research. Scand J Dent Res 1992;100: 52-9.

19. Morse DR. Endodontic microbiology in the 1970s. Int Endod J 1981; 14: 69-79.

20. Assed S,· Ito IY, Leonardo MR, Silva LAB, Lopatin DE. Anaerobic microorganisms in root canals of human teeth with chronic apical periodontics detected by indirect immuunofluoroscence. Endod Dent Traumatol 1996; 12: 66-68.

21. Cohen MM, Joress SM, Calisti LP. Bacteriologic study of infected deciduous molars.Oral Surg Oral Med Oral Pathol 1960; 13(11): 1382-1386.

22. Stevens RH, Grossman LI. Evaluation of the antimicrobial potential of calcium hydroxide as an Intracanal medicament. J Endodon 1983; 9(9): 372-74.

23. DiFiore PM, Peters DO, Setterstrom JA, Lortan L. The antibacterial effects of calcium hydroxide apexification pastes on Streptococcus sanguis. Oral Surg 1983;55(1): 91-94.

24. Canalda C, Pumarola J. Bacterial growth inhibition produced by root canal sealer cements with calcium hydroxide base. Oral Surg, Oral Med, Oral Pathol 1989;68:99-102.

25. Pumarola J, Berastegui E~ Brau E, Canalda C, Jimenez de Anta MT. Antimicrobial activity of seven root canal sealers: results of agar diffusion and agar dilution tests. Oral Surg, Oral Med, Oral Pathol 1992; 74: 216-220.

26. Barkhordar RA. Evaluation of antimicrobial activity in vitro of ten root canal sealers on Streptococcus sanguis and Streptococcus mutans.Oral Surg, Oral Med,Oral Pathol 1989; 68: 770-772.

27. Tchaou WS, Turng BF, Minah GE, Coll JA. In vitro inhibition of bacteria from root canals of primary teeth by various dental materials.Pediatr Dent 1995; 17(5):351-355.

28. Akdeniz BG, Koparal E, Sen BH, Ates M, Denizci AA. Prevalence of candida albicans in oral cavities and root canals of children.J Dent Child 2002; 289-292.

29. Pazelli LC, Freitas AC, Ito IY, Souza-Gugelmin MC, Medeiros AS, Nelson-Filho Prevalence of microorganisms in root canals of human deciduous teeth with necrotic pulp and chronic periapical lesions.Pesqui Odontol Bras 2003 Oct-Dec;17(4):367-71.

30. Bauer A, W, Kirby W, Sherris JC and Turch M. Antibiotic susceptibility testing by standardized single disc method. Amer J Clin Pathol 1966; 45: 493.

31. Rocas IN, Siqueira JF, Santos KRN. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endodon 2004; 30(5): 315-320.

32. Gomes BP, Pinheiro ET, Gade-Neto CR et al. Microbiological examination of infected dental root canals. Oral Microbiol Immunol 2004 Apr; 19(2):71-6.

33. Siqueria J.F and Gonclaves R.R Antibacterial activities of root canal against selected anaerobic bacteria.J Endod 1996; 22: 890.

34. Pabla T, Gulati MS, Mohan U. Evaluation of antimicrobial efficacy of various root

canal filling materials for primary teeth.J Ind Soc Pedod Prev Dent 1997;15(4): 134-40.

35. Amorin LFG, Toledo OA, Estrela CRA, Decurcio DA, Estrela C. Antimicrobial analysis of different root canal filling pastes used in pediatric dentistry by two experimental methods. Braz Dent J 2006; 17(4): 231-236.

36. Nurko C, Garcia-Goday F. Evaluation of a calcium hydroxide iodoform paste(vitapex) in root canal therapy for primary teeth. J Clin Ped Dent 1999; 23: 289-294.

37. Estrela C, Estrela CRA, HoUanda ACB, Decurcio DA, Pecora JD. Influence of iodoform on antimicrobial potential of calcium hydroxide. J Appl Oral Sci 2006;14: 33-37.

38. Wright KJ, Barbosa SV, Araki K and Spanberg LSW. In vitro antimicrobial and cytotoxic effects of Kri paste and zinc oxide eugenol used in primary tooth pulpotomies. Ped Dent 1994; 16(2): 102-106.

39. Estrela C, Pimenta FC, Yoko I, Bammann LL. In vitro determination of direct antimicrobial effect of calcium hydroxide. J Endodon 1998; 24(1): 15-17.

40. Gomes BPFA, Ferraz CCR, Garrido FD et al. Microbial susceptibility to calcium hydroxide pastes and their vehicles. J Endodon 2002; 28(11): 758-761.