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Effects of calcium hydroxide paste in different vehicles on bacterial reduction during treatment of teeth with apical periodontitis

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Abstract

Objective: Calcium hydroxide, Ca(OH)2, is a common intracanal medicament. Ca(OH)2 powder can be mixed with different vehicles and used as a paste for temporary intracanal treatment. The vehicle may influence the dissociation of calcium hydroxide into ions. We sought to evaluate the level of pH and to quantitatively estimate the release of proteins, hydroxyproline, and phosphorus from pieces of radicular dentin kept in different Ca(OH)2 solutions.

Study design: Twenty-eight extracted incisors were maintained for 35 days in Ca(OH)2 aqueous solutions prepared in chlorhexidine digluconate, propylene glycol (PG), anesthetic solution, camphorated monochlorophenol (CMCP), and CMCP-PG. The control solution contained Ca(OH)2 without vehicle.

Results: The pH values changed little during the experiment. The concentrations of proteins, hydroxyproline, and phosphorus rose for all the solutions under study. Statistical analysis of the data from the control and the experimental groups revealed an increase in the concentration of proteins when chlorhexidine, anesthetic solution, and PG were used; a rise in hydroxyproline levels when CMCP-PG, CMCP, and PG solutions were used; and an increase in phosphorus when PG and chlorhexidine vehicles were used.

Conclusion: The test solutions with the root dentin remained alkaline. A release of proteins, hydroxyproline, and phosphorus was observed.

Keywords: Calcium hydroxide, Propylene Glycol, proteins, hydroxyproline.

Introduction

Endodontics is the prevention or treatment of apical periodontitis. Endodontic research has shown a cause-and-effect relationship between bacteria and apical periodontitis[1,2]. Because bacteria are the cause of apical periodontitis, it is logical that the elimination of bacteria would cure apical periodontitis. A tooth without periapical radiolucency has a higher endodontic success rate upon treatment than a tooth with a radiolucency.[3,4] Likewise, teeth that are obturated after a negative culture have a better prognosis than those obturated following a positive culture.[5,6] Although there is substantial evidence that a negative culture does not equate to bacteria free[7,8], there is a threshold level of bacteria below that gives a negative sample and a success rate similar to teeth treated without apical periodontitis. The current technique for bacterial reduction includes instrumentation, irrigation and an intracanal antimicrobial medication. Bystrom et al[6]. showed that each step leads to less bacteria and eventually a canal that does not sample bacteria. In a 5 yr follow-up study, root canal samples that gave a negative culture following these steps were recalled to evaluate healing. Ninety-five percent of these cases showed complete radiographic healing or a decrease in the radiolucency size.[10]

It has recently been demonstrated that non-setting Ca(OH)2 pastes inserted into root canals can dissociate and Ca2 diffuses through dentin and reaches the root surface.10 A considerable increase in Ca2 concentration was observed in the surrounding media. This increase was directly related to the duration of the test. However, no increase in the pH of the surrounding media and a nonsignificant reduction during the test period were observed. Consequently, non-setting Ca(OH)2 pastes released Ca2 through root dentin but did not increase the pH of the media, which indicates that OH did not diffuse out of the dentin. The permeability and buffering capacity of dentin are key factors affecting the diffusion of OH^{-} through root dentin andcouldexplainthesefindings.[10]

Regretfully, there aren't many research discussing potential changes to dentin's chemical makeup following the use of Ca(OH)2 pastes in root canal therapy. In this work, we quantitatively assessed the in vitro release of proteins, hydroxyproline, and phosphorus from dentin fragments submerged in Ca(OH)2 solutions that were combined with various carriers and exposed to varying times. Additionally, the pH was assessed. To enable the determination of pH and chemical constituent concentration, this in vitro model uses a concentrated solution rather than a Ca(OH)2 paste.

Methodology

For this investigation, twenty-eight freshly excised maxillary incisors with single canals were chosen. We looked for cracks or fractures in the teeth. Gauze was used to get rid of the remaining soft tissue, and tap and distilled water were used to clean the teeth. Once used, they were kept in storage at -15°C. 7-8 and 11-12 Greys curettes (Hu-Friedy, Chicago, Ill.) were used to remove the cementum. At the cementoenamel junction, a high-speed handpiece with a water spray was used to remove the dental crown. Using files (for necrotic pulp) or barbed broaches (for vital pulp), the remaining pulp tissue in the canal was extracted. By inserting a No. 15 K-file until it was visible at the apex and then deducting 1 mm from this length, the working length of the root was ascertained. A step-back technique11 was used to instrument the canal up to a No. 60 apical master file. Following each instrumentation, 1 mL of distilled water was used to irrigate the root canal.

Solutions

Because Ca(OH)2 is usually used as a paste, it was necessary to prepare the test solutions so that they were of a more fluid consistency for pH measurements and chemical analysis. Therefore, the following test solutions were prepared in distilled water by mixing them with Ca(OH)2 to a final concentration of 0.1 mol/L:

1. Chlorhexidine digluconate to a final concentration of 0.07% (ICN Biomedicals Inc, Aurora, Ohio)
2. PG to a final concentration of 7.9 mmol/L (Anedra Lab, Buenos Aires, Argentina)
3. Carticainechlorhydrate to a final concentration of 0.05% (anesthetic solution: Totalcaina Forte 4%; Microsules-Bernabo S.A. Lab, Buenos Aires, Argentina)
4. CMCP to a final concentration of 20 mmol/L (Farmadental Lab, Buenos Aires, Argentina)
5. CMCP to a final concentration of 20 mmol/L combined with PG to a final concentration of 7.9 mmol/L
6. Distilled water and an aqueous mixture of 0.1 mol/L Ca(OH)2 was used as the control.

Determinations

A digital pH metre (Broadley-Yames Corp., Irvine, CA) was used to measure pH in small amounts (sensitivity, 0.01 pH units). Prior to use, the metre was calibrated to pH levels of 7 and 4 using standard buffer solutions. The electrode was submerged in a 10-µL sample on a slide for ten seconds in order to determine pH. Between readings, the electrode was cleaned with distilled water and dried with a cloth. Ca(OH)2 solutions with or without vehicles as well as vehicle solutions alone were subjected to pH assessments. To ascertain the total proteins in each solution—aside from those containing CMCP, which tampers with the Folin reagent—we applied the Lowry method12 to a 50-µL sample. The Bradford method 13 was applied to the same sample volume in this instance. The hy- droxyproline level was determined by using the method of Jammal et al14 on a 50-µL sample. Phosphorus was quantified by using the phosphomolybdate method on a 10-µL sample; to that end, a commercial kit (Wiener Lab, Rosario, Argentina) was used. Interferences be- tween Ca(OH)2 and vehicles with colorimetric reagents were also considered.

Experimental design

To improve contact with the Ca(OH)2 solutions, the root dentin was split into three sections. Each of these identical tooth fragments was weighed before being combined into a clean Eppendorf tube. Each of the tubes holding the three pieces of root dentin had one of the following added to it: 1.4 mL of either the Ca(OH)2 aqueous solution (control), the Ca(OH)2 solution with various vehicles, or deionized water devoid of both Ca(OH)2 and vehicles. Every component remained completely submerged in the solutions. Every experiment was run through four times. To stop light-induced chemical changes, tubes were maintained in complete darkness at 37°C. At days 0, 1, 3, 5, 7, 14, 21, and 35, samples were collected. To maintain a constant overall volume, each time, 300 µL was extracted with a micropipette and 300 µL of the same fresh solution was added.

Calculation

In terms of the weight of each piece of root, theresults for total proteins were expressed in milligramsper millilitre per gram, and the results for hydroxyprolineandphosphorusinmilligramsperdecilitrepergram. All the chemical determinations were recalculated with respect to the replacement of 300 µL ofsolutionforeachperiodoftime.

The values, if any, for the tubes with distilled water without $Ca(OH)_2$ or vehicles were subtracted from the

corresponding solutions of $Ca(OH)_2$ (control) and $Ca(OH)_2$ with vehicles1-5(listed in the Solutions subsection).

Statistics

Ph values were statistically evaluated by using analysis of variance for repeated measures and the DunnettT3 multiple-range test. The results for proteins, hydroxyproline, and phosphorus were also evaluated by using analysis of variance for repeated measures and by regression analysis. Statistical significance was set at P<.05.

Results

The average of 3 measurements of pH values re-corded for each of the vehicles was 7.13 for distilledwater, 6.60 for chlorhexidine, 5.30 for PG, 3.47 for the anesthetic solution, 6.61 for CMCP, and 5.49 for CMCP-PG.

Table1 shows the mean and standard error values for the pH, total proteins, hydroxyproline, and phosphorus determinations of each Ca $(OH)_2$ solution with vehicles through the experiment. Control solutions of 0.1 mol/L $Ca(OH)_2$ had an average pH value of 12.09, whereas the pH values of the different $Ca(OH)_2$ solutions addedto vehicles were 11.07for chlorhexidine, 12.47 for PG, 12.36 for the anesthetic solution,11.55forCMCP,and 11.57 for CMCP-PG. The amount of protein releasewas higher when anesthetic solution and chlorhexidinewere used, reaching values close to 2 mg/mL/g. PG, CMCP, and CMCP-PG had higher hydroxyproline values at all recorded intervals, reaching more than 3mg/dL/g at the 35 day. The highest phosphorus releasewas with PG, with a maximum value of 56 mg/dL/g at the end of the experiment.

The Figure illustrates variations in the times of thesemean values. All the groups maintained their alkalinityup to 35 days. PG solutions had higher pH values than did the other solutions at all intervals (Figure, A). The statistical analyses revealed no significant differencesfor each of the solutions through the times, but differences exist between them, except for CMCP versus CMCP-PG. Statistically significant differences were also observed between all solutions in comparison withthecontrols.

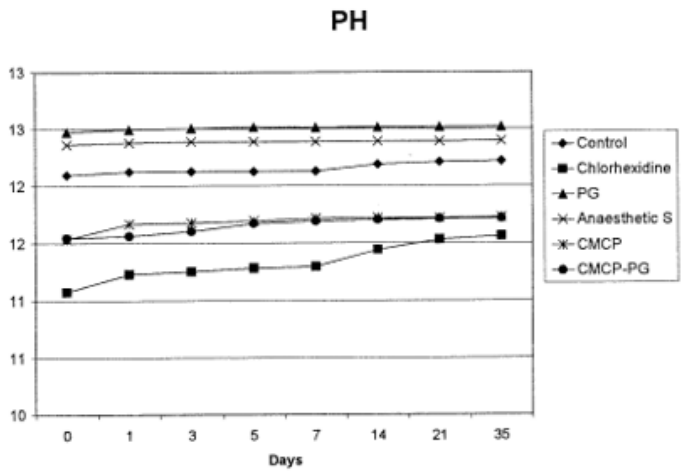
Table 2 shows the statistical results of the chemical content increase per day of each of the Ca $(OH)_2$ solutions. The proteins hydroxyproline and phosphorus were not detected at the onset of the experiment. All the solutions had a proteins release (Figure, B). The in-crease in proteins for $Ca(OH)_2$ with chlorhexidine, anesthetic solutions, and PG was statistically significant in comparison with that of the control; however, in solutions containing CMCP and CMCP-PG, the increase was not statistically significant.

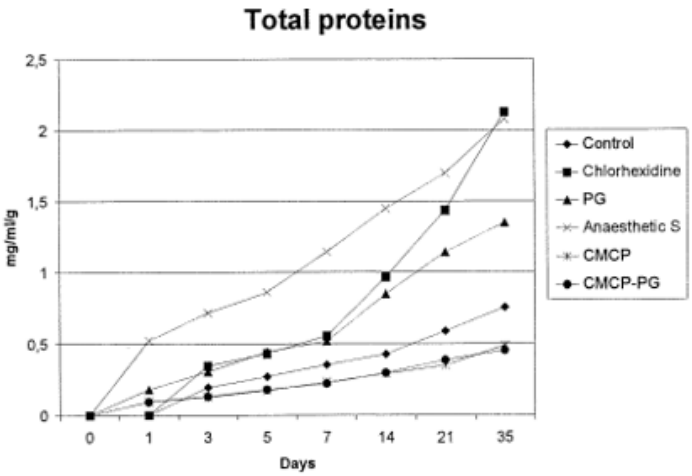
In the cases in which hydroxyproline was evaluated,the solutions had a gradual increase in concentration (Figure, C). $Ca(OH)_2$ with CMCP-PG, CMCP, and PG had a statistically significant increase in comparison with the control group; however, in the case of chlorhexidine and anesthetic solutions (Table II), the in-crease was not statistically significant. A gradual and significant increase in phosphorus was also observed (Figure, D). All the groups, with the exception of $Ca(OH)_2$ combined with PG and chlorhexidine, experienced a smaller increase than the control values Table 2. The differences were statistically significant only for these 2groups.

Table1 : Mean and SE of 4 determinations for pH, total proteins (mg/mL/g), hydroxyproline (mg/dL/g), and phosphorus (mg/dL/g)

Solutions	Time (d)	pH (mean±SE)	Total Proteins (mean±SE)	Hydroxyproline (mean±SE)	Phosphorus (mean±SE)
Control $Ca(OH)_2$ + distilled water	0	12.09±0.026	0.000 ±0.000	0.000±0.000	0.000 ±0.000
	1	12.12±0.014	0.000 ±0.000	0.122±0.034	0.240 ±0.074
	3	12.12±0.012	0.194 ±0.005	0.198±0.008	1.964 ±0.064
	5	12.12±0.008	0.272 ±0.013	0.284±0.010	3.143 ±0.090
	7	12.12±0.012	0.354 ±0.073	0.351±0.031	3.873 ±1.358
	14	12.18±0.003	0.429 ±0.038	0.527±0.008	15.144 ±2.134
	21	12.20±0.003	0.591 ±0.061	0.642±0.021	24.866 ±3.757
	35	12.21±0.003	0.758 ±0.049	0.849±0.034	40.530 ±2.588
$Ca(OH)_2$+ chlorhexidine	0	11.07±0.049	0.000 ±0.000	0.000±0.000	0.000 ±0.000
	1	11.22±0.036	0.000 ±0.000	0.000±0.000	2.137 ±0.380
	3	11.25±0.034	0.345 ±0.017	0.114±0.008	3.106 ±0.123
	5	11.28±0.036	0.434 ±0.020	0.201±0.007	4.817 ±0.078
	7	11.29±0.041	0.558 ±0.066	0.262±0.031	5.502 ±0.268
	14	11.43±0.036	0.969 ±0.041	0.466±0.033	9.335 ±0.522
	21	11.53±0.010	1.436 ±0.088	0.770±0.047	14.861 ±1.049
	35	11.55±0.002	2.127 ±0.134	1.145±0.046	33.283 ±0.724
$Ca(OH)_2$+ PG	0	12.47±0.017	0.000 ±0.000	0.000±0.000	0.000 ±0.000
	1	12.49±0.002	0.180 ±0.041	0.380±0.040	2.481 ±0.391
	3	12.50±0.005	0.303 ±0.055	0.557±0.041	5.247 ±0.565
	5	12.51±0.006	0.444 ±0.032	0.714±0.052	18.948 ±2.482
	7	12.51±0.007	0.519 ±0.035	0.959±0.088	27.999 ±2.966
	14	12.51±0.007	0.851 ±0.051	1.365±0.108	38.581 ±3.978
	21	12.51±0.006	1.140 ±0.045	1.978±0.206	47.797 ±4.793
	35	12.51±0.004	1.349 ±0.081	2.353±0.232	56.290 ±4.619
$Ca(OH)_2$+anesthetics olution	0	12.36±0.004	0.000 ±0.000	0.000±0.000	0.000 ±0.000
	1	12.37±0.007	0.524 ±0.034	0.048±0.010	0.479 ±0.119
	3	12.38±0.005	0.861 ±0.051	0.356±0.024	4.042 ±0.283
	5	12.38±0.005	1.036 ±0.098	0.455±0.057	5.862 ±0.772

	7	12.38±0.002	1.140 ±0.025	0.497±0.035	6.911 ±0.331
	14	12.38±0.002	1.447 ±0.046	0.586±0.026	9.168 ±0.452
	21	12.38±0.002	1.693 ±0.055	0.761±0.050	12.118 ±0.562
	35	12.39±0.002	2.075 ±0.058	1.152±0.023	17.585 ±0.280
$Ca(OH)_2$+ CMCP	0	11.55±0.006	0.000 ±0.000	0.000±0.000	0.000 ±0.000
	1	11.66±0.011	0.092 ±0.015	0.204±0.037	0.772 ±0.025
	3	11.67±0.006	0.128 ±0.011	0.483±0.111	1.562 ±0.134
	5	11.69±0.007	0.173 ±0.013	1.035±0.153	2.607 ±0.212
	7	11.71±0.010	0.234 ±0.027	1.459±0.158	3.812 ±0.374
	14	11.71±0.010	0.288 ±0.036	1.955±0.097	4.824 ±0.341
	21	11.71±0.010	0.352 ±0.039	2.308±0.105	7.566 ±0.325
	35	11.72±0.008	0.491 ±0.060	3.125±0.317	10.747 ±0.666
$Ca(OH)_2$+CMCP+PG	0	11.57±0.017	0.000 ±0.000	0.000±0.000	0.000 ±0.000
	1	11.57±0.028	0.094 ±0.010	0.293±0.021	4.250 ±0.190
	3	11.60±0.048	0.130 ±0.014	0.885±0.138	5.543 ±0.398
	5	11.67±0.010	0.180 ±0.021	1.198±0.099	8.322 ±0.172
	7	11.69±0.012	0.220 ±0.028	1.647±0.122	9.974 ±0.211
	14	11.70±0.011	0.299 ±0.024	2.351±0.139	11.510 ±0.421
	21	11.71±0.012	0.389 ±0.033	2.830±0.175	13.561 ±0.356
	35	11.71±0.010	0.457 ±0.035	3.367±0.275	16.564 ±0.878





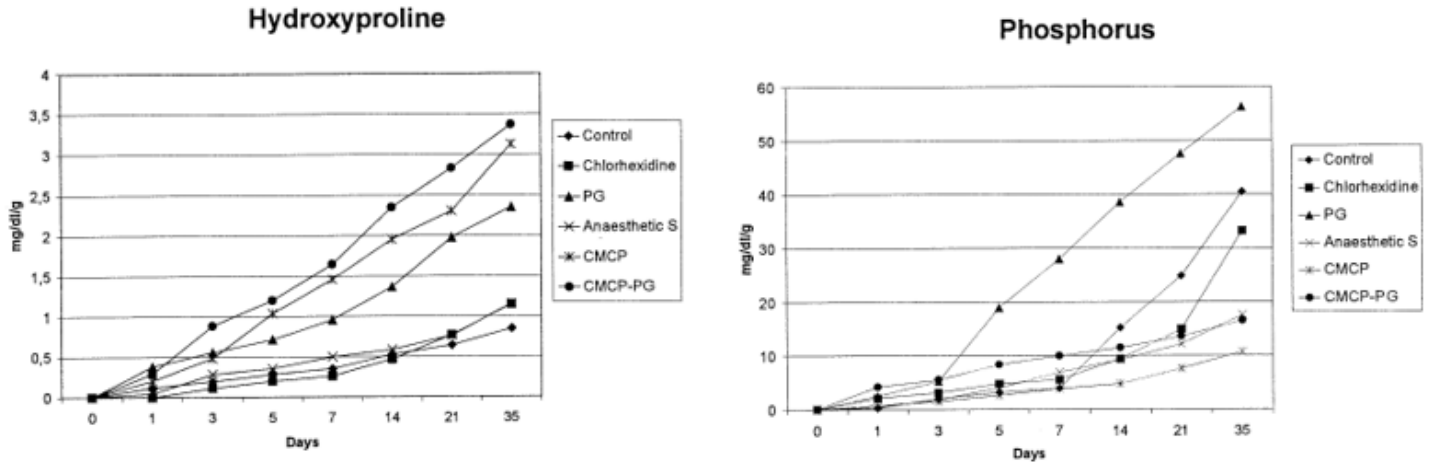


Figure 1: Means of 4 determinations performed with the various solutions, in which pieces of root dentin were kept for 35 days. A, pH; B, total proteins; C, hydroxyproline; D, phosphorus

Table 2: Regression analysis of chemical determinations of $Ca(OH)_2$ with the addition of different vehicles

$Ca(OH)_2$+ vehicles	Total protein increase perd(mg/mL/g)	Hydroxyproline increa seperd (mg/dL/g)	Phosphorusincrease perd(mg/dL/g)
Control	0.008687	0.017555	0.362520
Chlorhexidine	0.061386*	0.033620	0.909781*
PG	0.034327*	0.058029*	1.453130*
Anestheticsolution	0.042614*	0.0187561	0.358178
CMCP	0.010943	0.078292*	0.284187
CMCP+PG	0.010379	0.084018*	0.328448
*Statistical significance set at P<.05.			

Discussion

Strongly alkaline Ca(OH)2 is a chemical that is commonly used as an intracanal medication in various forms. It is crucial to ascertain whether the dentin may undergo a chemical change of some kind. As instrumentation time passes, the paste's composition could likewise change. On root dentin, the effects of solutions made with Ca(OH)2 and various vehicles were assessed.

Ca(OH)2 solutions' pH levels did not change over time, even when automobiles were present. Nonetheless, chemical substances from the root dentin were found. According to tests using tryptophane and tyrosine (the Lowry method) or alanine (the Bradford method), the protein level of each solution increased over time. The concentration of collagen, evaluated as hydroxyproline, also rose in the surrounding solutions—although in a lower concentration than total proteins, suggesting hat proteins other than collagen can be detected and could reveal a different behavior the presence of vehicles. The Ca $(OH)_2$ solution in this in vitro model also attracts phosphorus and forms soluble, detect able calciumphosphates.

The addition of vehicles (except for chlorhexidine and PG) seems to prevent dentin phosphorus release. The vehicles may form a protective film on hydroxyapatite crystals or combine with Ca(OH)2, thus reducing the attractive action on inorganic dentin components. The vehicles could affect Ca(OH)2 behavior in a different way. Anesthetic solutions, with or without vasoconstrictors, have been used as a vehicle of the paste because these solutions are readily available, sterile, and easy to handle. Most of these solutions have an acidic pH. How- ever, the final paste, prepared by mixing the vehicle with the Ca(OH)2 powder, has a high pH that is maintained over time and promotes rapid ionic release.[11]

A clear, colourless, and odourless liquid, PG has a distinct flavour similar toglycerin. The reason for its extensive use in endodontics as a delivery system for intracanal medications is its potent antibacterial activity against microorganisms often present in infected root canals.11 This ingredient also has the benefit of consistency, which enhances the paste's hand-holding properties. For Ca(OH)2 preparation, PG is the ideal vehicle, according to Simon et al. 8. Frank proposed the use of Ca(OH)2 and CMCP together.15 It has been suggested that this combination broadens the antibacterial range of Ca(OH)2, primarily against some facultative or anaerobic bacteria.12,Thirteen It has been demonstrated chemically that CMCP combined with Ca(OH)2 produces the weak salt calcium p-chlorophenolate. In a solution in water, the salt takes up the H+ ion and reconverts to p-chlorophenol, which gives off an excess of OH— ions from the water, thus maintaining the high pH.[14]

This formulation prolongs the antibacterial action because of the progressive release of p-chlorophenol from the calcium p-chlorophenolate complex. Although CMCP has strong cytotoxic effects,15studies have reported a favorable tissue response to a mixture of Ca(OH)2 and CMCP.[16] This response maybe caused by the small concentration of p-chlorophenol released, as well as because the high pH initiates a superficial denaturalization of the tissue it contacts. This area may act as a physical barrier to a deeper diffusion of p-chlorophenol into the tissue.[17]

Conclusion

We are unable to suggest a specific intracanal medication vehicle based on the current research. The alkalinity that provides antibacterial activity was retained in all of the cars. With a duration of less than 35 days often employed in clinical applications, the chemical structure of dentin might not be impacted.

References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germfree and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340 –9.
2. Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. Odontologisk Revy 1974;25:347–58.
3. Strindberg LZ. The dependence of the results of pulp therapy on certain factors. An analytic study based on radiographic and clinical follow-up examination. Acta Odontol Scand 1956;14(Suppl. 21):1.
4. Grahne´n H, Hansson L. The prognosis of the pulp and root canal treatment. A clinical and radiographic follow-up examination. Odontol Revy 1961;12:146 – 65.
5. Engstro¨m B, Hard AF, Segerstad L, Ramstro¨m G, Frostell G. Correlation of positive cultures with the prognosis for root canal treatment. Odontol Revy 1964;15:257–70.
6. Bystro¨m A, Happonen RP, Sjo¨ gren U, Sundqvist G. Healing of periapical lesions of pulpless teeth

after endodontic treatment with controlled asepsis. Endod Dent Traumatol 1987;3:58 – 63.

7. Sjo¨ gren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30:297–306.
8. Loesche WJ, Lopatin DE, Stoll J, van Poperin N, Hujoel PP. Comparison of various detection methods for periodontopathic bacteria: can culture be considered the primary reference standard? Clin Microbiol 1992;30:418 –26.
9. Conrads G, Gharbia SE, Gulabivala K, Lampert F, Shah HN. The use of a 16s rDNA directed PCR for the detection of endodontopathogenic bacteria. J Endod 1997;23: 433– 8.
10. Rolph HJ, Lennon A, Riggio MP, Saunders WP, MacKenzie D, Coldero L, Bagg J. Molecular identification of microorganisms from endodontic infections. J Clin Microbiol 2001;39:3282–9
11. FavaLR, SaundersWP. Calciumhydroxide pastes: classification and clinical indications. Int Endo dJ1999;32:257-82.
12. Leonardo MR, Almeida WA, Ito IY, da Silva LA. Radiographicand microbiologic evaluation of posttreatment apical and periapicalrepair of root canals of dogs' teeth with experimentally inducedchroniclesion.OralSurgOralMedOralPathol 1994;78:232-8.
13. Siqueira JFJr, Uzeda M. Intracanal medicaments: evaluation of the anti bacterial effects of chlorhexidine, Metronidazole and calciumhydroxide associated with three vehicles.JEndod1997;23:167-9.
14. Anthony DR, Gordon TM, delRioCE. The effect of three vehicles on the pH of calcium hydroxide. Oral Surg Oral Med Oral Pathol1982;54:560-5.
15. Spångberg L, Rutberg M, Rydinge E. Biologic effects of end-odonticantimicrobial agents. JEndod1979;5: 166-75.
16. A. Effect of intra canal dressing on repair and apical bridging of teeth with incomplete root formation. Endod DentTrauma-tol1993;9:25-30
17. Siqueira JF Jr, Uzeda M. Disinfection by calcium hydroxide pastes of dentinal tubules infected with two obligate and one facultative an aerobic bacteria. JEndod1996; 22: 674-6.