

Diffusion of calcium hydroxide through dentinal tubules of retreated root canals: An *in vitro* study

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ABSTRACT

Objective: Studies about the dispersion of hydroxyl ions through dentin of endodontically retreated teeth are scarce. The present study aims at examining the diffusion of calcium hydroxide *in vitro* by recording the pH changes of retreated root canal using two types of calcium hydroxide preparations. **Materials and Methods:** A total of 45 recently extracted single-rooted mandibular first premolars were collected and cleaned from calculus and remains of periodontal tissue. The teeth so collected were stored in thymol 10% solution till use. During collection, teeth with cracks, open apices, and restorations were excluded. Root canal preparations were performed using Protaper rotary system files, till #F3 file. All canals were obturated using cold lateral condensation and zinc oxide-based sealer. After 7 days, all gutta-percha were removed and the canals were irrigated with normal saline. The teeth were then randomly distributed into three groups. Canals in Group I were kept empty without any dressing to serve as a negative control group, Canals in Group II were filled with a freshly prepared mixture of calcium hydroxide powder with normal saline solution, and Group III was filled with Metapex. Periodically, the calcium hydroxide material was removed and the pH was measured using pH meter at 7, 10, 14, and 30 days. **Results:** The pH values of Group II and III ranged between 9.2 and 11.2. This was found to be highly significant against the negative control group. After 7 and 10 days, freshly mixed Ca(OH)₂ showed the higher pH than the creamy Metapex, and statistically the difference was highly significant after 10 days ($P < 0.001$). After 14 days, Metapex group pH was higher than freshly mixed Ca(OH)₂, but it was not statistically significant as they reached nearly the same pH after 30 days ($P > 0.05$). **Conclusion:** All Ca(OH)₂ preparations had high pH around the roots after 7 and 10 days. Metapex continued to have higher pH after 14 days.

Key words: Calcium hydroxide, Dentinal tubules, Metapex, Retreated root canals

INTRODUCTION

Root canal therapy eradicates bacteria from infected root canals and prevents reinfection. Inappropriate mechanical debridement or persistence of bacteria in the root canal structure due to poorly performed

root canal therapy may lead to failure.^[1] Failure of root canal treatment can be managed by nonsurgical retreatment. Calcium hydroxide has been widely

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used in endodontics as an intracanal medicament because of its bactericidal activities, especially in retreatment cases. This has also been suggested for use as intracanal medicament because of its antiresorptive and tissue dissolving properties.^[2]

It was in 1920 that Herman introduced calcium hydroxide (Ca(OH)₂) as a pulp-capping agent, to the field of endodontics.^[3] Ca(OH)₂ is a white odorless powder having a molecular weight of 74.08. It has less solubility in water (about 1.2 g/L at 25°C). Although it does not have property of bonding to dentin, it has antibacterial property.^[4] Ca(OH)₂ exhibits lethal effects on bacteria as a result of damage to DNA, protein denaturation, and the damage caused to cytoplasmic membranes. On common endodontic pathogens, it is found to exhibit an extensive range of antimicrobial activities. Further, it maintains high pH after setting, as the material dissolves readily in aqueous solution and liberating hydroxyl ions. This high pH delivers an incentive for tooth to repair himself in nonexistence of bacterial infection.^[5]

Ca(OH)₂ has low solubility in tissue fluids, even when there is direct contact with vital tissues. It has an alkaline pH (about 12.5–12.8), which is chemically categorized as a strong base. The material has the ability to quickly separate into hydroxyl ions and calcium ions and maintain a high pH, and lead to bactericidal effects in the root canal system.^[6,7]

For Ca(OH)₂ to perform efficiently as an intracanal medicament, hydroxyl ions must diffuse through dentine. It is expected that this would occur in a similar manner with water because dispersion through dentine is principally determined by molecular weight. Various studies have attempted to identify the quantity of diffusion of hydroxyl ions into the dentin using a diversity of experimental methods, comprising pH indicating solutions or papers, pH values of the surrounding medium, and pH measurement of ground dentin.^[5-7]

Ca(OH)₂ controls infection and decrease the incidence of unfavorable symptoms. It is also used in various clinical situations as an intracanal medicament, been shown to decrease the pathogens associated with pulp necrosis, and limit root resorption and promote the repair of periapical tissues.^[8-10]

Kazemipoor *et al.*,^[11] have stated that the difficulties which are more challenging in the retreated canals are the openings of dentinal tubules which may be blocked with residual sealer and gutta-percha. This

phenomenon can have an influence on the diffusion and penetration of hydroxyl ions into the dentinal tubules.

Pashley and Livingston had suggested that hydrogen and hydroxyl ions like water should diffuse readily through dentine since permeability was in general inversely related to molecular size, and in this study where molecular charge was examined as a variable it did not appear to influence the rate of diffusion.^[12]

There are only limited studies that have examined the diffusion dynamic of hydroxyl ions through dentinal tubules of retreated teeth. The present study was done to examine the diffusion of Ca(OH)₂ through dentinal tubules of retreated root canals using two different types of Ca(OH)₂ preparations.

MATERIALS AND METHODS

A total of 45 extracted single-rooted mandibular first premolars were taken for the study. The teeth were cleaned from calculus and any remains of periodontal tissue and kept in a thymol 10% solution till use.

Inclusion criteria

The inclusion criteria were as follows:

1. The premolars of patients aged between 16 and 20 years old, with single canal extracted for orthodontic purpose were collected for this study
2. In the selected tooth, the presence of single root canal was verified using two digital radiographs in both buccolingual and mesiodistal directions.

Exclusion criteria

1. Teeth with restorations, cracks, and open apices were excluded from the study.

Ethical approval was obtained from the Department of Conservative Dental Sciences, College of Dentistry, Prince Sattam bin Abdulaziz University, before the study. All the teeth used in this study were cleaned using running tap water and then stored in normal saline. The crowns were transversally sectioned with a diamond-cutting disc at the cemento-enamel junction level.

The length of the root canals was recorded at 1 mm short of the main apical foramen and was standardized to nearly the same lengths (13 ± 1 mm) for all the sample teeth as shown in Figure 1. Glyde (DENTSPLY, Maillefer) has been used as a lubricant. Root canals were prepared using Protaper (DENTSPLY, Tulsa) rotary system files

using an electric motor (X-Smart; DENTSPLY, Maillefer), till #F3 file. Irrigation during cleansing and shaping was accomplished using distilled water. After instrumentation, a #30 file was introduced up to the total length of the root canal for apical cleaning, and the root canal was then filled with a solution of ethylenediaminetetraacetic (EDTA) (ULTRADENT, IndiSpense) for 3 min. The root canals were then cleansed with normal saline solution and then absorbent paper points were used for drying. Then, all canals were obturated using cold lateral condensation technique. Each tooth was placed in a container with unbuffered isotonic saline soaked in cotton wool, which allows the sealer to set, kept in an oven at 37°C, with 100% humidity for 7 days. After that, the gutta-percha was removed from the canals using chloroform and Protaper Universal Retreatment file # D3 (Protaper® Universal Retreatment, DENTSPLY), along with the removal of certain amount of sound dentin. The dentinal thickness in apical, middle, and coronal third was measured using digital radiograph in mesiodistal and buccolingual direction as shown in Figure 1. Then, the selected teeth were randomly distributed into three groups, each group comprising 15 teeth:

- Group I: 15 root canals will not be filled with anything to be used as control group, as shown in Figure 2
- Group II: 15 root canals will be filled with a fresh mixture of calcium hydroxide powder (Pulpdent Corporation, USA) and distilled water solution, as shown in Figure 3
- Group III: 15 root canals will be filled with oily readymade calcium hydroxide Metapex (Meta Biomed, Korea), as shown in Figure 4.

The coronal access cavities and the apical openings were sealed with amalgam restoration. Each specimen was kept in small vial with 30 ml of deionized water kept in an oven at 37°C, with 100% humidity; to be used for pH measurements after 7, 10, 14, and 30 days using pH meter.

When measuring the pH, the tooth was removed from the vial and rinsed with saline. At the end of each stage, the teeth were placed back in the same vial with freshly placed cotton saturated with saline.

RESULTS

The average baseline pH in control group was similar (pH 6.9–7.4). The pH values of both Ca(OH)₂ was between 9.2 and 11.2 when measured in all the



Figure 1: Peri-radiographic showing the dentinal wall width of a sample

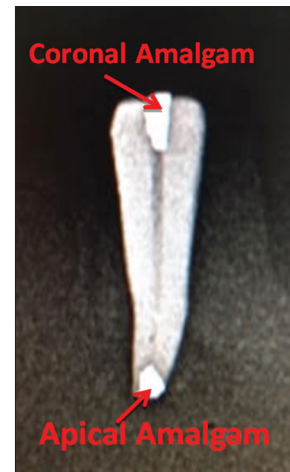


Figure 2: Peri-radiographic showing a sample from control group



Figure 3: Peri-radiographic showing freshly mixed Ca(OH)₂ inside the canal with the coronal and apical amalgam seals of a sample from Group II

four periods which were highly significant than the negative control group.

Table 1 shows the mean of pH readings of the control group after 7, 10, 14, and 30 days. The pH value dropped after 14 and 30 days, while no significant change in the pH after 7 and 10 days.

Table 2 shows mean of pH readings of freshly mixed calcium hydroxide group after 7, 10, 14, and 30 days. The pH reading was shown an increase after 10 and 14 days, while no significant increase in pH after 30 days.

Table 3 shows the mean of pH readings of Metapex group after 7, 10, 14, and 30 days and no significant change in the reading in the pH was noticed.

After 7 and 10 days, freshly mixed Ca(OH)₂ showed the higher pH than the creamy Metapex, and statistically, the difference was highly significant after 10 days ($P < 0.001$).

After 14 days, Metapex group pH was higher than freshly mixed Ca(OH)₂, but it was not statistically significant, they reached the same pH after 30 days ($P > 0.05$). Descriptive statistics using one-way ANOVA test was used to compare the pH values for different types of Ca(OH)₂ at different time periods and multiple comparisons *post hoc* test was used in this study to get the results [Table 4].

Graph 1 presents the comparison of the changes in the pH reading of all the three groups after 7, 10, 14, and 30 days. The control group does not show any significant change in the pH reading, while the Group III has shown changes in the pH reading, but it was less as compared to the Group II [Graph 1].

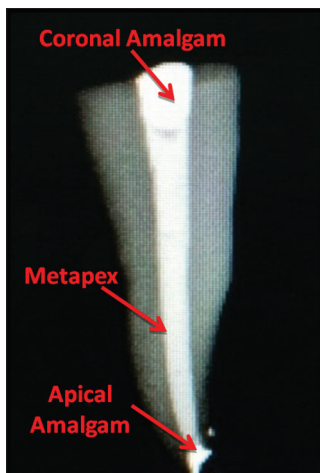


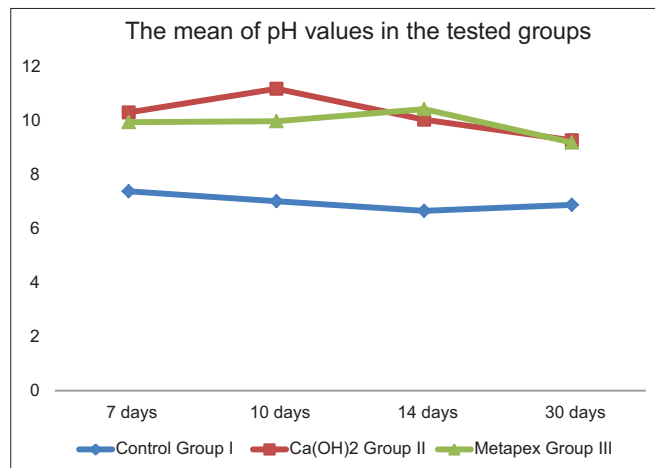
Figure 4: Peri-radicular radiograph showing Metapex inside the canal with the coronal and apical amalgam seals of a sample from Group III

DISCUSSION

The desired beneficial effect of Ca(OH)₂ is dependent on its separation into hydroxyl ion and calcium ion, and their diffusion through the dentinal tubules and the apical foramen. When the hydroxyl ions diffused, the pH will be raised causing destruction of bacteria, reduction of osteoclastic activity, inactivation of bacterial enzymes, and activation of alkaline phosphatase which is involved in mineralization.^[10] Diffusion of Ca(OH)₂ through dentinal tubules has been studied in the recent years.^[13,14]

According to Ba-Hattab *et al.*,^[15] The hydroxyl ions release in an aqueous environment is necessary for antimicrobial activity of calcium hydroxide. Hydroxyl ions react intensively with biomolecules because of their free radicals. As this reactivity is unspecified, the free radicals most likely gathered at the sites of generation.

For the medications to show complete efficacy, they have to be in contact with the dentin for certain period and also be able to enter the dentinal tubules. Therefore, the intracanal medicaments' proper wetting with the radicular dentine and contact angle between the hard tissue and the paste should be considered.^[16] According to Kontakiotis *et al.*,^[17] wetting of aqueous liquid means that, an edge contact is being molded between a liquid and a solid with a concurrent exclusion of air. The propensity of a liquid to wet a solid surface is conveyed with the development of a contact angle. Principally, the lower the angle, the faster the liquid medium will wet on the tooth surface, and a contact angle of $< 90^\circ$ designates good wetting properties.



Graph 1: The mean of pH values of the three test groups (Group I = control group, Group II = calcium hydroxide, and Group III = Metapex) in the four tested periods (7 days, 10 days, 14 days, and 30 days)

Table 1: Mean of pH readings of Group I (Control Group)

pH readings of group I				
Sample no.	Mean			
	After 7 days	After 10 days	After 14 days	After 30 days
1	7.336	8.236	6.808	6.928
2	7.420	8.034	6.484	6.798
3	7.296	7.986	7.004	6.726
4	7.420	8.236	6.388	6.720
5	7.570	8.002	6.688	7.770
6	7.276	7.950	6.528	6.608
7	7.362	7.762	6.802	6.716
8	7.360	8.034	6.484	6.798
9	7.296	7.986	7.004	6.726
10	7.420	8.236	6.388	6.720
11	7.570	8.002	6.688	7.770
12	7.276	7.950	6.528	6.608
13	7.362	7.762	6.802	6.716
14	7.296	8.236	6.808	6.798
15	7.420	8.034	6.484	6.726

Table 2: Mean of pH readings of Group II (Calcium Hydroxide Group)

pH readings of group II				
Sample no.	Mean			
	After 7 days	After 10 days	After 14 days	After 30 days
16	10.555	11.764	9.866	8.250
17	10.320	11.250	10.932	9.678
18	11.086	11.778	10.956	8.452
19	10.450	11.778	10.000	8.786
20	10.088	11.778	11.072	10.550
21	10.498	11.246	10.702	9.170
22	10.650	11.720	10.028	10.204
23	9.854	10.822	10.402	9.558
24	9.532	10.548	9.724	8.994
25	10.436	11.550	10.802	8.878
26	9.458	9.844	7.826	7.646
27	10.766	11.674	10.178	8.862
28	8.356	9.200	7.994	9.156
29	11.280	11.222	10.822	10.896
30	11.594	11.918	9.536	8.124

Table 3: Mean of pH readings of Group III (Metapex Group)

pH readings of group III				
Sample no.	Mean			
	After 7 days	After 10 days	After 14 days	After 30 days
31	8.902	7.166	9.144	8.192
32	8.180	8.594	8.194	10.160
33	11.098	10.484	10.346	9.308
34	10.184	10.270	10.832	9.258
35	10.260	8.882	10.772	9.374
36	10.130	10.648	11.356	9.722
37	9.890	10.154	11.124	9.310
38	11.008	10.444	11.218	9.380
39	10.962	10.062	10.772	9.186
40	10.126	10.256	10.440	9.560
41	9.198	8.202	10.356	8.038
42	8.958	7.212	9.458	9.984
43	10.290	10.192	10.268	8.548
44	9.862	9.722	11.156	8.922
45	10.446	9.686	11.308	9.186

Madarati *et al.*,^[18] conducted a study, in which participants were aware that the main function of intracanal medicaments is disinfection of the root canal system, where Ca(OH)₂ was used as an intracanal medicament in necrotic pulp cases.

The study conducted by Rizvi *et al.*,^[19] states that MTAD is effective in the eradication of *Enterococcus faecalis*, around neutral pH, the effective inhibition of *E. faecalis* is better than the other root canal irrigants investigated in this study such as sodium hypochlorite, EDTA, and chlorhexidine.

Yücel *et al.*,^[20] stated that for proper wetting of the canal wall, Ca(OH)₂ powder has been used as a mixture for root canal dressing and can be mixed with vehicles such as olive oil, glycerin, propylene glycol, iodoform plus saline solution, camphorated parachlorophenol plus glycerin, camphorated parachlorophenol, methylcellulose, metacresylacetate, saline, distilled water, anesthetic solutions, Ringer's solution, and camphorated monochlorophenol cresatin.

Besides permitting diffusion of the Ca(OH)₂, the vehicle can enhance the antimicrobial capacity of the paste.^[21] According to Gomes *et al.*,^[22] chlorhexidine can be used as a vehicle for attempting an increase in the antimicrobial property to be effective against aerobic and facultative anaerobic microorganisms, Gram-positive and Gram-negative microorganisms, yeasts, and viruses.

Only a few studies have evaluated the dissolution capacity of Ca(OH)₂ preparations in retreated cases. During the root canal retreatment procedure, the dentinal tubules openings may be obstructed with gutta-percha and the remaining sealer. This can impact on the penetration and diffusion of hydroxyl ions through dentinal tubules. Hence, we intended to remove sound dentin during removal of the gutta-percha. The permeability of dentin is directed mostly by dentin tubule anatomy, diameter, density, and length as well as features of the solute such as charge and size. Dentin is a substrate, whereas calcium hydroxide is a material. The size of the

Table 4: Descriptive Statistic one way ANOVA test and multiple comparisons *post hoc* test

n	Descriptive (one way)			Multiple comparisons (<i>post hoc</i> test)			
	Group I (Control)	Group II Ca (OH) ₂	Group III (Metapex)	P	PI and II	PI and III	PII and III
	Mean±SD	Mean±SD	Mean±SD				
Day 7	7.398±0.108	10.328±0.804	9.966±0.838	0.000	0.000	0.000	0.193
Day 10	7.029±0.166	11.206±0.796	10.00±1.171	0.000	0.000	0.000	0.000
Day 14	6.672±0.217	10.056±0.998	10.450±0.898	0.000	0.000	0.000	0.222
Day 30	6.895±0.398	9.295±0.906	9.209±0.591	0.000	0.000	0.000	0.815

Ca(OH)₂: Calcium hydroxide, SD: Standard deviation

dentin tubules correlates with the size of the Ca(OH)₂ particles.^[23,24]

In the present study, in one group, we used Ca(OH)₂ powder mixed with distilled water; and in the other group, we used oily Metapex composed of Ca(OH)₂, iodoform, and silicon oil to fill the canals of retreated roots. To simulate the clinical condition, samples were kept in incubator at 37°C during the testing periods. According to the condition of our study, the normal baseline pH in negative control group was similar (pH 6.9–7.4). Both Ca(OH)₂ types showed pH values between 9.2 and 11.2, when measured in all the four periods which were highly significant than the negative control group and these are consistent with most studies.^[25-27]

After 7 and 10 days, freshly mixed Ca(OH)₂ showed higher pH than the creamy Metapex. Statistically, the difference was found to be highly significant after 10 days. This means that the diffusion of hydroxyl ions of freshly mixed Ca(OH)₂ is more than that of oily Metapex, and this result is consistent with Larsen and Horsted-Bindslev.^[28] It was also observed that oil paste containing Ca(OH)₂ was having low solubility and were mainly deficient in both ion release and antimicrobial properties. The aqueous suspension showed the highest pH and calcium ion liberation.

After 14 days, Metapex group pH was higher than freshly mixed Ca(OH)₂ but was not statistically significant. This could be due to Metapex comprising of Ca(OH)₂ and iodoform mixture in silicon oil. The low solubility and diffusibility could preserve the high pH till 14 days. The pH of both Ca(OH)₂ preparations reached nearly the same after 30 days.

CONCLUSION

The freshly mixed Ca(OH)₂ showed the higher pH after 7 and 10 days than that of the Metapex. Significant statistical difference was observed after 10 days. For Metapex group, pH was higher than freshly mixed

Ca(OH)₂ after 14 days. However, it was not statistically significant. After 30 days, Metapex group attained the same pH as that of the Ca(OH)₂. Thus, Ca(OH)₂ preparations showed higher pH after 7, 10, 14, and 30 days, while the Metapex in our study continued to have higher pH after 14 days but achieved nearly the same pH after 30 days as that of the Ca(OH)₂.

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Conflicts of interest

There are no conflicts of interest.

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