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Analyzing the mechanical properties of intracanal calcium hydroxide formulations on radicular dentin

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Abstract

Aim: Evaluation of calcium ion release and pH alteration across root dentin after using three types of calcium hydroxide intracanal medicaments over a period of 28 days.

Methods: Sixty single rooted mandibular premolars were decoronated for the study. Working length was determined, canals enlarged up to Size F2 (Protaper) and irrigated with 5.25% NaOCl. External defects of 3 mm depth and 1 mm diameter were prepared on the coronal third of the mesial root surface. The 17% EDTA was used to remove the smear layer from root canals and external defects. The specimens were divided into four groups (n=15): Group I (Control): no medicament; Group II: Met apex; Group III: RC Cal; and Group IV: Hygenic calcium hydroxide [Ca $(OH)_2$] points. At 3, 7, 14, and 28 days, calcium ion release and pH value were measured using an atomic absorption spectrophotometer and digital pH meter respectively. Statistical tests, One-way ANOVA followed by post-hoc Bonferroni test ($p<0.05$) was applied.

Results: All groups allowed ion diffusion, although aqueous paste RC Cal exhibited the most (57.240.90ppm / Day 28) and maintained a high pH (9.26 0.21 / Day 28), followed by Met apex.

Conclusion: RC Cal and Met apex aqueous pastes had greater Ca+ ion release and alkaline properties than Ca $(OH)_2$ points as an intracanal medicament vehicle.

Keywords: Calcium hydroxide; Intracanal Medi cement; Ion diffusion; Root canal therapy.

Introduction

The purpose of treating infected root canal system is to disinfect the root canals. In certain instances, root canal instrumentation and irrigation with antibacterial solutions are insufficient, and intracanal medications may be necessary for a supportive effect. Calcium hydroxide [Ca $(OH)_2$] has a wide array of uses in endodontics like root canal sterilization, controlling recalcitrant exudates, eliciting a calcific reaction, stimulating the production of reparative dentin, arresting inflammatory root resorption, promoting apexification and healing of larger periapical lesions. Ca $(OH)_2$ is highly bactericidal and induces desirable soft tissue reaction due to its high alkaline pH and substantial Ca^{2+} release concentrations. $Ca(OH)_2$ role includes its antibacterial action, ability to induce hard tissue formation, intratubular occlusion, and its tissue dissolving capacity(1). An alkaline environment (high pH)(2) is bactericidal and reverse the acidic resorptive activity favoring alkaline phosphatase activity. Ca^{+2} dependent adenosine triphosphatase may be initiated by calcium in hard tissue formation(3). The property of alkalinity depends on the percentage of extractable ions which further depend on the intrinsic property of the material such as vehicle, preparation form and method of placement.

The optimal vehicle will have no negative effects on eliciting hard tissue deposition and would allow slow and gradual release of Ca2+ and OH- ions, sustain diffusion in the tissues with low solubility in fluids(2). When used as an intracanal medicament the effect of calcium hydroxide is related to the diffusibility and solubility of the preparation as well as the buffering of the medium, while the diffusion capacity and the efficiency improves by reducing the surface tension of the vehicles(4).

Therefore, the aim of this study was to evaluate the calcium ion diffusion and pH change through radicular dentin after the use of Ca $(OH)_2$ preparations i.e. Met apex, RC Cal and Hygienic Gutta percha points over a 28-day period.

Materials and methods

Institutional Ethical Committee (Ref: DMR/IMS-SH/SOA/16025) provided ethical clearance for the study. Sixty single rooted mandibular premolars extracted for orthodontic purpose were selected for the study**.** The teeth chosen were free of caries, restorations, cervical abrasions and fractures. In order to obtain root specimens, the crowns were resected at the Cemento-enamel junction with a carborundum disk (Ukam, USA) in a high-speed handpiece under water spray. A barbed broach (Mani Inc, Japan) was used to extirpate the contents of the canal. X-Gates (Dentsply Maillefer, Switzerland) was used for coronal flaring and the working length of the root canal was determined by inserting a no. l0 K file (Mani Inc, Japan) until it was visible at the apex and then subtracting 1 mm from it. Apical enlargement was done up to F3 rotary Protaper (Dentsply Maillefer, Switzerland). 5ml of 5.25% sodium hypo chlorite (Prime Dental, India) was used for irrigation.

External defects of 1mm diameter and 3mm depth were prepared in the coronal third of root surface using tissue protective end cutting diamonds (ISO No.10, 680C-1, Shofu Inc, Japan) at high speed. The approximate size of the fenestration was controlled with a millimeter graph paper. The root canals and external defects were then flushed with 17% EDTA (Prevest Denpro, India) which was left in place for 4 minutes to remove the smear layer. They were then rinsed with copious amounts of distilled water to remove the EDTA completely and dried with sterile paper points (Dentsply Maillefer, Switzerland)

before placement of any preparation. Irrigation protocol followed was after understanding the interactions, advantages and limitations(5)

The teeth were then randomly allotted using online software (www.randomizer.org) to the control and experimental groups(n=15). Group I served as control with no medicament**.** Group II [Met apex (Meta Biomed Co. Ltd, Republic of Korea)] and Group III [RC Cal (Prime Dental, India)] a lentulospiral (Mani Inc, Japan) was used to apply the paste followed by use of paper points to adapt the material. Teeth in group IV were filled with Ca $(OH)_2$points, size 30 (Coltene Whale dent Inc, Germany)**.**

The coronal access was then sealed with 4mm of temporary cement (Cavit, 3M ESPE, Germany). All the surfaces of the roots except for the defects were coated with two coats of nail varnish and samples were placed in an airtight glass vial containing 20 ml of distilled water (pH = 6.8) and kept in an oven at 37^0C with 100% humidity. A digital pH meter, (Model L-I-120, Elico Private Ltd, India) was used to measure the pH after calibrating to a pH of 4, 7 and 9.2 with a standard buffer. The solution in each vial was stirred with a clean glass rod for 10 seconds and the pH was then determined at different time intervals (3,7,14 and 28 days) by placing the completely immersed probe into each sample bottle. An average of five readings were taken for one pH value. Between each reading, the electrode was washed with distilled water and wiped dry. Precision of the apparatus was verified with constant measurements with the standard buffer.

To measure the calcium ion concentration, a GBC 932 AA Atomic Absorption spectrophotometer (GBC Scientific Equipment Private Ltd, Australia) was used. The instrument was calibrated at zero absorbance using a blank solution. Absorbance values of the standard solutions of known concentrations were then obtained. A standard curve on plotting absorbance and concentrations was obtained.

Results

Calcium release was calculated using the line equation of the standard curve after absorbance of each sample was noted. For each sample the calcium ion concentration and pH values were recorded on 3, 7, 14 and 28 days. The calcium ion concentration and pH changes through the root dentin for and all groups were recorded and the data was analyzed for intergroup comparison and intra-group comparison using SPSS 23.0 software through one way ANOVA followed by post-hoc Bonferroni test. The observations are tabulated in tables 1, 2,3 and 4.

All the groups showed a gradual rise in the calcium ion concentration indicating an accumulation of calcium ions in the surrounding medium over the test period. pH values for all groups were above 7 indicating an alkaline environment throughout the study period. The control group showed the lowest values for both the variables, while among the experimental groups, the calcium ion measurements recorded were highest for Group III (57.24±0.9ppm/ day 28), and lowest for Group IV (16.53±0.66ppm/Day 3). The overall pH value was highest for Group II (9.51± 0.09/Day 3), closely followed by Group III (9.26±0.21/ Day 28) and lowest for group IV (7.52±0.15/Day 28). RC Cal (Group III) showed more Ca+ ion diffusion (57.24±0.90ppm) and maintained a high pH (9.26 ±0.21) even after 28 days depicting better support for calcium hydroxide action.

Discussion

The success of Ca $(OH)_2$as intracanal medicament depends on its dissociation into calcium ions and hydroxyl ions. Alkanization of environment by hydroxyl ions activates the alkaline phosphatase enzyme, which

indices mineralized tissue formation and thus helps in repair process(6)

For a medicament to be considered effective, its diffusion is a prime factor of consideration. Dentin does not have constant diffusion surface which is 1% near the amelodentinal junction but increases to up to 22% near the pulp(7). Also, diffusion is directly proportional to the dentinal tubule density and the square of the tubule radius(8). Additionally, the diffusion of OH^- ions may slow down by decreasing tubule diameter as the cementum is approached.

In order to alter the disease, process the intracanal medicament must diffuse well, traverse the remaining dentinal tubules and reach the external surface by simple diffusion. Dentin is a very reactive tissue which is capable of binding or trapping a variety of substance as they move through the tubules. Dentin has a buffering capacity to acids and this property although limited is present with alkalis too. Buffering may occur when proton donors such as H_2PO_4, H_2CO_3 and HCO_3, in the hydrated layers of hydroxyapatite, furnish additional protons to keep the pH unchanged. OH^- ions may also be adsorbed into the hydrated layer, thus slowing their diffusion along the dentinal tubules(9). When the whole thickness of dentin is saturated with OH^- ions, it is indicated by detection of raised pH at the outer dentin surface(9).

It is evident that if antibacterial activity is required, calcium hydroxide should have the greatest diffusion of OH^- ion(10).

The beneficial effects of calcium hydroxide are partly attributed to the hydroxyl alkalinizing properties and partly to the enhancing effects of Ca^{2+} in the formation of mineralized tissue. Very little is known about Ca^{2+} diffusion through the dentin. The phenomena of Ca^{2+} diffusion as for bio mineralization are caused by the presence of intra tubular glycosaminoglycans and phosph oproteins. Hydrophilic glycosamino glycans enhance calcium fixation while anionic phosphoproteins have a great affinity for calcium resulting in penetration and diffusion of calcium(11).

20 ml of distilled water was chosen to suffice the requirement for submergence of pH meter probe as well as the amount of liquid required for spectrophotometer analysis. In this study, AAS (atomic absorption spectrophotometer) was used for measuring calcium ion concentration as it is highly sensitive method and can measure down to parts per billion of a gram ($\mu g\ dm^{-3}$) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another, higher, energy level.

The results of the present study emphasize the fact that calcium hydroxide preparations sealed within the root canal can dissociate and diffuse into the surrounding medium and cross the dentinal barriers. All groups showed an alkaline ph. The value of pH was noted to be above that of the surrounding medium (6.8) prior to placement of the specimens. It is an established fact that the pH of dentin increased after placing calcium hydroxide(12), and that there happens a consistent rise in pH of the surrounding medium till day 30(13).

The control group showed an alkaline pH(14). The pH variation observed amongst all the groups during the testing period is highly significant. The pH of Groups II, III and IV was significantly higher($p \leq 0.05$) than the control group. The pH value for all groups was noted to be higher on day 3.

The highest pH value on day 3 was shown by Met apex (Group II) followed by RC Cal (Group III). In all the groups the pH then dropped by day 7. A slight rise over the next 7 days followed by tendency to stabilize in the

following 14 days was a general pattern observed in all groups(15). Some authors observed a rapid increase of pH change in the first 3 days(16). There was an unpredictable decrease of pH from 3rd day to 28th day as maintenance of high pH may be unlikely due to tissue fluid circulation and high dissolution of the calcium hydroxide preparations(17). Neutralized substances may adhere to walls preventing further release of hydroxyl ions.

$Ca(OH)_2$ has a tendency to undergo rapid carbonation and inactivation by absorption of atmospheric CO_2 and formation of insoluble calcium carbonate which results in decrease of pH and stabilization of ionic release(18).

In this study, there is no statistically significant difference between Group II and III on day 7 indicating a very similar behavior of aqueous pastes. In general, RC Cal significantly maintained a higher pH throughout the testing period. It has been reported that hydroxyl ions derived from the calcium hydroxide dressing diffuse in a matter of hours into the dentin and maintain it for 120 days(1). $Ca(OH)_2$ has poor solubility in water (0.16 gm in 100 grams of water at 30°C) and high alkalinity in aqueous solutions (pH 12.0 at 37°C)(19). The activity depends on the concentration of hydroxyl ions in the solution. In less soluble substances, as long as undissolved solute is present in contact with the saturated solution, the concentration of ions remains constant. As the ions are consumed, the dissolution of Ca $(OH)_2$continues to maintain the ionic concentration. An aqueous preparation potentially maintains high pH for a long time(19).

Ca $(OH)_2$ points displayed a significantly lower pH value than the other experimental groups. The higher pH in the initial days of use may be due to increased amount of $Ca(OH)_2$ in the composition (50-54 wt%). These points contain agents that leach out in the moist environment to influence dissociation of $Ca(OH)_2$(19).

After placing Ca $(OH)_2$ paste, there is an appreciable increase in Ca^{2+} concentration in the surrounding medium. The increase in Ca^{2+} concentration was directly related to the duration of test period(14). Greater release of calcium ions from non-setting pastes (Groups II and III) was observed. The simple structure of non-setting pastes allowed the release of suspended active agent followed by its dissolution in the surrounding(20).

The results in the experimental group indicate that different vehicles permit the release of ions to different degrees. This observation is of clinical significance as it may be possible to have some control on the OH^- release and Ca^{2+} release(13).

RC Cal had high value of pH as well as Ca^{2+} ions indicating high solubility, high dissolution and diffusion of Ca $(OH)_2$ in an aqueous medium. A continuous release of ions from the undissolved solutes maintains pH and result in accumulation of Ca^{2+} making it useful for use over a longer duration.

In applying the findings of an in vitro study to a clinical situation, it is important to realize that the three are widely different and may not co-relate completely. This study provides an experimental model that may reflect the probable cause-effect relationship.

Conclusion

Under the limitations of this study it was concluded that irrespective of duration of exposure, the aqueous Ca $(OH)_2$ suspension resulted in higher pH as compared to Ca $(OH)_2$ gutta percha points and the control groups, showing greater ability of aqueous suspensions to cause diffusion and rise in total ionic concentration. Use of RC Cal closely followed by Met apex was more beneficial than Ca $(OH)_2$ points, as an intracanal dressing materials in terms of alkalization and calcium ion release.

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Table Legends

Table 1: Comparison of Calcium ion concentration on Day 3 (Calcium ion concentration in ppm)

Group	Mean	S. D.	F	P
Group I	14.65	0.70	179.27	0.000
Group II	19.33	1.02		
Group III	20.36	0.57		
Group IV	16.53	0.66		
Comparison of Calcium Ion concentration on day 7				
Group I	16.87	0.44	309.94	0.000
Group II	26.16	1.16		
Group III	28.19	0.93		
Group IV	22.69	1.56		
Comparison of Calcium and Ion concentration on day 14				
Group I	17.22	0.57	529.64	0.000
Group II	31.21	1.18		
Group III	31.42	1.72		
Group IV	25.03	0.63		
Comparison of Calcium Ion concentration on day 28				
Group I	17.25	0.55	7531.69	0.000
Group II	51.88	1.03		
Group III	57.24	0.90		
Group IV	35.61	0.65		

Table 2: Comparison of pH Change on day 3

Group	Mean	S. D.	F	P
Group I	7.66	0.22	149.02	0.000
Group II	9.51	0.09		
Group III	9.26	0.29		
Group IV	8.49	0.38		
Comparison of pH Change on day 7				
Group I	7.13	0.05	393.92	0.000
Group II	9.21	0.11		
Group III	9.11	0.31		
Group IV	8.02	0.20		
Comparison of pH Change on day 14				
Group I	7.13	0.05	505.33	0.000
Group II	9.27	0.07		
Group III	9.21	0.30		
Group IV	7.69	0.20		
Comparison of pH Change on day 28				
Group I	7.11	0.07	541.69	0.000
Group II	8.49	0.19		
Group III	9.26	0.21		
Group IV	7.52	0.15		

Figure Legends

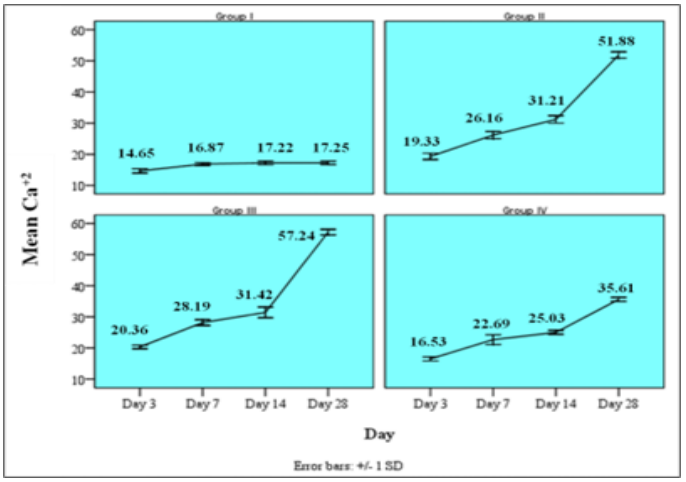

Figure 1: Evaluation of Calcium ion concentration at different days – Intra group comparison.

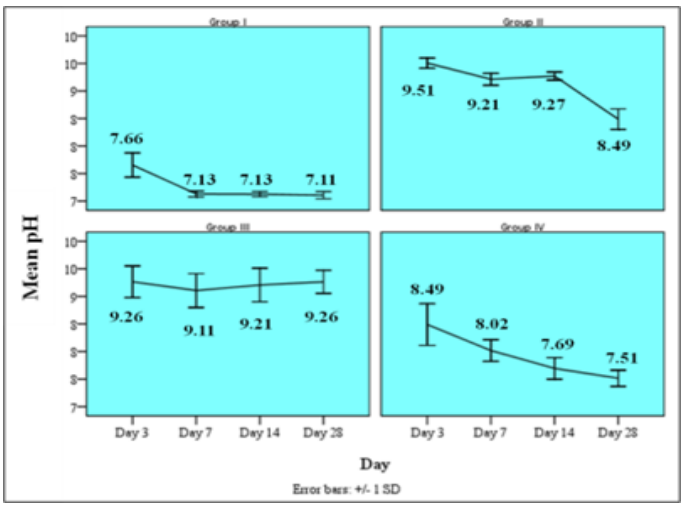

Figure 2: Evaluation of pH change at different days- Intra group comparison.