A Comparative Assessment of Hydroxyl and Calcium Ion Diffusion from Calcium Hydroxide Based Intracanal Medicaments in Primary Teeth - An In Vitro Study

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Abstract

Aim

The aim of the present study was to compare and determine quantitatively the diffusion of hydroxyl ions and calcium ions through radicular dentin and cementum from three different vehicles of calcium hydroxide pastes when used in primary teeth.

Material and Methods

60 roots of extracted primary molar teeth (20 per group) were collected. The chemo-mechanical preparation was done. The teeth were divided into 3 groups of different vehicles: (1) Group A (n=20) - Calcium hydroxide with distilled water; (2) Group B (n=20) - Calcium hydroxide with 0.2% chlorhexidine solution and (3) Group C (n=20) - Calcium hydroxide with propylene glycol in form of premixed syringes (Diapaste). The specimens were suspended in deionised water. Diffusion of the Calcium ions and Hydroxyl ions was determined using a digital pH meter and atomic absorption spectrometer at 24 hours, 7 days and 14 days respectively.

Results

Two-way analysis of variance and Turkey’s test revealed that the difference between the study groups was statistically not significant with regards to pH and hydroxyl ion diffusion at end of study period. However, for Group A, B achieved significantly higher pH and hydroxyl ion values (P<.001), followed by Group C in the seven day time period. In the calcium ion diffusion, a statistically significant difference was observed with group C showing highest release at the end of the study period.

Conclusion

All three formulations showed favorable results in terms of pH and hydroxyl ion concentrations. However, propylene glycol proved better as a vehicle in terms of slow and sustained action.

Keywords: Calcium Hydroxide; Endodontics; pH; Vehicle

Introduction

Endodontic treatment constitutes the last clinical resort for maintaining primary teeth in the oral cavity. In order, to achieve success with endodontic treatment it is necessary that all phases be carried out with the aim of healing the periradicular tissues and elimination of infection from the root canal system. The temporary nature of the primary tooth does not justify conducting precarious treatments where biologic principles are overlooked or neglected [1].

Due to the tortuous and ribbon shaped anatomy of the primary teeth, the clinician must rely primarily on chemical cleansing and sterilization and secondarily on mechanical instrumentation [2]. In these clinical situations, the use of intra-canal medication is required to complement the disinfection of the root canal system, dentin tubules, and external surface of the root apex, which cannot be achieved by chemo-mechanical preparation alone[3].

Calcium hydroxide is the most commonly used intra-canal medicament. The role of calcium hydroxide in endodontics as a medicament includes its ability to induce hard tissue formation, intra-tubular occlusion, antibacterial actions and tissue-dissolving capability [4]. It is suggested that calcium hydroxide placement in the root canal elevates the pH, producing an alkaline environment in the surrounding tissues by the diffusion of hydroxyl ions through the dental tubules [5]. When dissolved in water, calcium hydroxide dissociates into hydroxyl ions and calcium ions. Both Calcium and hydroxyl ions have a role in the healing process. The presence of hydroxyl ions in a solution makes it alkaline and thus antimicrobial and inhibits osteoclastic activity as well [6].

To be effective, calcium hydroxide has to be adequately placed and condensed into the root canal space, in combination with a carrying vehicle. Therefore, the type of vehicle used may either facilitate or inhibit the ionic dispersion of the paste. Vehicles commonly used are classified as aqueous, viscous, and oily. The aqueous vehicle promotes a high degree of solubility. Viscous water-soluble substances release ions more slowly for extended periods. Oily vehicles that are non-water soluble provide the lowest solubility and diffusion of the paste [7].

Considering the sparse studies reported in literature regarding...
effects of calcium hydroxide based intra-canal medicaments in primary teeth, the present study was undertaken. Thus the aim of the present study was to compare and determine quantitatively the diffusion of hydroxyl ions and calcium ions through radicular dentin and cementum from three different vehicles of calcium hydroxide pastes. The objectives were to determine maximum pH obtained and time taken to reach the maximum pH, to quantify the diffusion of hydroxyl and calcium ions. The hypothesis suggested was null hypothesis that there is no difference in the diffusion of hydroxyl and calcium ions from the three different calcium hydroxide formulations in the primary teeth.

**Methods**

This study was approved by Institutional Ethics Committee, Kasturba Hospital, Manipal (process no. IEC 509/2012).

**Sample size calculation.**

The study was based on demonstrating a 5% difference of diffusion of ions in between the groups, which was thought to be significant. To give 80% power and a 5% significance level using a two sided ANOVA test, a sample size of 15 in each group was calculated as sufficient to detect the difference between the groups.

**Sample Selection**

To generate minimum of 15 samples per group a convenient sample of 60 (20 in each group) was decided. For this purpose, 120 freshly extracted primary molar teeth were collected. The soft tissue remnants on the tooth were removed with a soft brush and stored in saline. The teeth were examined under stereomicroscope (20 x magnifications, Speedfair company, Hong Kong) to rule out surface resorption and furcation defects.

2. Roots with single patent root canals. Exclusion criteria: Teeth with perforation, resorption on their apical, middle or cervical thirds of the roots

The specimens were obtained by transverse sectioning of the crown 2.0 mm coronal to the cement-enamel junction using a double-face diamond disk (DENTSPLY, India) under water coolant. The preparation of the entrance of the canal was done with Endo Access bur. (DENTSPLY, India). The patency of the canals was ensured using no 10K file. At this stage, 20 roots which had more than one canal were excluded. Hemi-sectioning was carried out using a double-face diamond disk under water coolant. Each root was considered as a new specimen. The samples were stored in saline solution at room temperature until further use.

**Chemo-Mechanical Preparation**

Each canal was individually shaped with 21mm K files (DENTSPLY, India). Bio-mechanical root canal preparation was done using series of files till no 35 K files. Irrigation was done during the chemo mechanical preparation with 2.5 ml of 1% sodium hypochlorite solution (Medilise chemicals, India) in each canal. At the end, 1ml of 17% EDTA (Pulp dent, India) for 3 minutes was used for removal of smear layer and thereafter washing with saline[8]. The samples were then randomized using simple randomization into three groups based on the formulation to be used in the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Root canal filling material</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Calcium hydroxide with distilled water</td>
</tr>
<tr>
<td>B</td>
<td>Calcium hydroxide with 0.2% chlorhexidine</td>
</tr>
<tr>
<td>C</td>
<td>Calcium hydroxide mixed with propylene glycol in form of premixed syringes (Diapaste)</td>
</tr>
</tbody>
</table>

**Preparation of Medicament**

Electronic high precision balance (Sartorius, Germany) (Figure2) was used for weighing of the medicament -Calcium hydroxide powder (Deepthi Dental Products, India). After the calibration of the instrument, Calcium hydroxide powder was weighed to 0.5 gms and packed into sachets of butter paper. The paste was prepared in the three groups as follows ; Group A: The paste was prepared by mixing 0.5 gm of calcium hydroxide and 0.5 ml of distilled water (Manipal Pharmaceutical Products, India) just prior to placement of medicament.; Group B: The paste was prepared by mixing 0.5 gm of calcium hydroxide and 0.5 ml of a 0.2% chlorhexidine (Icpa Health Products Ltd, India) solution just prior to placement of medicament; Group C: Diapaste(Diadent international, USA & Canada) is an iodo form-free premixed calcium hydroxide, barium sulfate paste with propylene glycol base.

Figure 1: High precision electronic balance

80 intact roots of extracted primary molar teeth were selected based on the following inclusion criteria:

1. Primary molar teeth which are grossly decayed and un-restorable with root intact.
**Placement of the medicament**

Pre weighing of the samples before the placement of the medicament was done using high precision electronic balance. The samples were dried with paper points. Medication was then carried into the root canal with 20K file for groups A and B. Premixed syringe was used for samples of group C. The paste was further condensed with an endodontic plugger. Complete filling of the root canals was checked by the extrusion of paste through the apical foramen and reflux through the root canal opening. Post weighing of samples was then done. The mean weight of medicament inside the root canal obtained was 0.215 Gms.

**Apical and Coronal Sealing**

The apical foramen was sealed with sticky wax to prevent any liquid from entering the canal space and a layer of nail varnish was applied over the sticky wax (DENTSPLY, India) to make the tooth more impermeable, so that the diffusion of ions only occurred through the dentin of the roots and not through the apical foramen [9]. The medicament was compressed with a cotton ball until there is a 2-mm space mm for the gutta-percha (DiaDent Group International Inc, India). Using composite resin (VOCO, India), the roots were individually attached to a metal rod fashioned from orthodontic wire (0.7-mm thick and 5-cm long) and suspended from rubber caps of the vials containing 30 mL of double deionised water. (Manipal pharmaceutical products, India)

**Determination of pH, Hydroxyl and calcium ions concentrations in the solution**

The readings were obtained at 24 hrs, 7 days and 14 days. The solution was changed at each observation period to prevent super saturation of the medium. Then, the samples were frozen at 4°C till the readings were taken [8].

**Measurement of pH**

Digital pH meter (Cyberscan, Eutech Instruments Pvt Ltd, Singapore) [Figure 3] was employed for measurement for pH. The pH meter was calibrated with distilled water of a known pH 7, before and after measurements of each time period. Before each reading, the medium was homogenized by shaking the vial to ensure uniform distribution of the hydroxyl ions. The pH was determined by placing the pH electrode fully immersed in the deionized water in each sample bottle, stirring the solution for 10 seconds and then recording the pH level. For each measurement, the electrode of the pH meter was carefully rinsed with deionized water and dried with absorbent paper to eliminate any residues that might interfere with the measurements.

**Digital pH meter**

Calculation of the Hydroxyl Ion Concentration

From the data of measurement of pH in different time period in all the groups, pOH was tabulated using the equation – [10].

\[
\text{pH} + \text{pOH} = 14 \quad \text{.... (Eq 1)}
\]

or \[\text{pOH} = 14 - \text{pH} \quad \text{.... (Eq 2)}\]

Once pOH was determined \([\text{OH}^-]\) i.e. Hydroxyl ion concentration was tabulated by the reverse mathematical operation employed to find the pOH.

\[
[\text{OH}^-] = \text{antilog} (- \text{pOH})
\]

or \[[\text{OH}^-] = 10^{\text{pOH}}\]

or \[[\text{OH}^-] = 10^{(-\text{pOH})}\]

or \[[\text{OH}^-] = 10^{(-(14-\text{pH})} \quad \text{.... (Eq 3)}\]

**Measurement of Calcium ions**

Atomic absorption spectrometry (AAS, GBC 902 Australia) (Figure 4) was employed for the determination of \(\text{Ca}^{2+}\) concentrations in the medium. Prior to the analysis, a reading was performed of the double deionized water used in the study to determine whether it contains \(\text{Ca}^{2+}\), thereby establishing the baseline for the equipment. The amount of \(\text{Ca}^{2+} \, (\mu g/ml)\) in the double deionized water of the samples was then determined for subsequent analysis.
Results

Graph 1 shows intergroup comparison of pH across the three time periods. On application of repeated measures of ANOVA with post hoc Tukey test, there was highly significant differences (p<0.001) at 7th day between group A and Group B with Group C, with Group C showing lower pH values compared to other 2 groups. But at the end of the study period, the difference between the groups was statistically non-significant.

Graph 1: shows intergroup comparison of pH across the three time periods

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean pH Value</th>
<th>24 Hrs</th>
<th>7 Days</th>
<th>14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>7.12</td>
<td>7.32</td>
<td>7.35</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>7.11</td>
<td>7.40</td>
<td>7.41</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>7.06</td>
<td>7.18</td>
<td>7.41</td>
<td></td>
</tr>
</tbody>
</table>

Graph 2: shows intergroup comparison of hydroxyl ion diffusion across the three time periods

Legend

Group A: Calcium hydroxide with distilled water
Group B: Calcium hydroxide with chlorhexidine solution
Group C: Calcium hydroxide with propylene glycol in form of premixed syringes (Diapaste)

Graph 3 shows intergroup comparison of calcium ion diffusion across the three time periods. There was highly significant difference between group B and Group A at 24 hrs with Group A showing lower Calcium ion values than group B. On the 7th day, there was no statistically significant difference between the three groups (P 0.67).

Graph 3: shows intergroup comparison of calcium ion diffusion across the three time periods

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Ca ion (mg/mL)</th>
<th>24 Hrs</th>
<th>7 Days</th>
<th>14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>1.67</td>
<td>3.22</td>
<td>2.34</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>4.68</td>
<td>3.23</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>3.16</td>
<td>3.58</td>
<td>2.71</td>
<td></td>
</tr>
</tbody>
</table>
Legend

**Group A** Calcium hydroxide with distilled water

**Group B** Calcium hydroxide with chlorhexidine solution

**Group C** Calcium hydroxide with propylene glycol in form of premixed syringes (Diapaste)

**Discussion**

This study was an experimental type of in vitro study conducted in the Department of Pedodontics and Preventive Dentistry, Manipal College of Dental Sciences, Manipal, Karnataka, in collaboration with National Institute of Technology, Surathkal, Karnataka and Jawaharlal Nehru Technological University, Hyderabad.

The medicament used was calcium hydroxide. Since the introduction of Calcium hydroxide for use in dentistry by Herman in 1920 [2] This medicament has been reported to promote healing in many clinical situations. Calcium release and an alkaline pH are extremely important for the biological and microbiological performance of the material. The diffusion of calcium hydroxide through dentinal tubules has been evaluated in several studies. The diffusion through dentinal tubules, apical foramen, secondary and accessory canals allows the calcium hydroxide to reach regions contaminated by microorganisms, areas of root resorption and surrounding tissues, promoting its antimicrobial and anti-resorptive action.

It has been asserted that all biological actions of calcium hydroxide will be progressed by the ionic dissociation of Ca$^{2+}$ And OH ions (Leonardo et al. 1982, Estrela 1997) [11, 12]. The differences in the velocity of ionic dissociation are related directly to the vehicle employed to obtain the paste. Our study used three vehicles: distilled water, 0.2% chlorhexidine solution and a premixed formulation containing calcium hydroxide with propylene glycol.

Our first vehicle was distilled water. One of the most common and easiest ways to prepare a calcium hydroxide formulation is to mix calcium hydroxide powder with water. Advantages of this formulation being, easier to prepare and reduced chairside time [11].

The usefulness of mixing 0.2% chlorhexidine with Calcium hydroxide still remains unclear and controversial (Athanassiadis et al. 2007) [13]. Chlorhexidine is a cationic bisguanide whose optimal antimicrobial activity is achieved within a pH range of 5.5–7.0. It has been shown that addition of chlorhexidine digluconate to calcium hydroxide forms calcium digluconate and a precipitate of chlorhexidine molecules (which is not soluble). (Signoretti et al., 2011) [14]. However studies have shown that the combined use of chlorhexidine and calcium hydroxide in the root canal generates excessive reactive oxygen species, which explains why this mixture shows more efficacy in eliminating endotoxin, even though non soluble chlorhexidine precipitate is formed. (Yeung et al,2007) [15]. However, it has also been demonstrated that the alkalinity of Calcium hydroxide when mixed with chlorhexidine remained unchanged [Haenni S, 2003] [16]. Chlorhexidine was considered as one of the vehicles in our study, to assess if the combination had any advantage with respect to pH, hydroxyl and calcium ion release.

Propylene glycol is a dihydric alcohol and has been used as a vehicle and pharmaceutical solvent in several products. Its hygroscopic nature permits the absorption of water, which ensures a good sustained release of calcium hydroxide for long periods. Another advantage of this substance is its consistency, which improves the handling qualities of the paste (Bairy et al. 1993, Simon et al. 1995) [17, 18].


In primary molar teeth, furcal radiolucency is commonly seen unlike primary anterior teeth where radiolucency is seen at the periapical region. Therefore, to assess the lateral diffusion of intracanal medicament along the dentinal tubules, it was imperative to collect primary molar teeth without any resorption, perforation or surface defect. To rule out perforation/ resorption, a high precision instrument i.e. stereomicroscope has been used. The advantages of stereomicroscope over the traditional ones being great working distance and depth of field thereby reducing the chance of human error.

In the present study, the coronal portion of the tooth sample was sectioned transversely 2mm above the CEJ to obtain a coronal seal which more closely simulates a clinical scenario thereby ensuring that medicament penetration occurred only through the radicular dentine. Also the samples were suspended till the level of CEJ in the deionized water so the diffusion of ions through the radicular dentin was assessed. Which is according to study conducted by Ximenes et al 2012 [8].

It is suggested that the removal of the smear layer may facilitate the diffusion of hydroxyl ions through the dentinal tubules and improve the ability to kill bacteria [27]. The removal of the smear layer from the interior of the root canal also facilitates the diffusion of ions through the dentin tubules, thereby enhancing the action of the medication (Mori et al,2009; Foster et al,1993; Garcia-Godoy,1987) [27,28,29]. Therefore in the present study, prior to using an intracanal Calcium hydroxide based medication, irrigation was performed with 1 % sodium hypochlorite and 17% EDTA for 3 minutes to allow greater action of the medication. The samples were there afterwards washed with saline to prevent any residual chelating action of EDTA. (Saif et al, 2008) [30].
In our study, the root apex was sealed with sticky wax and nail varnish for greater impermeability (Nunes et al, 2005) [9] and coronally with gutta-percha and composite (Ximenes et al 2012) [8] to ensure that the diffusion of ions occurred only through radicular dentin.

The diffusion of ions is dependent on the volume of the medicament in the canal. Keeping all other factors (the vehicle, length and diameter of dentinal tubule) constant, it was hypothesized that the more the volume, more will be the diffusion. Therefore, standardization was done at every step of the study in terms of ratio of powder and liquid, weight of medicament, and the biomechanical preparation hence minimizing the possibility of bias.

For the assessment of OH– and Ca2+ diffusion, the roots remained immersed in double deionized water for the time periods established (24 hours, 7 days, and 14 days). Double deionised water is ultrapure water free of ions like Mg2+, Ca2+, Na+, Cl– which can interfere with readings of the three variables (pH, hydroxyl and calcium ions), thereby reducing the confounding factors and error [31]. Unlike in previous studies, (Nerwich et al, 1993; Simon et al, 1995; Nunes et al, 2005) [4], the double deionised water in which the roots were immersed was replaced on every measurement day to obtain the maximum diffusion of ions without saturating the medium. The samples were frozen at 4 degrees Celsius till analysis. It was in accordance to the study done by Ximenes et al, 2012 [8].

We have assessed the diffusion of ions at 24hrs, 7 days and 14 days. The period needed for calcium hydroxide to optimally disinfect the root canal system is still unknown. Byström et al in 1985 [32] demonstrated that calcium hydroxide effectively eliminated all the microorganisms when the medicament was maintained for 4 weeks. However, Jorgen et al in 1991 [33] have reported that an intracanal medication with calcium hydroxide for 1 week effectively eliminated bacteria in the root canal in 100% of the cases. In a clinical situation, since the obturating material in the primary teeth is strongly antibacterial and it was assumed that an intermediate dressing of 14 days could be employed. So, a period of 14 days was chosen in our study. Also, a change of dressing was not performed between the time periods.

Atomic absorption spectrometer was used to determine Ca2+ ion concentration. It makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration. It can detect the concentrations of analyte up to as low as 0.1µg/ml. Unlike colorimetric method, the chances of human error and contamination is almost nil [34].

In the present study, all three formulations of calcium hydroxide facilitated a gradual increase in pH of the solution above neutral pH but at the same time did not show a large rise either. There may be various explanations for this marginal rise of pH. Primary molar teeth with ribbon shaped canals do not accommodate as much medicament as in permanent tooth. In our present study, the cementum was left intact on the root surface and the two ends of the specimen were sealed. However, in a clinical scenario, presence of open apical foramina, areas of resorption and cementum loss may result in greater rise of pH. Also, in this study the primary molar teeth were sectioned and each root was considered as one sample. But in clinical situations, the multirooted teeth when filled with medicament may result in a greater diffusion of ions into periapical region.

The minimal amount of hydroxyl ions capable of making a medium unviable for bacterial proliferation by neutralizing the acid environment remains unknown. However, as seen by the results of the present study the medium into which the specimens were suspended showed a steady rise in towards alkaline pH signifying that ionic release takes place through dentin of primary roots. These results are in accordance with Nerwich et al, 1999 [4] who reported that hydroxyl ions required 1-7 days to reach the outer root dentine and 2-3 weeks to reach peak levels. All agents showed peak pH and hydroxyl ion concentration at 7 days.

Although combining calcium hydroxide and chlorhexidine has been viewed skeptically, the results of this study show it was capable of raising the pH of the solution comparable to distilled water and this result is accordance to that reported by Haenni S, 2003 [16] that pH remained unchanged on combination with chlorhexidine.

With respect to calcium release, the greatest release was demonstrated by Calcium hydroxide with propylene glycol followed by calcium hydroxide and distilled water.

Calcium hydroxide with chlorhexidine performed poorly when compared to other two groups, in terms of the calcium ion release. It was observed that there was an initial burst of calcium ion followed by steep decrease to negligible amount (1.51µg/ml) at end of study period. It has been reported that Calcium hydroxide when mixed with chlorhexidine forms calcium digluconate and a precipitate of chlorhexidine [14]. In our study, the medicament was freshly mixed just prior to obturation of each sample and the roots were suspended in double deionized distilled water immediately following obturation. Hence it can be assumed that the initial burst was due to the unbound calcium ions present in the medium and the steep decrease of diffusion was due to decreased calcium available for diffusion following the formation of calcium digluconate. The nature and chemistry of calcium digluconate is still unclear and needs further investigation.

Overall, the results of this study confirmed to findings reported in literature that distilled water vehicle shows faster ionic dissociation and viscous vehicles cause slow, constant and prolonged release of ions. Based on results of our study, the null hypothesis was rejected.

The limitation of the present study is that, in vivo conditions were not simulated. The presence of the open apex (which was closed in this study) which is also responsible for diffusion of ions to periapical region, the presence of physiologic and pathologic root resorption (present in most endodontic treatment cases) and the presence of an inflammatory process which reduces the external pH around the root all affect the action of intracanal medicaments.
Therefore more in-vitro studies with larger sample size need to be conducted with simulated in vivo conditions.

**Why This Study Is Important To Pediatric Dentists**

- This study has shown that hydroxyl and calcium ions from the different formulations of calcium hydroxide diffused through the dentin and cementum of the roots of primary teeth.
- This study has shown that peak concentrations for pH, Hydroxyl ion observed at 14 days in all three groups.
- With given parameters, the vehicle of propylene glycol performed the best in length and duration of action while the distilled water vehicle caused rapid ionic release. Therefore in cases where patient follow up is difficult, the vehicle of oil can be preferred.

**References**

