

## Evaluation of pH and calcium ion diffusion from calcium hydroxide pastes and MTA

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### ABSTRACT

The aim of this *ex vivo* study was to evaluate changes in pH and calcium ion diffusion through root dentin from calcium hydroxide (Ca(OH)<sub>2</sub>) and mineral trioxide aggregate (MTA) pastes at 7, 30 and 60 days; and the relationship between pH and ion diffusion. Thirty-two human premolars were used. Crowns were sectioned and root canals instrumented and filled in with the following preparations: 1) Ca(OH)<sub>2</sub> + distilled water (n=7); 2) Ca(OH)<sub>2</sub> + 0.1% chlorhexidine gluconate (n=7); 3) MTA + distilled water (n=7); 4) MTA + 0.1% chlorhexidine gluconate (CHX) (n=7); 5) distilled water (n=2) (control); 6) 0.1% chlorhexidine gluconate (n=2) (control). The apex and coronary opening were sealed with IRM. Roots were placed in Eppendorf

tubes with 1 ml distilled water at 37°C and 100% humidity. At baseline, 7, 30 and 60 days, pH was measured with pH meter, and calcium ion content in the solution was analyzed by atomic absorption spectrophotometry. The data were statistically analyzed using ANOVA, simple linear regression analysis and Pearson's correlation test. The highest pH values were achieved with calcium hydroxide pastes at 60 days ( $p \leq 0.05$ ). Calcium ions were released in all groups. The calcium hydroxide paste with distilled water at 60 days had the highest calcium ion value ( $p \leq 0.01$ ). There was a positive correlation between calcium and pH values.

**Key words:** pH; calcium hydroxide; mineral trioxide aggregate.

## Evaluación del pH y la difusión de iones calcio de pastas de hidróxido de calcio y MTA

### RESUMEN

El objetivo de este estudio *ex vivo* fue evaluar los cambios en el pH y la difusión a través de la dentina radicular de iones calcio a partir de pastas de hidróxido de calcio (Ca(OH)<sub>2</sub>) y trióxido mineral agregado (MTA), durante 7, 30 y 60 días; y la relación entre el pH y la difusión de iones. Se utilizaron 32 premolares humanos. Las coronas fueron seccionadas, los conductos radiculares fueron instrumentados y obturados con las siguientes preparaciones: 1) Ca(OH)<sub>2</sub> + agua destilada (n = 7); 2) Ca(OH)<sub>2</sub> + gluconato de clorhexidina (CHX) al 0,1% (n = 7); 3) MTA + agua destilada (n = 7); 4) MTA + gluconato de clorhexidina al 0,1% (n = 7); 5) agua destilada (n = 2) (control); 6) gluconato de clorhexidina al 0,1% (n = 2) (control). El ápice y la apertura coronaria se sellaron con IRM. Las raíces se

colocaron en tubos Eppendorf con 1 ml de agua destilada a 37°C y 100% de humedad. Se midió el pH inicial y a los 7, 30 y 60 días, con pHmetro, y se analizó el contenido de iones calcio en la solución por espectrofotometría de absorción atómica. Los datos fueron analizados estadísticamente con ANOVA, análisis de regresión lineal y correlación de Pearson. Los valores de pH más altos se obtuvieron con las pastas de hidróxido de calcio a los 60 días ( $p \leq 0,05$ ). Todos los grupos mostraron liberación de iones calcio. La pasta de hidróxido de calcio con agua destilada mostró el valor más alto de iones calcio a los 60 días ( $p \leq 0,01$ ). Hubo una correlación positiva entre los valores de pH y calcio.

**Palabras clave:** pH, hidróxido de calcio; compuesto de trióxido mineral.

### INTRODUCTION

Calcium hydroxide (Ca(OH)<sub>2</sub>) has been widely used as an intracanal medication<sup>1</sup>. It is a strongly alkaline substance (pH 12.5-12.8) with high antibacterial activity against oral bacteria. The effectiveness of Ca(OH)<sub>2</sub> for inactivation of microorganisms and tissue healing is directly related to its dissociation

into calcium and hydroxyl ions. Hydroxyl ions diffuse through the dentinal tubules and inactivate Gram-negative bacterial lipopolysaccharide (LSP)<sup>2</sup>. In addition, their high pH activates tissue enzymes, causing a mineralization effect<sup>3</sup>.

Drug vehicles play an important role in the ion dissociation rate, causing the paste to be solubilized

and resorbed by the periapical tissues from within the root canal<sup>4</sup>. Calcium hydroxide was pioneered by Heithersay<sup>5</sup> and Frank<sup>6</sup> in apexification treatment, which provided adequate apical healing through the induction of an apical barrier. However, long-term calcium hydroxide treatment lasting more than 30 days may reduce dentin fracture strength<sup>7</sup>. Due to its alkaline nature, calcium hydroxide denatures some of the acidic dentin proteins which act as bonding agents, thereby weakening the dentin and increasing risk of fracture<sup>8</sup>.

Mineral trioxide aggregate (MTA) powder is basically a mixture of 75% Portland cement, 20% bismuth oxide and 5% gypsum<sup>9</sup>. It was developed in 1990 by Torabinejad at Loma Linda University<sup>10</sup>. MTA has been recognized as a bioactive<sup>11</sup>, hard tissue conductive<sup>12</sup>, hard tissue inductive, biocompatible material<sup>13</sup>. It has been used for apical barriers, root-end fillings, perforation repairs, regenerative therapy, pulp capping and pulpotomies<sup>14</sup>. During the setting process, MTA pH is initially 10.2, increasing to 12.5 during the first few hours. MTA has also been shown to have antibacterial activity<sup>15</sup>.

Chlorhexidine gluconate (CHX) is widely used as an irrigant for the treatment of infected root canal systems to reduce endodontic microbiota<sup>16</sup>. Many studies suggest the use of a combination of CHX with Ca(OH)<sub>2</sub> for greater antimicrobial action<sup>17</sup>. Chlorhexidine is a broad spectrum antibacterial agent with effectiveness against *Enterococcus faecalis* and *Candida albicans*<sup>18</sup>. CHX can be adsorbed onto dental tissue, resulting in substantive antibacterial activity<sup>19</sup>. Stowe et al.<sup>15</sup> demonstrated that the substitution of sterile water by 0.12% CHX in tooth-colored ProRoot MTA enhanced the antimicrobial effect of the MTA *in vivo*.

MTA could be an alternative to Ca(OH)<sub>2</sub> to halt resorption without the disadvantage of weakening tooth structure<sup>20</sup>. Use of MTA must be considered permanent because it is difficult to remove once it has set<sup>21</sup>.

The aim of this *ex vivo* study was to evaluate the relationship between pH and diffusion of calcium ions through root dentin using Ca(OH)<sub>2</sub> and MTA pastes with different vehicles for 7, 30 and 60 days.

## MATERIALS AND METHODS

### Preparation of specimens

Thirty-two single-root human mandibular premolars, recently extracted for orthodontic reasons, were

selected on the basis of their similarity in morphology and size. Crowns were amputated at cemento-enamel junction level using a high speed bur #2200 (KG Sorensen, SP, Brazil) and water irrigation. The cementum was then removed using Gracey curettes. Root canals were enlarged up to file #45 (Maillefer, East Lansing, MI, USA), and cleaned and shaped using the step-back technique. After each instrument change, root canals were irrigated with 2 ml 1% sodium hypochlorite, and 17% EDTA was used as final irrigation for 2 minutes. Then root canals were rinsed with distilled water, dried with absorbent paper points, randomly divided into six groups and filled with the following preparations: Group I: 1 g calcium hydroxide (Farmadental Lab, Buenos Aires, Argentina) with 1.5 ml distilled water (Ca(OH)<sub>2</sub> + DW); Group II: 1 g calcium hydroxide with 1.5 ml 0.1% chlorhexidine (Ca(OH)<sub>2</sub> + CHX); Group III: MTA (CPM™, Egeo S.R.L., Buenos Aires, Argentina) with distilled water (MTA + DW). The powder/liquid ratio was 0.33/1 (w/w). Group IV: MTA with 0.1% chlorhexidine (MTA + CHX). The powder/liquid ratio was 0.33/1 (w/w). Group V: distilled water as a control group (DW); Group VI: 0.1% chlorhexidine (CHX), prepared from a 20% chlorhexidine solution, as a control group.

In the experimental groups, the paste was placed using a Lentulo spiral (Dentsply, Mailfer, Switzerland). Once the root canal was filled completely, the apical foramen and root canal openings were sealed with restorative temporary cement, IRM™ (Dentsply, USA). The roots were then stored in individual plastic tubes (Eppendorf) containing 1 ml distilled water (Fig. 1). They were kept at 37 °C and 100% relative air humidity throughout the testing period. After 7, 30 and 60 days, the water was assessed for pH and calcium ion release.

### Analyses of pH and calcium ion release pH readings

The pH was determined with a digital pH meter (Broadley-James Irvine, California, USA) for small volumes (sensitivity: 0.01 pH units), calibrated to pH 7 and 4 with standard buffer solutions before use. The pH was determined by placing the refillable calomel electrode in 30 µl of sample on a slide for 10 seconds. The electrode was washed with distilled water and wiped dry between readings.



Fig. 1: Experimental groups were stored in individual plastic tubes (Eppendorf).

### Calcium ion release readings

To determine the concentration of calcium ions, 970  $\mu$ l of each sample were transferred to a 5 ml flask, and 5 g/l of potassium chloride and 5g/l of lanthanum chloride were added to eliminate interferences by acidifying the samples with hydrochloric acid. The final volume was completed with distilled water. A calibration curve was prepared with standard solutions of 1.5, 2.5 and 5 mg/l of Ca (Certipur Merck to NIST).

Atomic Absorption Spectroscopy (Perkin Elmer AAnalyst 100) with acetylene flame was used. Calcium in the samples was determined at 422.7 nm wavelength. Each value was expressed in mg/l. Measurements were performed at 7, 30 and 60 days.

### Statistical analysis

Differences between study groups were statistically analyzed by ANOVA at a significance level of 5%.

Simple linear regression analysis and Pearson's correlation test were used to evaluate the relationship between calcium ion release and pH over time.

### RESULTS

Table 1 shows mean pH values for the pastes at the different experimental times. The pH values increased over time for all groups. At 7 days, all test groups differed significantly from the control groups. There was no statistically significant difference between treatments and controls at 30 days. At 60 days, Group I presented the highest pH value. There was a considerable difference between  $\text{Ca}(\text{OH})_2$  and MTA groups, but no difference between Groups I and II ( $p > 0.05$ ).

Groups III and IV did not differ significantly either at different time intervals or from each other (statistical data not shown).

Mean values for calcium ions released for all groups are shown in Table 2. Calcium ion diffusion occurred in all groups. At 7 days, there were statistically significant differences between Groups I and II and the control Groups ( $p \leq 0.05$ ). Group II presented the highest value for calcium ion release at 7 days and was the only group that differed significantly from the rest of the experimental groups ( $p \leq 0.05$ ).

At 30 days, Group I showed the highest value for calcium. There was no significant difference between Group II and Group IV ( $p > 0.05$ ).

Moreover, Groups III and IV did not differ significantly from the control group.

Group I released the most calcium ions and achieved the highest mean at 60 days, differing significantly from all the other groups ( $p \leq 0.05$ ). Group II also differed considerably from the rest of

Table 1: Mean values for pH and standard deviations for all periods and groups.

Groups	Initial	7 days	30 days	60 days
I. $\text{Ca}(\text{OH})_2$ + DW	6.48 $\pm$ 0.00	7.55 $\pm$ 0.05 <sup>b</sup>	8.93 $\pm$ 0.78 <sup>a</sup>	12.2 $\pm$ 0.26 <sup>b</sup>
II. $\text{Ca}(\text{OH})_2$ + CHX	6.48 $\pm$ 0.00	7.66 $\pm$ 0.23 <sup>b</sup>	8.64 $\pm$ 0.84 <sup>a</sup>	11.4 $\pm$ 1.00 <sup>b</sup>
III. MTA + DW	6.48 $\pm$ 0.00	7.72 $\pm$ 0.11 <sup>b</sup>	7.89 $\pm$ 0.24 <sup>a</sup>	8.27 $\pm$ 0.26 <sup>a</sup>
IV. MTA + CHX	6.48 $\pm$ 0.00	7.84 $\pm$ 0.11 <sup>b</sup>	8.00 $\pm$ 0.30 <sup>a</sup>	8.00 $\pm$ 0.23 <sup>a</sup>
V. DW	6.48 $\pm$ 0.00	7.17 $\pm$ 0.14 <sup>a</sup>	7.78 $\pm$ 0.02 <sup>a</sup>	7.53 $\pm$ 0.08 <sup>a</sup>
VI. CHX	6.48 $\pm$ 0.00	7.18 $\pm$ 0.10 <sup>a</sup>	7.83 $\pm$ 0.05 <sup>a</sup>	7.47 $\pm$ 0.05 <sup>a</sup>

DW: distilled water;  $\text{Ca}(\text{OH})_2$ : calcium hydroxide; CHX: chlorhexidine; MTA, Mineral trioxide aggregate. Different letters in the column indicate statistically significant differences between the groups in each study period ( $p \leq 0.05$ ).

the pastes. MTA pastes did not differ significantly from each other or from the control group.

There was no significant difference between 7 and 30 days in Group II, although a difference appeared at 60 days (statistical data not shown).

At all the experimental times, the MTA groups showed the lowest values for calcium ion release (Table 2).

There was a positive correlation between calcium ions and pH in experimental groups I, II, III and IV (Fig. 2), with calcium ions increasing as pH values increased. According to Pearson's correlation test, the correlation between pH and calcium ion diffusion was 69% at 7 days, 98% at 30 days and 97% at 60 days.

Control groups V and VI showed a negative correlation, with calcium ions decreasing as pH values increased (Fig. 2).

## DISCUSSION

The therapeutic effects of calcium hydroxide depend on the dissociation of calcium and hydroxyl ions and the availability of hydroxyl ions to increase the pH of the medium<sup>22</sup>. The greater the number of hydroxyl ions, the higher the pH. Many components are mixed with Ca(OH)<sub>2</sub> including various vehicles, such as propylene glycol, distilled water, anesthetic solution, saline solution, chlorhexidine, chitosan and

antibiotics<sup>23</sup>. Propolis<sup>24</sup> and *Aloe vera*<sup>25</sup> have also been proposed as vehicles for Ca(OH)<sub>2</sub>.

Considering that the efficacy of distilled water (DW) as a vehicle for Ca(OH)<sub>2</sub> has been demonstrated in the literature<sup>3</sup>, it was used in this study as reference group for comparisons.

Nerwich et al.<sup>26</sup> measured pH changes in root dentin over a four-week period, which they considered to be a reasonable time interval in which to expect effective therapeutic benefits from Ca(OH)<sub>2</sub> based materials. However, long-term and short-term

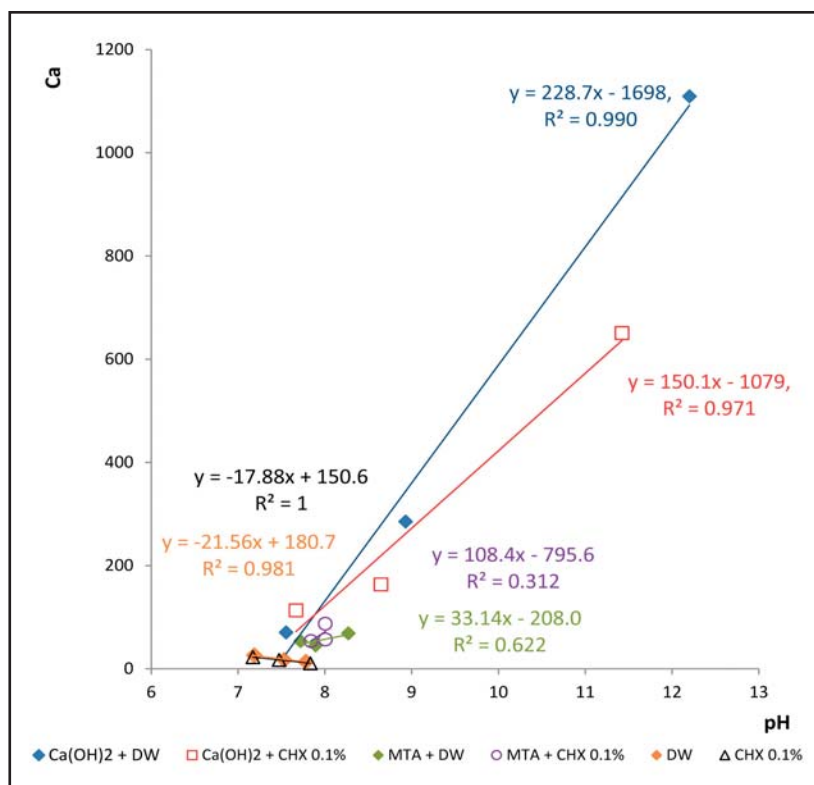


Fig. 2: Correlation between pH and calcium ion diffusion.

**Table 2: Mean values and standard deviations for calcium ions (mg/l) for all treatments and periods.**

Groups	7 days	30 days	60 days
I. Ca(OH) <sub>2</sub> + DW	70.47 ± 24.64 <sup>b</sup>	285.27 ± 82.47 <sup>c</sup>	1109.33 ± 79.41 <sup>c</sup>
II. Ca(OH) <sub>2</sub> + 0.1% CHX	113.27 ± 11.97 <sup>c</sup>	163.47 ± 63.22 <sup>b</sup>	650.67 ± 157.60 <sup>b</sup>
III. MTA + DW	53.47 ± 8.81 <sup>ab</sup>	45.40 ± 12.94 <sup>a</sup>	68.67 ± 26.31 <sup>a</sup>
IV. MTA + 0.1% CHX	53.70 ± 13.46 <sup>ab</sup>	57.03 ± 4.35 <sup>ab</sup>	87.33 ± 11.15 <sup>a</sup>
V. DW	26.20 ± 0.36 <sup>a</sup>	13.57 ± 3.21 <sup>a</sup>	17.33 ± 3.21 <sup>a</sup>
VI. 0.1% CHX	22.47 ± 2.67 <sup>a</sup>	10.60 ± 1.37 <sup>a</sup>	17.00 ± 5.57 <sup>a</sup>

DW: Distilled water; Ca(OH)<sub>2</sub>: calcium hydroxide; CHX: chlorhexidine; MTA, mineral trioxide aggregate. Different letters in the column indicate statistically significant differences between treatments in the same time period ( $p \leq 0.05$ ).

Ca(OH)<sub>2</sub> treatment may reduce dentin fracture strength<sup>7,8</sup>. MTA could be a treatment option in cases with external inflammatory root resorption, particularly when root fracture could be a risk<sup>20</sup>.

The diffusion of Ca(OH)<sub>2</sub> through dentinal tubules has been evaluated in several studies<sup>22, 27-29</sup>. In this study, calcium ion release from MTA and Ca(OH)<sub>2</sub> was evaluated over two months. The accuracy of the technique used is important for precise assessment of the amount of calcium ion released. Several methods such as atomic absorption spectrometry, ultraviolet spectrophotometer, fluorometry, flame photometry and complexometric titration with EDTA have been used<sup>30</sup>. The present study used an atomic absorption spectrometer.

In the present study, all the experimental groups showed an increase in pH over time. This is in agreement with Duarte et al.<sup>31</sup>, who found that all pastes behaved similarly in terms of pH and calcium ion release for the different study periods.

The vehicles employed in calcium hydroxide pastes and MTA influence their diffusion capacity. Estrela et al.<sup>3</sup> explained that distilled water allows the fastest and most significant dissociation. This is consistent with our study, where the highest levels of calcium ion release were obtained when distilled water was used as a vehicle. The highest pH values were also observed with distilled water at 60 days.

Hansen et al.<sup>32</sup> found different pH values at different levels of the root, possibly related to the number and direction of dentinal tubules at each level. Perez et al.<sup>33</sup> showed that the pH of dentin depends on the type, location and duration of Ca(OH)<sub>2</sub> application. In agreement with this, our study found higher pH and calcium ion values at 60 days.

Evans et al.<sup>34</sup> reported that *Enterococcus faecalis* was resistant to Ca(OH)<sub>2</sub> at pH 11.1, but at pH 11.5, 99.9% were killed. In a previous study, we found that the pH values of the experimental groups were not alkaline enough to eliminate *E. faecalis* from the root canal in the lapse of a week or even a month<sup>35</sup>. However, in the present study, Ca(OH)<sub>2</sub> with DW at 60 days had a pH value (12.2 ± 0.26) high enough to kill *E. faecalis*.

Due to the antimicrobial effectiveness of CHX, it has been suggested as a vehicle for Ca(OH)<sub>2</sub> pastes. The antimicrobial activity of the association of Ca(OH)<sub>2</sub> and CHX has already been evaluated, revealing effectiveness against several bacterial

populations<sup>17,36</sup>. Stowe et al.<sup>15</sup> observed that adding CHX to MTA could enhance antimicrobial activity. According to our results, CHX would be an efficient vehicle for Ca(OH)<sub>2</sub> allowing the diffusion of calcium ions. Moreover, for MTA pastes using CHX as vehicle, calcium ion diffusion was similar or higher than for MTA with distilled water.

Mori et al.<sup>37</sup> concluded that the concentration of CHX influenced calcium diffusion. They demonstrated that 2% CHX allowed higher diffusion than 0.2% CHX. In contrast, the present study found higher calcium ion diffusion with 0.1% chlorhexidine than the results reported by Mori et al.<sup>37</sup>. Another interesting factor to consider is the higher viscosity and easier preparation of the pastes with CHX compared to those prepared with distilled water.

This study showed that at 30 days there was no difference in the pH values between Ca(OH)<sub>2</sub> pastes and MTA pastes. However, Heward et al.<sup>20</sup> found that after four weeks, intracanal placement of MTA resulted in significantly higher pH than did Ca(OH)<sub>2</sub>. Differences in methodologies might explain this apparent disparity.

Ozdemir et al.<sup>38</sup> showed diffusion of calcium through the defects in the dentin in MTA-filled roots with a significant increase in concentration over time. Each drug presents different diffusion characteristics, which are directly related to its interaction with the tooth structure<sup>39</sup>. In the present study, MTA pastes present the lowest calcium ion release values at all experimental times. In contrast, Tonomaru-Filho<sup>40</sup> reported that MTA and Sealer 26 presented the highest values of calcium ion release after 28 days, out of six sealer materials evaluated. Similarly to our results, Tonomaru-Filho<sup>40</sup> also reported that control group released calcium ions, even though the teeth were unfilled, because the tooth itself can release calcium ions from its structure.

In this *in vitro* study, all the pastes analyzed increased the pH values over time, although calcium hydroxide and distilled water pastes showed the highest pH values and calcium ion diffusion. The greatest diffusion of ions took place between 30 and 60 days. Calcium hydroxide paste groups showed better diffusion capacity than MTA. Nevertheless, further studies should be undertaken to determine whether MTA could be a good option for teeth at risk of fracture.

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