

# EFFECTS OF CORONAL LEAKAGE ON CONCENTRATION OF HYDROGEN IONS AND CALCIUM RELEASE OF SEVERAL CALCIUM HYDROXIDE PASTES OVER DIFFERENT PERIODS OF TIME

## ABSTRACT

**PURPOSE:** To evaluate the effects of coronal leakage on concentration of hydrogen ions (pH) and calcium release of several calcium hydroxide pastes, over different periods of time. **MATERIAL AND METHODS:** Fifty extracted human mandibular central incisors (n=10) were instrumented up to the F2 instrument and assigned to the following intracanal dressing: G1- Calen, G2- Calen with 0.4% chlorhexidine (CHX), G3- Calcium hydroxide with camphorated paramonochlorophenol (CPMC) and glycerin, G4- Calen, but temporary filling material maintained during all test (positive control) and G5- Root canal without intracanal dressing (negative control). All groups were immersed in distilled water for 7 days. In sequence, the temporary filling materials were removed, except in controls groups. All specimens were individually mounted on a specific device and only its root again immersed in distilled water. Concentration of hydrogen ions and calcium release by calcium hydroxide pastes in distilled water were evaluated in 24h, 7, 14 and 28 days. The results were submitted to ANOVA test ( $p = 0.05$ ). After 28 days, root canals from experimental groups were examined in SEM. **RESULTS:** G1, G2, G3 and G4 presented similar pH values and calcium release and did not differ from each other ( $p > 0.05$ ), up to 7 days. After this time G1, G2 and G3 presented values lower values than G4 ( $p < 0.05$ ). In SEM analysis, calcium hydroxide residues were observed in all experimental groups. **CONCLUSIONS:** After 7 days, coronal leakage decreased the concentration of hydrogen ions and calcium ion release provided by all calcium hydroxide pastes.

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## KEYWORDS

Calcium. Camphorated parachlorophenol. Chlorhexidine. Hydrogen. Scanning electron microscopy.

## INTRODUCTION

Calcium hydroxide (CH) is recommended as intracanal dressing, because of its adequate biologic and antimicrobial effects.<sup>1</sup> To facilitate the handling, several vehicles have been proposed, providing different concentration of hydrogen ions (pH) values and calcium ion release.<sup>2-5</sup> Combination of CH with other antimicrobial substances, such as chlorhexidine (CHX) or camphorated paramonochlorophenol (CMPC), to promote a synergistic effect have also been proposed.<sup>5,6</sup> The Calen paste (S.S. White, Rio de Janeiro, RJ, Brazil) is a CH-based medication associated with a vehicle (polyethylene glycol 400) that permits slower liberation of hydroxyl ions, maintaining its action for a longer period.<sup>5</sup>

On the other hand, CHX is a molecule which form positively charged ion that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and has a wide use as endodontic irrigation solution.<sup>5-7</sup> Currently have been suggested that CHX could be mixed with CH to improve antimicrobial efficacy against CH-resistant microorganisms.<sup>8,9</sup>

The combination of CH and CMPC was proposed also to promote an antimicrobial synergistic effect against some facultative and anaerobic bacteria.<sup>10</sup> This combination forms calcium para-chlorophenolate, which maintain a high pH value and allows a controlled released of calcium and hydroxyl ions.<sup>3,11</sup>

The efficacy of CH paste as an intracanal medication is due to its ionic effect based on chemical dissociation into calcium and hydroxyl ions in aqueous solution, which promotes alkalization of the medium.<sup>12,13</sup> The high concentration of hydrogen ions (pH) induces hard tissue formation through mineralization and is also responsible for its bactericidal effect.<sup>14</sup> Studies have been performed to assess the pH changes on the external surface of apical root dentin when CH is used as intracanal medication.<sup>15</sup> However, the higher pH change on the external root surface occurs near the apex when the canal is completely filled with CH, in comparison to when filled 3 or 5 mm shorter of the apical foramen.<sup>16</sup> For intracanal dressing have beneficial effect is necessary that calcium hydroxide paste filled all root canal.

After intracanal CH removal procedures frequently is observed residues on dentin root canal, but the long-term effects these residues are controversial, not know its impact on prognosis root canal treatment.<sup>17</sup> On the other hand, in coronal unsealed teeth but with intracanal CH paste, contamination of the root canal is retarded due to the low solubility of CH.<sup>18</sup> In several clinical situations, there is displacement of temporary filling material and root canal is exposed to oral environment. In these conditions, CH residues are maintained intracanal but its effects beyond the radicular apices are unknown. Therefore, no previous

study was evaluated if calcium hydroxide intracanal dressing maintains the same concentration of hydrogen ions and calcium ion release after coronal leakage.

The aim of this study was evaluate the concentration of hydrogen ions and calcium ions release of intracanal dressing with CH paste, alone or mixed with 0.4% chlorhexidine digluconate or CMPC and glycerin, in the periods of 24 h, 7, 14 and 28 days, after coronal leakage in the absence of a temporary filling material. , compared to root canal filled with CH paste (Calen) and coronal access closed. The hypothesis of this study was that there were statistically differences among concentration of hydrogen ions and calcium ion release provided by CH intracanal dressing after coronal leakage, with different chemical formulation, when compared to CH intracanal dressing without coronal leakage, in several periods of time.

## MATERIAL AND METHODS

After the approval by Ethics Committee (protocol 373486/2010), fifty similar extracted human mandibular incisors kept in a solution of 0.1% thymol, were selected and standardized with a length of 16 mm from the root apex. The canals were initially explored with the K file # 15 and prepared with ProTaper rotary instruments (Dentsply Maillefer - Ballaigues - Switzerland) up to the F2 instrument. The working length was established until its tip to be visible at the root apex. During instrumentation of all teeth, 5 mL of

2.5% NaOCl (Rioquímica - São José do Rio Preto - SP - Brazil) solution was used for irrigation, at each change of instrument.

After biomechanical preparation, the root canals were then filled with 17% EDTA (Biodinâmica - Ibiporã - PR - Brazil) for 3 min. All the canals were finally rinsed with 5 mL of 2.5% NaOCl, aspirated and dried with absorbent paper points. In sequence, the apical foramen was covered with wax and two coats of waterproofing agent (Resina Multiuso - Hydronorth - Londrina - PR - Brazil) were applied over the radicular surface.

The specimens were divided into three experimental groups (n = 10, each) and two control groups (n = 10, each). The experimental groups were: G1- CH paste (Calen; - SS White - São Paulo - SP - Brazil) consisting of calcium hydroxide, zinc oxide, colophony (pine resin) and polyethyleneglycol; G2- CH paste (Calen - SS White - São Paulo - Brazil) with chlorhexidine digluconate 0.4% (Farmácia Arte e Ciência - Araraquara - SP - Brazil), at a proportion of 1.96 g of paste mixed with 0.04 mL of 20% chlorexidine digluconate (Farmácia Arte e Ciência, Araraquara, SP, Brazil), according to Silva et al.<sup>5</sup>; and G3- CH paste (Biodinâmica Ind. Com. - Ibiporã - PR - Brazil) with camphorated paramonochlorophenol (Biodinâmica Ind. Com. - Ibiporã - PR - Brazil) and glycerin (SS White - São Paulo - SP, Brazil), respectively in the proportion of 1 g: 0.5 mL: 0.5 mL, according to Vianna et al.<sup>13</sup>. The control groups were: G4 (positive control) - CH paste (Calen - SS White - São Paulo - SP, Brazil) maintained intracanal during all experimental periods and coronal access sealed and G5 (negative control) - Root canal without intracanal dressing.

The intracanal dressing was placed in root canal using Lentulo spiral (Maillefer, Ballaigues, Switzerland) until the paste extrude apically. The teeth were radiographed to confirm the complete filling of the root canal. The coronal access was temporarily filled with glass ionomer cement (Maxion R; FGM, Joinville, SC, Brazil) and then kept immersed in a plastic flask containing 10 mL of distilled water for 7 days. In positive control group (G4), root canal was filled only with CH paste (Calen), which was maintained throughout the study and coronal access also filled with glass ionomer cement but covered by two coats of waterproofing agent and nail polish.

After this period, the temporary filling material was removed, except in G4 and G5 (positive and negative control groups). In sequence, an apparatus similar to described by Siqueira et al.<sup>18</sup> was mounted to each specimen and only radicular portion was again individually immersed in 10 mL of distilled water. The coronal portion of the apparatus was filled with 3 mL of distilled water, replaced daily. After 24 hours, 7, 14 and 28 days the specimens were transferred to new flasks, containing other 10 mL of distilled water. A total of 120 samples of the experimental groups, 40 positive control samples and 40 samples from negative control group were obtained. All specimens were maintained in 37 °C during all experiment.

In each period, the concentration of hydrogen ions (pH) measurement was made directly in distilled water, using pHmeter (DM23; Digimed, São Paulo, SP, Brazil) with pH electrode (DME-CV1; Digimed, São Paulo, SP, Brazil), at ambient temperature of 25°C. The calcium ions released (in mg/L) was obtained using a calcium ion-selective electrode (Q838; Quimis, Diadema, SP,

Brazil), in a specific unit to ions dosage (Q400I; Quimis, Diadema, SP, Brazil). To all measures, the equipment was calibrated in accordance to manufacturer.

Previous to the immersion of specimens, the pH and calcium ions concentration of distilled water were verified, attesting pH 7.1 and total absence of calcium. The data obtained for the concentration of hydrogen ions (pH) and calcium ion release, in all periods, were submitted to ANOVA, at 5% significance.

To confirm the presence of residues of the CH paste, 6 teeth from each experimental were analyzed in SEM (LEO, model 1450VP; Carl Zeiss, UK), in 120X, at 10 kV after 28 days, as described by Kuga et al.<sup>17</sup>

## RESULTS

In the negative control group (G5), the pH of the distilled water was kept at 7.1 and calcium ions were not detected in any of the periods, and all periods the values were lower than other groups ( $p < 0.05$ ). In 24 hours and 7 days, G1, G2 and G3 presented similar pH and calcium release to G4 and did not differ from each other ( $p > 0.05$ ). After 7 days, experimental groups presented lower pH and ions calcium release than G4 ( $p < 0.05$ ). The mean value and standard deviation of pH and calcium ions released by the experimental and control groups are described in Tables 1 and 2, respectively.

The SEM images showed the presence of residues of calcium hydroxide paste on radicular dentin in all specimens of the experimental groups. Figure 1 show the presence of calcium hydroxide dressing residues in experimental groups.

Table 1: Means and standard deviation of concentration of hydrogen ions (pH) for experimental and controls groups, in different times.

	Groups				
	G1	G2	G3	G4	G5
24 h	11.37 <sup>a</sup>	11.30 <sup>a</sup>	11.07 <sup>a</sup>	11.50 <sup>a</sup>	7.10 <sup>b</sup>
	(0.31)	(0.28)	(0.29)	(0.25)	(0.0)
7 days	10.32 <sup>a</sup>	10.19 <sup>a</sup>	10.22 <sup>a</sup>	10.51 <sup>a</sup>	7.10 <sup>b</sup>
	(0.24)	(0.13)	(0.17)	(0.15)	(0.0)
14 days	9.29 <sup>b</sup>	9.11 <sup>b</sup>	9.12 <sup>b</sup>	10.32 <sup>a</sup>	7.10 <sup>c</sup>
	(0.19)	(0.22)	(0.13)	(0.10)	(0.0)
28 days	8.21 <sup>b</sup>	8.10 <sup>b</sup>	8.22 <sup>b</sup>	10.30 <sup>a</sup>	7.10 <sup>c</sup>
	(0.14)	(0.20)	(0.16)	(0.15)	(0.0)

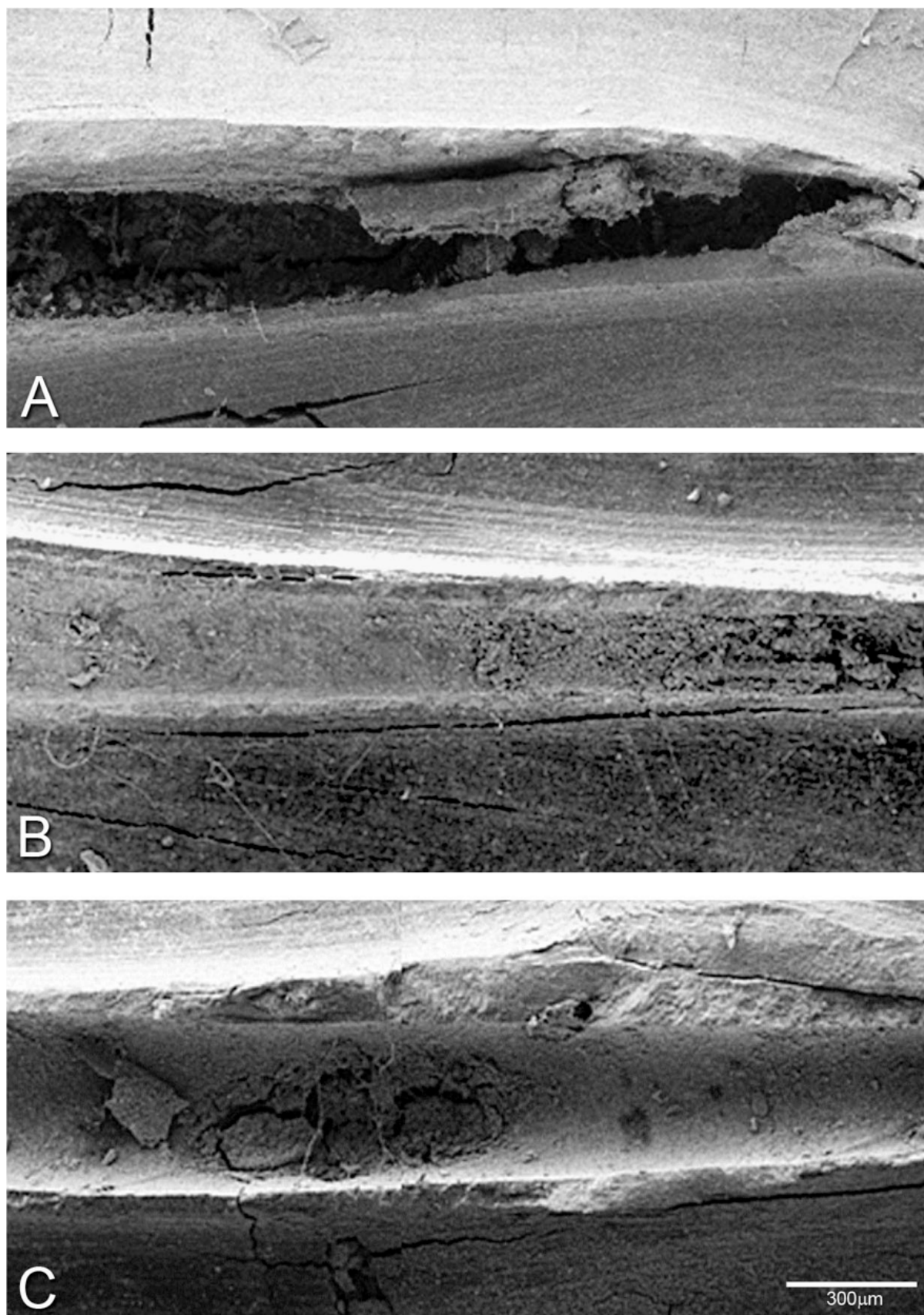
a,b,c Different letters indicate statistically significant differences in same line ( $p < 0.05$ ). G1- Calen, G2- Calen + 0.4% chlorhexidine digluconate, G3- Calcium hydroxide + camphorated paramonochlorophenol + glycerin, G4- positive control, G5- negative control.

Table 2: Means and standard deviation of calcium ion release (mg/L) for experimental and controls groups, in different periods.

	Groups				
	G1	G2	G3	G4	G5
24 h	9.49 <sup>a</sup>	8.87 <sup>a</sup>	9.42 <sup>a</sup>	9.51 <sup>a</sup>	0.00 <sup>b</sup>
	(1.22)	(1.71)	(1.07)	(0.02)	(0.0)
7 days	7.38 <sup>a</sup>	6.78 <sup>a</sup>	6.92 <sup>a</sup>	7.37 <sup>a</sup>	0.00 <sup>b</sup>
	(0.83)	(1.11)	(0.97)	(0.15)	(0.0)
14 days	3.19 <sup>b</sup>	3.46 <sup>b</sup>	3.48 <sup>b</sup>	5.31 <sup>a</sup>	0.00 <sup>c</sup>
	(0.98)	(1.29)	(1.39)	(0.25)	(0.0)
28 days	2.34 <sup>b</sup>	2.82 <sup>b</sup>	2.51 <sup>b</sup>	4.52 <sup>a</sup>	0.00 <sup>c</sup>
	(1.14)	(1.16)	(1.17)	(0.12)	(0.0)

a,b,c Different letters indicate statistically significant differences in line ( $p < 0.05$ ). G1 - Calen, G2 - Calen + chlorhexidine digluconate, G3 - Calcium hydroxide + camphorated paramonochlorophenol + glycerin, G4 - positive control, G5 - negative control.

Figure 1: SEM images (120X) of experimental groups: (A) G1- Calen, (B) G2- Calen + chlorhexidine, (C) G3- Calcium hydroxide + camphorated paramonochlorophenol + glycerin.



## DISCUSSION

The hypothesis this study was partially accepted, because concentration of hydrogen ions and calcium ions release provided by calcium hydroxide intracanal dressing when there was coronal leakage was lower than presented by positive control group, but only after 7 days.

The calcium ion participates in the mineralization healing process and the hydroxyl ion promotes an alkaline environment in the surrounding tissues.<sup>19,20</sup> There are various studies evaluating the alkalinizing potential and calcium ion release by calcium hydroxide pastes.<sup>3,20,21</sup> However, only in present study was evaluated the effects of coronal leakage on concentration of hydrogen ions and calcium ions release provided by calcium hydroxide pastes with several chemical compositions.

Only after 7 days, coronal leakage due to absence of a temporary filling material interfered negatively on concentration of hydrogen ions and calcium ions release of CH pastes. After this time, possibly occurred partial dissolution of the CH dressing, as observed in figure 1. Thus, probably there was reduction in the volume of CH in root canal and, as the higher pH value on the external root surface near the apex is obtained when the canal is completely filled, were observed these results.<sup>16</sup> Another factor that possibly

contributed to these results avoiding the immediate pH and calcium ions release decrease was due to low dissolution of the CH pastes.<sup>2,20,22</sup>

The pH evaluation methodology used has already been established in literature.<sup>4,24,25</sup>

The atomic absorption spectroscopy is used to evaluate the calcium release from CH pastes, but this method quantified the total calcium present in the sample.<sup>24</sup> Due to the participation of mainly the ionized calcium in biological processes, our study used electrodes selective to calcium ions which quantify only the ions present in sample.<sup>19,20</sup>

The tooth length was standardized to 16 mm and instrumented to the F2 instrument. This procedure aimed to standardize the root canal diameter, and consequently the amount of CH paste placed inside the canal. All external root surfaces were coated with nail polish, except in apical foramen region. The apical preparation was performed at the working length, as recommend by Verissimo et al.<sup>26</sup>

The vehicle mixed with CH interferes in its physical and chemical properties and clinical applications.<sup>14</sup> Additives, such as chlorhexidine and CMPC, are often mixed with CH to use in infected root canal.<sup>6,12,14</sup> The use of CH with CHX is unclear and controversial, but it has been demonstrated that the alkalinity of CH remained unchanged after mixing.<sup>14,23</sup> In our study, the CH with 0.4% CHX was chosen

because of its low cytotoxic effect.<sup>5</sup> The CH with glycerin and CMPC is another mixture to be used as intracanal dressing for cases of endodontic infections, and provides an adequate alkalization and calcium ion release.<sup>6,13,14</sup> Chemically, it has been shown that the combination of CH and CMCP produces p-chlorophenolate.<sup>11</sup> In present study, all vehicles showed the similar behavior. If there was no coronal leakage, such as proposed in G4, the concentration of hydrogen ions and calcium ion release decrease but significantly lower than other groups.

Considering the result obtained in present study is important to emphasize that coronal leakage through the intracanal dressing after 7 days decreased the concentration of hydrogen ions and calcium ion release provided by several CH pastes. After this time, due to dissolution of CH dressing is desirable to replacement a new CHdressing, regardless of vehicle previously used.

### CONCLUSION

After 7 days, coronal leakage through intracanal dressing decreased the concentration of hydrogen ions and calcium ion release provided by calcium hydroxide paste, alone or mixed with 0.4% chlorhexidine digluconate or CMPC and glycerin.

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