Original Research Article

**In vitro** analysis of the pH alteration of the dentine after using different calcium hydroxide-based pastes

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**Abstract**

**Introduction and Objective:** To analyze the pH increase at the external root surface after the use of different calcium hydroxide pastes (Calen, calcium hydroxide associated with 2% chlorhexidine gel, calcium hydroxide associated with saline) with and without EDTA as chelating agent before the topical application of the intracanal medication. **Material and methods:** One-hundred single-rooted extracted teeth were cleaned and shaped. They were randomly divided into six experimental groups (n = 15) and one control group (n = 10), according to the medication to be used. The teeth were kept immersed in saline solution and the pH measurements were weekly verified with the aid of a pH meter. **Results:** It was verified the pH increasing at the first week in almost all groups. Only the groups in which Ca(OH)₂ was associated with 2% chlorhexidine gel exhibited a significant evolution in the pH increasing over time (p = 0.0116). The use of EDTA did not result in higher pH values (p = 0.2278). **Conclusion:** i) the pH increased in all associations used; ii) 2% chlorhexidine gel allowed the gradual pH increasing over time; iii) the smear layer removal did not influence on the pH increasing.
Introduction

The aim of the endodontic treatment is the complete removal of the pulpal tissues and/or microorganisms within this root canal system, enabling a good quality filling and consequently the regeneration/repairing of the periapical tissues [9, 20]. For this purpose, combinations of endodontic techniques of instrumentation, irrigation/aspiration, use of intracanal medicaments and pulp cavity filling which eliminates the microorganisms within the infected root canals [5, 31].

The success rate of the conventional endodontic treatment reported by the literature ranges from 80% to 85% [26]. Therefore, the failure quite frequently occurs and demands the execution of the endodontic retreatment, because there is an increasing need of the maintenance of the teeth on the arches [5]. The main causes of endodontic failure are coronal infiltration, incomplete cleaning and filling, anatomic alterations and occlusal trauma [9, 28].

Teeth exhibiting endodontic failure have a considerable diversity of microorganisms [20, 27]. Compositions of calcium hydroxide-based pastes have been proposed over time and they have shown very positive antiseptic activity against these bacterial strains [1, 5, 15, 31].

Studies utilizing calcium hydroxide pastes as intracanal medications have demonstrated that at minimum intervals of seven days there is a significant reduction in the endodontic microbiota [25], consume of CO₂ [11], alkalization of the dentinal tissue [2, 4, 29] and hydrolysis of the lipopolysaccharide [22]. Although the antibacterial activity of calcium hydroxide is dependent on the direct contact with the bacteria [23], Ørstavik and Haapasalo [18] demonstrated that calcium hydroxide is not effective in eliminating the bacterias deeply colonizing the dentinal tubules. Thus, calcium hydroxide depends on other characteristics to act against the present infection, such as its capacity of increasing the pH through the dissipation of calcium ions [16, 29].

Because of this failure in the antibacterial activity and its low solubility, many studies have searched to find other efficient substances that could be associated with calcium hydroxide [24, 25, 32]. Chlorhexidine has been proposed as antiseptic agent in Dentistry for more than two decades and for some years as endodontic irrigating agent and intracanal medication [8, 24]. Chlorhexidine is biocompatible and it has a large antibacterial spectrum and action against lipoteichoic acid [12] associated with calcium hydroxide, demonstrating excellent results in clinical and laboratorial studies [7, 13-15, 30].

Several studies have demonstrated the good efficacy of the association of calcium hydroxide with several vehicles [19, 32]. Its association with chlorhexidine gel has been little studied and reported in literature regarding the releasing of hydroxyl ions. Thus, this study aimed to analyze in vitro the capacity of pH increasing of the external root surface of different associations of calcium hydroxide used as intracanal medications in extracted teeth.

Material and methods

This was an experimental, quantitative, transversal study of prospective character which aimed to analyze in vitro the pH increasing in the external surface of the root after the use of different calcium hydroxide pastes (Calen® paste, calcium hydroxide associated with 2% chlorhexidine gel and calcium hydroxide associated with saline solution), with and without the use of EDTA prior to the topical application of the intracanal medication.

Obtainment and selection of the specimens

This study was executed on 100 single-rooted natural teeth that were extracted and kept in 10% formalin solution until their use. The origin data of the specimens are seen in a donation consent form attached to the research project referred to the Ethical Committee in Research of the University of Fortaleza. The project was approved under protocol number 364/2006.

Preparation of the specimens

The cleaning and shaping procedures of the specimens were based on the crown-down technique described Maniglia-Ferreira et al. [13]. The irrigation
procedures were standardized with the use of 5 ml of 1% sodium hypochlorite (Biodinâmica, São Paulo, Brazil) at every change of instrument. The irrigation in all groups of teeth was executed with the aid of a disposable syringe (5 ml) associated with a BD needle (20X0.55 mm). All canals were dried with absorbent paper points previously to the topical application of the calcium hydroxide pastes.

**Preparation and composition of the calcium hydroxide – Ca(OH)\(_2\) pastes**

**Calen\(^\circledast\) paste (S.S. White, Rio de Janeiro, Brazil)**

Product kept in tubes with the following composition: Ca(OH)\(_2\), polyethylene glycol 800 and colophony.

**Association of calcium hydroxide with 2% chlorhexidine gel (CX)**

The paste was obtained by mixing Ca(OH)\(_2\) PA (Biodinâmica, São Paulo, Brazil) with 2% chlorhexidine gel (Endogel, Endosupport, Itapetininga, SP, Brazil), at the proportion of 1 ml vehicle/1 gram of powder, up to a paste consistency.

**Association of calcium hydroxide with saline solution**

The paste was obtained by mixing Ca(OH)\(_2\) PA (Biodinâmica, São Paulo, Brazil) with saline solution (0.9% sodium chloride) (Gaspar Viana S.A., Fortaleza, CE, Brazil), at the proportion of 1 ml vehicle/1 gram of powder, up to a paste consistency.

**Study groups**

After the instrumentation procedures, all teeth were randomly divided into six experimental groups of 15 specimens. The teeth of each group were filled with a different calcium hydroxide-based (Ca(OH)\(_2\)) paste, according to table I. Ten specimens were used to compose the control group, in which there is no application of the intracanal medication.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Pastes used</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15</td>
<td>Calen (S.S. White, Brazil)</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>Calen after EDTA use</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>Ca(OH)(_2) + CX</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>Ca(OH)(_2) + CX after EDTA use</td>
</tr>
<tr>
<td>V</td>
<td>15</td>
<td>Ca(OH)(_2) + saline</td>
</tr>
<tr>
<td>VI</td>
<td>15</td>
<td>Ca(OH)(_2) + saline after EDTA use</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>Canals instrumented and kept empty</td>
</tr>
</tbody>
</table>

For groups II, IV and VI, EDTA was used as auxiliary chemical agent, for 4 minutes, to remove the smear layer, prior to the final irrigation.

The filling of the root canals with the different calcium hydroxide pastes was carried out with the aid of irrigation syringes for the specimens of the groups III to VI, while the specimens of groups I and II (Calen paste) a special syringe suitable for the application of this medicament was employed according to the manufacturer’s instructions.

**Verification of the pH alteration**

All teeth received provisional restorations with Super Bonder glue (3M do Brazil, Campinas, SP, Brazil), suspended onto individual holders in Becker flasks containing saline solution for the analysis of the pH alteration. The pH meter (Micronal\(^\circledR\) – model B474) was connected to the liquid involving the specimens of each group through its terminal portion, so-called electrode. The readings were performed at every week for 4 weeks. All values were recorded in sheets for statistical analysis.
Results

The results were statistically analyzed with the aid of Bioestat 5.0 software and did not follow the normal curve distribution. Data was submitted to Kruskal-Wallis test, with level of significance of 5%. The pH value means for the different groups and time periods studied are seen in table II.

### Table II - pH value means of the different groups and time periods analyzed

<table>
<thead>
<tr>
<th>Groups</th>
<th>pH 1(^{st}) week</th>
<th>pH 2(^{nd}) week</th>
<th>pH 3(^{rd}) week</th>
<th>pH 4(^{th}) week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calen</td>
<td>8.03</td>
<td>8.20</td>
<td>8.23</td>
<td>7.45</td>
</tr>
<tr>
<td>Calen + EDTA</td>
<td>8.12</td>
<td>8.40</td>
<td>8.18</td>
<td>7.94</td>
</tr>
<tr>
<td>CX Ca(OH)(_2)</td>
<td>7.69</td>
<td>8.76</td>
<td>8.90</td>
<td>8.37</td>
</tr>
<tr>
<td>CX Ca(OH)(_2) + EDTA</td>
<td>8.07</td>
<td>8.81</td>
<td>8.98</td>
<td>8.68</td>
</tr>
<tr>
<td>Saline Ca(OH)(_2)</td>
<td>8.84</td>
<td>8.32</td>
<td>8.01</td>
<td>7.34</td>
</tr>
<tr>
<td>Saline Ca(OH)(_2) + EDTA</td>
<td>8.98</td>
<td>8.67</td>
<td>8.27</td>
<td>8.01</td>
</tr>
<tr>
<td>Negative control</td>
<td>7.39</td>
<td>7.57</td>
<td>7.56</td>
<td>7.71</td>
</tr>
</tbody>
</table>

Except for group III, all materials used promoted significant increasing in pH values at the first week (p = 0.0095) in comparison with the control group which did not exhibited pH alteration at the different time periods analyzed (p = 0.3789).

Groups III and IV showed significant evolution in the pH increasing over time (p = 0.0116), differing from the other groups which demonstrated a decrease in the pH values over time. The use of EDTA did not provided higher pH values (p = 0.2278).

Discussion

The hypothesis that the diffusion of hydroxyl ions from calcium hydroxide through the dentinal tubules would increase the pH of the dental tissues was evaluated by Tronstad et al. [29], by using an experimental animal model. The authors verified that the insertion of calcium hydroxide within root canal has a direct influence on the pH increase of the external root surface, mainly in areas of root resorption or incomplete root formation, making impossible the osteoclastic activity and stimulating the repairing of the local tissues. In this present study, a previous analysis of the provisional restorative materials was executed so that they did not influence on the results. Because Super Bonder glue did not show any influence on pH, it was the option of choice for the coronal and apical sealing. In a pilot study with different provisional restorative materials, we found many influences on pH, both separately and when they were applied as restoration of teeth with intracanal medication of calcium hydroxide. It is known, however, that the provisional restoration with ideal properties of neutral pH is almost impossible in Dentistry, but a substance closer to that desired conditions must be searched and if possible, found. The time intervals selected for the measurement of the pH were based on technical reasons related to the routine of the dental office, determined by studies in the literature [8, 24, 25].

The methodology used is valid once the hydroxyl ions on the external root surface are immediately noted by the electrode of the pH meter. Some studies [21, 29] were carried with similar methodology. The results of this present study differ from those of the literature [6, 21], which demonstrated that there was not pH alteration at periods shorter than 14 days with use of viscous vehicles, such as Calen paste and chlorhexidine gel. The dissociation of calcium hydroxide is directly proportional to the vehicles used, because the aqueous vehicles enable to reach a pH close to 12.6, due to a faster dissociation and diffusion velocity of the hydroxyl ions. Because the viscous vehicles exhibit smaller dissociation and diffusion velocity of hydroxyl ions they require longer times to reach high pH thresholds [3].

Concerning to the seven groups of the study, each one with a different intracanal medication, they were tested as aforementioned described. The sealing of the apical foramen was carried out to avoid to the direct contact of the medication with the solution (saline) in which the specimens were immersed, therefore avoiding influences on the results. This occurs because which causes the pH
alteration of the samples would be, according to the literature, the diffusion of the intracanal medication through the dentinal tubules of the dentinal tissue, reaching the periodontal tissues consequently with pH increase of the external root surface.

It is necessary to emphasize that for the medication diffusion through dentinal tubules to occur and consequently alter the pH of the external environment, the tubules should be the most preserved as possible and this was obtained with the maximum conservation of the teeth through their washing with saline just after the extraction and storage in 10% formalin solution.

The results of the pH measurements revealed that the most beneficial association to increase the pH progressively over time was calcium hydroxide with 2% chlorhexidine gel, regardless of the use of EDTA after the ending of the instrumentation procedures of the root canals.

The use of EDTA prior to the application of the intracanal medication has a chelating function of the removal of the smear layer, opening of the dentinal tubules and facilitation of the diffusion of the intracanal medication inside the canals [5, 6, 8, 18]. However, the results of this present study demonstrated there was no influence of EDTA on the process of the hydroxyl ion diffusion through the dentinal tissue.

Although the Calen® paste is for many dentists more practical for usage and demonstrated adequate initial results it did not show the maintenance of the pH increasing, as expected with pastes with viscous vehicles. Probably, the fact of the paste is ready for use, i. e., it had been mixed long time ago, the calcium and hydroxyl ions react internally and promote the formation of calcium carbonate, which is stable.

There is no ideal intracanal medication, that is, one that is able to influence directly on the clinical signs and symptoms of the patients. Notwithstanding, the researches must be conducted to guide the best choices of the existing materials as well as their associations. The association of biocompatible substances with high antimicrobial capacity directs the ideal intracanal medication because the endodontic treatment aims to eliminate of the aggressive agent within the root canals and to promote its tridimensional sealing. Further studies analyzing the profile of the medicaments over time and which verify their antimicrobial activities and formation of compounds should be executed with the goal of searching the ideal substances and associations for dental and endodontic purposes.

Conclusion

According to the results obtained, it can be concluded that:
• All calcium hydroxide pastes used demonstrated the capacity of releasing hydroxyl ions at the initial period of use, with pH increasing;
• The use of 2% chlorhexidine gel provided the gradual pH increasing over time;
• The removal of the smear layer did not influence on the pH of the root surface of teeth with canals filled with calcium hydroxide paste.

References


