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Influence of dentin on pH of 2% chlorhexidine gel and calcium hydroxide alone or in combination

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Abstract – The aims of endodontic treatment in cases of apical periodontitis are to reduce as much as possible the number of microorganisms inside the root canal system and to inactivate toxins produced by them. Most of the times, these objectives are not achieved solely by chemomechanical preparation, and intracanal dressing may be necessary. In these cases, calcium hydroxide is used as a root canal dressing due to its well-known and recognized antimicrobial activity. Chlorhexidine has a wide spectrum of antimicrobial activity and its association with calcium hydroxide has been recommended in an attempt to amplify antimicrobial effects of calcium hydroxide. It is also known that dentin exerts a buffering effect under wide pH variations, and may be responsible for decreasing the antimicrobial activity of drugs inside the root canal. The objectives of this study were to assess the pH of 2% chlorhexidine gel and calcium hydroxide alone or in combination, as well as the influence of dentin on the pH of these compounds. Dentin powder was obtained from bovine teeth and added as 1.8% to the volume of the medications. All substances were individually stored in plastic flasks, in triplicate. A pH meter was used at five different moments to assess pH in viscous medium: immediately after preparation and after 24 h, and 7, 14, and 21 days. Results were analyzed by paired Student's *t*-test. Statistically significant differences were observed in the 2% chlorhexidine gel group alone or associated with calcium hydroxide and added of dentin powder ($P < 0.05$). Mean pH values indicated the influence of dentin powder because of a significant increase in pH. Calcium hydroxide with propylene glycol as the vehicle always showed high pH, demonstrating that this compound was not affected by the presence of dentin.

Microorganisms are main etiological agents of pulp and periradicular disorders (1). Studies on endodontic microbiota show that anaerobic microorganisms predominate in primary apical periodontitis (2). The objective of intracanal dressing in endodontic infections associated with apical periodontitis is to complete decontamination process produced by root canal preparation (3).

Calcium hydroxide is the most commonly used intracanal medicament due to its well-known and recognized antimicrobial activity. This activity is influenced by the speed of dissociation of hydroxyl ions, which create a high pH environment that inhibits most microorganisms (4–8), inactivates endotoxins, stimulates mineralization, dissolves organic material, and produces a chemical and physical barrier (9). Especially, in cases of dental trauma, calcium hydroxide is the first-choice intracanal medication because its high pH positively contributes for resorption control and prevention, which are very common consequences of traumatic injuries (8). However, Haapasalo et al. (10), in a recent review of the literature, concluded that calcium hydroxide is not equally effective against all microorganisms found in the root canal system. This occurs because the buffering effect of the dentin structure under wide pH variations may decrease the antimicrobial activity of the calcium hydroxide (10–12).

Other agents have been added to calcium hydroxide in an attempt to amplify its antimicrobial effects and to achieve some beneficial synergism, and the use of chlorhexidine, a large spectrum antimicrobial agent, has been suggested (13, 14). The antimicrobial effect of chlorhexidine is based on attraction and binding of its cationic molecule to the anionic molecules of the bacterial cell membrane (15, 16). Optimal pH ranges from 5 to 7. At pH higher than 8, chlorhexidine base may precipitate in aqueous solution (17). When associated with calcium hydroxide, 2% chlorhexidine gel may precipitate because of high pH produced by calcium hydroxide in aqueous medium, forming sub-products of unknown effects (18, 19). Therefore, the objectives of this study were to evaluate the pH of 2% chlorhexidine gel and calcium hydroxide used alone or in combination, as well as the influence of dentin on the pH of these products.

Materials and methods

Ten bovine incisor teeth were cleaned and autoclaved at 121°C for 15 min, as described by Haapasalo et al. (11). Then, teeth were cut perpendicularly to their axes immediately below the enamel–cement junction using carborundum abrasive disks (Moyco Union Broach, York, PA, USA). Dentin powder was obtained by using spherical dental burs #4 (KG-Sorensen, São Paulo, Brazil) inside the root canal, without coolant, in low speed hand piece. All dentin power was stored in plastic flasks.

Medications were prepared using a glass plate and spatula, and pH was determined in 1 ml of each of them. Dentin powder was added as 1.8% of the medication volume (10). Mixtures were individually placed in plastic flasks, in triplicate, and divided into the following groups: G1 – 2% chlorhexidine gel (Fórmula & Ação, São Paulo, Brazil); G1d – 2% chlorhexidine gel added of dentin powder; G2 – 2% chlorhexidine gel associated with calcium hydroxide P.A. (Fórmula & Ação, São Paulo, Brazil). Group 2d –2% chlorhexidine gel associated with calcium hydroxide P.A. added of dentin powder; G3 – calcium hydroxide P.A. using propylene glycol 600 as vehicle; G3d – calcium hydroxide P.A. using propylene glycol 600 as vehicle added of dentin powder.

A pH meter for viscous medium with electrodes sensitive to hydrogen ions was used to determine the pH of the mixtures (pH Meter, Model E520, Metrohm Herisau, Switzerland). The microelectrode was calibrated at pH 7 using standardized solutions before any measurement, and after use, it was washed with plenty of distilled water. pH was assessed at five different moments: immediately after preparation and after 24 h; 7, 14, and 21 days.

Results

Samples were analyzed in triplicate and pH values were recorded. Means pH values of the different groups at the five moments are shown in Table 1 and Fig. 1.

Results of paired Student's *t*-test showed statistically significant differences in G1d and G2d ($P < 0.05$), in which 2% chlorhexidine gel was used alone and associated with calcium hydroxide.

pH of 2% chlorhexidine gel associated or not with calcium hydroxide was significantly modified by dentin; pH of calcium hydroxide in propylene glycol was not affected by the presence of dentin.

Discussion

The difficulty in eliminating microorganisms, remain in the root canal system and dentinal tubules, even after cleaning and filling procedures, underscores the need for complete root canal preparation with intracanal dressing (4, 12, 14, 19, 20).

Calcium hydroxide is the most commonly used intracanal medication due to its antimicrobial activity, which is influenced by the speed of dissociation of hydroxyl ions that generate a high pH environment (4–7). This alkaline environment is very important to reduce the number of microorganisms, dissolve organic tissues, inactivate endotoxins, and prevent root resorption (8, 9).

The combination of calcium hydroxide and chlorhexidine has been widely studied and recommended as intracanal dressing, especially in cases of persistent apical periodontitis (13–21). The main objective of this combined treatment is the synergism between the two compounds. However, studies show that, in terms of antimicrobial action, the combination of calcium hydroxide and 2.0% chlorhexidine is at least as efficient as calcium hydroxide alone in sterile deionized water (4, 12, 18, 19, 21).

Vianna et al. (9), in an *in vivo* study, determined the amount of bacteria and endotoxins in primary endodontic infections before and after chemomechanical preparation of root canals, and after intracanal dressing with calcium hydroxide and chlorhexidine alone or in combination. These authors did not observe any synergism against endotoxins or microorganisms between sessions, after 7 days of intracanal dressing. On the contrary, some studies concluded that this therapeutic combination is more efficient than calcium hydroxide paste in sterile water against *Enterococcus faecalis* cultures found inside dentinal tubules (14, 22).

Because of such contradictory reports, it certainly seems that the synergistic effect of intracanal medications may involve more complex interactions than simply the mixture of drugs. As there are optimal conditions for the action of any chemical compound, pH plays a determinant role in their use, alone or in combination.

Table 1. Mean pH of the medications at five periods

Medication	Immediate	24 h	Days 7	Days 14	Days 21	*P*-value
G1: CHX	6.0	6.0	6.4	6.2	6.2	0.0008
G1d: CHX + dentin	8.2	7.7	8.1	7.7	7.3	
G2: $Ca(OH)_2$ + CHX	13	12.9	12.8	12.7	12.5	0.0326
G2d: $Ca(OH)_2$ + CHX + dentin	13.2	12.9	12.9	12.8	12.7	
G3: $Ca(OH)_2$ + PEG	14	14	14	14	14	0.0605
G3d: $Ca(OH)_2$ + PEG + dentin	14	13.8	14	13.8	13.5	

CHX, 2% chlorhexidine gel; $Ca(OH)_2$, calcium hydroxide; PEG, propylene glycol 600.

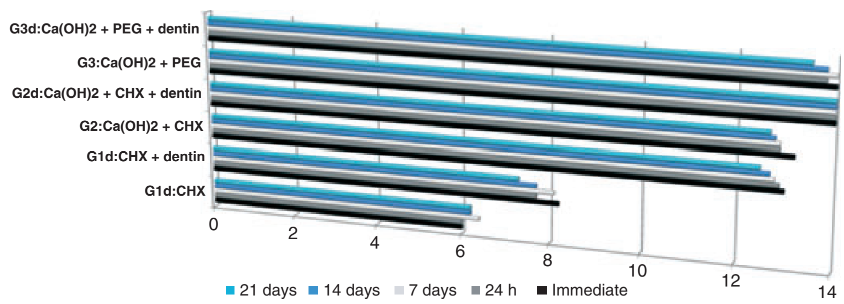

Fig. 1. Mean pH of the medications at five periods.

Therefore, pH of chlorhexidine and calcium hydroxide alone or in combination was studied at different moments, and the influence of dentin on pH was also assessed.

Results demonstrated that pH of calcium hydroxide was always high, either using propylene glycol as vehicle or associated with chlorhexidine, and in the absence or presence of dentin powder. pH of calcium hydroxide ranged from 12.5 and 14.0, during the 21 days of the study (Table 1 and Fig. 1).

Although the usual residence time of intracanal medication between sessions is 7 days, studies suggest that this period does not enable a significant reduction in microorganism counts or endotoxin concentrations. Camargo et al. (6) compared the pH and calcium ions liberation after use of calcium hydroxide pastes with different vehicles in human or bovine teeth, and they showed that it takes at least 14 days for those ions to penetrate the dentin and reach the external surface of the root. In 2007, a clinical study by Vianna et al. (9) demonstrated that after chemical and mechanical preparation, a 7-day residence time of calcium hydroxide gel did not lead to any significant improvement either in the microbial load or in endotoxin concentration. On the contrary, Nerwich et al. (8) carried out an *in vitro* study of teeth dressed with calcium hydroxide in which pH was measured using microelectrodes in small cavities at apical and cervical levels in the inner and outer dentin and showed that although pH of inner dentin increased in a few hours, reaching 10.8 at the cervical level and 9.7 at the apical level, it took 1–7 days for these high values to be reached in the outer root dentin, and 2–3 weeks for peak levels. This study was designed to last 21 days to determine if pH remained high after a longer treatment.

Ethical reasons and the unavailability of human teeth make alternative models necessary (10, 11, 15). This study was based on bovine dentin because its composition is compatible with that of humans. Camargo et al. (6) did not observe any statistical difference in the pH of bovine and human groups. These results showed that bovine teeth may be used in studies in this area, especially when involving pH changes.

Bovine teeth from which dentin powder was obtained were autoclaved according to the methodology by Haapasalo et al. (11), because the specimens must be handled without contamination. However, some studies consider that this sterilization method may change organic and inorganic components of teeth structure because of the denaturation of proteins in the collagen matrix (23–25). Other studies showed that sterilization do not change either dentin permeability or its adhesion resistance, properties that are sensitive to alterations in the collagen fiber network (26, 27). It is not known if this putative change in organic components would influence the buffering capacity of dentin in this study, making the pH of some groups remain high. Further studies would be necessary to better understand this relationship.

The small particles of dentin powder enable the use of standardized quantities and greater control of its mixture with the medications. Many studies with therapeutic agents have been carried out to evaluate inhibition of their antimicrobial activity caused by dentin (10–12).

Dentin powder was added as 1.8% of the medication volume based on the study by Haapasalo et al. (10), who showed that this percentage was enough to inhibit antimicrobial activity of therapeutic agents.

pH of 2% chlorhexidine gel alone was inside the optimum range for its antimicrobial action, which is from 5 to 7 (17). In this study, minimum pH was 6.0 and maximum, 6.4. However, when dentin powder was added to chlorhexidine, pH was significantly increased during the 21 days of the study, reaching a minimum of 7.7 and a maximum of 8.2, much higher than the optimum for its action.

When 2% chlorhexidine gel was associated with calcium hydroxide, pH was increased and remained high, reaching 13.0 immediately after preparation and 12.5 after 21 days.

Therefore, dentin power significantly increased the pH of 2% chlorhexidine gel associated or not with calcium hydroxide (Table 1 and Fig. 1). In the other group (G3d), pH values remained unchanged after the addition of dentin powder (Table 1).

Haapasalo et al. (11) showed that calcium hydroxide may be inactivated by the buffering capacity of dentin. However, this effect was not observed in this study, once pH of calcium hydroxide remained high for 21 days, even after dentin powder was added.

As for chlorhexidine, results of this study support those of other trials (10–12) that showed that dentin and its components, such as hydroxyapatite and collagen, may diminish the antimicrobial activity of chlorhexidine.

Antimicrobial activity of calcium hydroxide is not lost when it is mixed with chlorhexidine (5, 28) because alkalinity is not changed. pH is essential to microbial control because most microorganisms do not survive pH values higher than 9.0. Even *E. faecalis*, a facultative Gram-positive bacterium extremely resistant to high pH and able to survive values as high as 11.5, would not resist direct contact with the environment generated by calcium hydroxide (3). The greatest difficulty in eliminating *E. faecalis* inside dentinal tubules in cases of persistent apical periodontitis is the low diffusion rate of calcium hydroxide pastes.

In this study, the combination of calcium hydroxide and chlorhexidine (G2) did not change the ability of calcium hydroxide to dissociate in hydroxyl ions, and pH remained high even in the presence of dentin powder (G2d).

Chlorhexidine action in this formula is controversial (29) because under high pH conditions the compound may be hydrolyzed by hydroxyl ions produced by the dissociation of calcium hydroxide (12, 17, 19).

Barbin et al. (19) carried out the chemical analysis of 0.2% chlorhexidine digluconate by means of mass spectrometry and liquid chromatography. Chlorhexidine was not found both in the initial analysis and after 7 and 14 days, indicating it was totally dissociated since the moment of preparation. Yeung et al. (18) reported similar results using UV absorption. They observed that $>99\%$ of the chlorhexidine mixed with calcium hydroxide precipitated. This finding suggests that high pH generated by calcium hydroxide would promote the precipitation of chlorhexidine digluconate. Other authors state that chlorhexidine undergoes deprotonation of guanidine groups at pH >8 generated by calcium hydroxide in aqueous medium, and that this fact would be responsible for the reduction of antimicrobial action of chlorhexidine and calcium hydroxide mixtures.

Antimicrobial effects of medications that combine calcium hydroxide and chlorhexidine may not be due to chlorhexidine itself, but to the action of different sub-products of its fragmentation (13, 14, 21, 22). Antimicrobial power of these sub-products may be related to their pro-oxidant action (19). However, the chemical properties of these compounds should be further analyzed to better understand their effects on bacteria and live tissue.

In this study, pH of calcium hydroxide in propylene glycol was always high – concluding that this paste was not affected by the presence of dentin. On the contrary, pH of 2% chlorhexidine gel associated or not with calcium hydroxide increased with the addition of dentin power.

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