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Ions Release and pH of Calcium Hydroxide-, Chlorhexidine- and Bioactive Glass-Based Endodontic Medicaments

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This study evaluated pH and release of calcium, sodium and phosphate ions from different medications in human dentin. Fifty premolars were prepared and randomly divided into groups: (CHX) – 2% chlorhexidine gel; (CHX + CH) - CHX + calcium hydroxide PA; (CH) – CH + propylene glycol 600; (NPBG) – experimental niobium phosphate bioactive glass + distilled water; (BG) – bioactive glass (Bio-Gran) + distilled water. The specimens were immersed in deionized water and the pH variations were measured. The quantification of ions in the solutions was made by inductively coupled plasma - atomic emission spectroscopy (ICP/AES) at 10 min, 24 h, 7, 14, 21 and 30 days. The results were analyzed by ANOVA and Tukey`s test, with a significance level of 5%. CH had the highest level of calcium ions release at 30 days, while CHX and BG released more sodium ions. BG, NPBG and CHX released a higher amount of phosphate ions. The pH of CH was significantly higher compared with the other groups. CH favored the greatest increase of pH and calcium ions release. The bioactive glasses released more sodium and phosphate ions and presented an alkaline pH immediately and after 30 days.

Key Words: bioactive glass 45S5, calcium hydroxide, chlorhexidine, endodontics, niobium phosphate glass.

Introduction

The intracanal medication for cases of pulp necrosis should be effective against bacteria that survived the chemomechanical preparation and be capable of controlling the persistent exudation and destructive action of osteoclasts when external root resorption occurs (1).

The success of calcium hydroxide as an endodontic medication is related to its ionic effect, produced by the chemical dissociation of hydroxide and calcium ions that act on the tissue and bacteria. These ions are responsible for the antimicrobial and biological properties of the medication (2), but calcium hydroxide is not equally effective against all microorganisms found in the root canal system (3). It has been reported that *Enterococus faecalis* shows resistance to high pH, ability to penetrate dentinal tubules and adaptation to different environmental conditions (4). Moreover, a buffering effect of calcium hydroxide alkalinity by the dentin has been demonstrated (5) and long-term calcium hydroxide as a root canal dressing may increase the risk of root fracture (6). Therefore, more effective alternatives to calcium hydroxide are still required.

Bioactive glass is a material that is able to mineralize dentin, has antimicrobial effects in closed systems and may be used as endodontic medication (7). Unlike the calcium hydroxide, it has been reported that the antibacterial efficacy of bioactive glass increases when it is mixed with dentin (7,8). In addition, these glasses release calcium, phosphate, sodium, silica and depending on liquid exchange in the environment, slowly transform into calcium phosphate (9). The bioactive glass suspension has shown to affect the mechanical properties of human root dentin in a lesser extent than calcium hydroxide (10).

An ideal preparation of bioactive glass suspensions for root canal disinfection should combine the induction of high pH with the capacity for continuing release of alkaline species (11). Thus, the objectives of this study were to evaluate the pH and ion release of calcium hydroxide, 2% chlorhexidine gel and bioactive glass medications.

Material and Methods

Preparation of the Niobium Phosphate Bioactive Glass

The experimental niobium phosphate glass was prepared by melting mixtures of chemical compounds in an electric furnace, as previously described (12). The molten mixture was poured into a stainless steel mold and cooled to room temperature. The glass was then crushed in a vibrating system with a tungsten ball (Pulverisette; Fristsch, Idar-Oberstein, RP, Germany) for 30 min. The resultant powder passed through a series of sieves until 20 µm particles were obtained (Hogentogler & Co., Inc, Columbia, MD, USA).

Samples of the experimental glass and Bio-Gran (Bio-Gran®; Orthovita, Implant Innovations, Palm Beach Gardens, FL, USA) were analyzed by Energy Dispersive X-ray fluorescence spectrometry (EDX-720, Shimadzu, Tokyo,

Japan) to verify the final composition and for possible contaminants. The EDX analyses were performed on the surfaces of the glass powders used in this study. A CCD (charge-coupled device) camera was used to select a 10 mm diameter area (12).

The particle size distribution of the glasses was observed by scanning electron microscopy (SEM - LEO 440; Electron Microscopy Ltd, Cambridge, UK).

Preparation of the Teeth

After the approval of research protocol by the Dental School Research Ethics Board, USP, were selected fifty single-rooted completely formed mandibular premolar teeth, extracted for various reasons and measuring approximately 15 mm long. The specimens were submitted to a radiographic exam to verify the presence of a single root canal, absence of any signs of local or extended calcification, internal resorption or previous endodontic treatment.

After removal of the organic material from the root surface, they were submitted to 18,5 KGy of gamacell radiation (Centro Tecnológico de Radiação, Instituto de Pesquisa de Energia Nuclear - IPEN, São Paulo, SP, Brazil) for microbiologic control.

Once sterilized, the teeth were rehydrated and kept in sterile saline solution (0.9% sodium chloride, Aster Produtos Médicos Ltda., Sorocaba, SP, Brazil) in an oven at 37 °C for 48 h. To establish standard hydration equilibrium, the specimens were stored in a refrigerator at 4 °C with a daily change of solution until use.

The tooth crowns were removed in a cutting machine (Isomet 100 Precision Saw; Buehler Ltd, Lake Bluff, IL, USA) and the root lengths were standardized at 10 mm. The working length for all teeth was 9 mm.

The canals were prepared by rotary instrumentation using the conventional multiple-file rotary ProTaper System (Dentsply Maillefer, Ballaigues, Switzerland) up to a master apical file size F5 (size 50, 0.05 taper). The canals were irrigated with 2 mL of 1% sodium hypochlorite solution (Fórmula & Ação, São Paulo, SP, Brazil) for 1 min between each instrument.

The diameter of the greatest dentin constriction in the apical region was standardized at 0.56 mm. For this purpose, a K type file #50/.02 taper was inserted into the root canal until it passed 3 mm beyond the apical foramen. Next, rotary movements were performed to remove any remaining residue in the most apical portion of the canal. This procedure was repeated with progressive caliber files until a #50.02 taper instrument.

The smear layer was removed by irrigating the canal with 5 mL of 17% EDTA solution for 1 min (Fórmula & Ação). Next, the canals were dried with suction cannulas and absorbent paper points (ProTaper Paper Points; Dentsply Maillefer).

The external root surfaces of all teeth were dried with filter paper sheets and later rendered water proofed. A #40 gutta-percha cone (Maillefer, Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil) previously lubricated with hydrosoluble gel (Johnson & Johnson Com. e Dist. Ltda., São José dos Campos, SP, Brazil) was inserted into the root canal until it passed the apical foramen. The roots were rendered water proofed with two layers of ultra-fast drying nail polish, from the point of exposure of the gutta-percha cone to the entire root length. After 24 h, the root canals were irrigated with 10 mL distilled water for complete removal of the hydrosoluble gel and stored in individual glass flasks containing the same solution, in an oven at 37 °C for 48 h, in order to maintain a standard hydration level.

Next, the access cavities were sealed with composite resin (Z350, 3M/ESPE St Paul, USA) and cyanoacrylate adhesive (SuperBonder Instant Adhesive; Loctite Corp, Cleveland, OH, USA) for the first pre-filling analysis without the medications.

Pre-Analysis of pH and Ions of the Teeth without Medication

Ten specimens from each group were immersed in plastic tubes containing 10 mL deionized water (Permution, Curitiba, PR, Brazil), sealed with parafilm (American National Can, Menasha, WI, USA) and stored in an oven at 37 °C until the established analysis time. The pH and ion concentrations were measured without any medication at six different moments: 10 min, 24 h, 7, 15, 21 and 30 days. These first readings were the baseline measurements.

After each analysis, the specimens were removed from the tubes and allocated in new plastic tubes containing 10 mL deionized water. The solutions from each experimental time were shaken in a vortex for 5 s and the pH levels were measured with a pH meter (QM-400; Quimis, São Paulo, SP, Brazil). The solutions in the tubes were also analyzed for the presence and quantification of calcium, sodium and phosphate ion release, using inductively coupled plasma-atomic emission spectroscopy (ICP-OES, model Arcos; Spectro Analytical Instruments, Kleve, Germany), according to the manufacturer's instructions for each ion analysis.

Analysis of pH and Ions of the Teeth after Medication

After the pre analysis of the ions from the teeth, the sealing was removed from the roots, and canals were irrigated with 10 mL distilled water and dried following the same procedure.

The 50 roots were randomly divided into the following experimental groups (n=10): CHX – 2% chlorhexidine

gel (Fórmula & Ação); CHX+CH - 2% chlorhexidine gel + calcium hydroxide PA (Fórmula & Ação); CH - calcium hydroxide + propylene glycol 600; NPBG – niobium phosphate bioactive glass + distilled water; BG – bioactive glass (Bio-Gran®; Orthovita, Implant Innovations) + distilled water.

The medications were weighted on a precision scale at 1 gram of powder to 1 mL liquid rate and mixed using a glass plate and spatula.

Next, the pastes were inserted in the root canals using a plastic syringe with a needle. Complete canal filling was verified by extrusion of the medication from the apical foramen and by reflux from the canal entrance. The access cavities were sealed with composite resin (Z350, 3M/ESPE) and cyanoacrylate adhesive (SuperBonder Instant Adhesive, Loctite Corp) and analyzed as previously described.

Statistical analysis of the ion release was performed with regards to the difference between the measurements obtained in the pre - and post intra-canal medication time intervals. Negative values were considered null; in this case, there was no release of ions. The data were submitted to ANOVA and Tukey tests, at a 5% significance level.

Results

The EDX analyses showed quantitative result of the composition of NPBG and BG (Table 1). The particle size distribution in SEM showed particles with 300-350 µm for the BG (Fig. 1A) and a large number of particles smaller than 10 µm for NPBG (Fig. 1B).

The presence of Nb, P, Ca and Na ions was observed, which was expected for NPBG. The Al ion originated from the crucible. For the BG analysis the presence of Si, Ca, Na and P ions was evident and expected.

Table 1. EDX analysis of the different types of glasses; data are expressed as weight percentages (%) of constituent elements

Glass	Oxides	Result (%)
NPBG	Nb_2O_5	41.8
	P_2O_5	35.2
	CaO	18.8
	Al_2O_3	2.7
	Na_2O	1.5
BG	SiO_2	42.3
	CaO	28.3
	Na_2O	22.8
	P_2O_5	6.6

pH Analysis

CH presented alkaline pH in all experimental times, with maximum value obtained at 10 min. Both glasses demonstrated a slightly alkaline pH. Figure 2 shows the mean pH values of the medications at the different study times. The pH of pre-analysis remained at 6.9 in all experimental periods.

Ion Release Analysis

CH presented a significantly higher level of Ca^{2+} release ($p<0.05$) than the other medicaments. Table 2 describes the amount of Ca^{2+} released at the different experimental times. BG and CHX showed a significantly higher Na^+ release ($p<0.05$) than other medicaments, in alternating time intervals. Table 3 describes the amount of Na^+ released at the different experimental times. NPBG, BG and CHX presented a significantly higher level of PO_4^{3-} release ($p<0.05$) than other medicaments, with significant ion release starting from 7 days of use of the medications. Table 4 describes the amount of PO_4^{3-} released at the different experimental times.

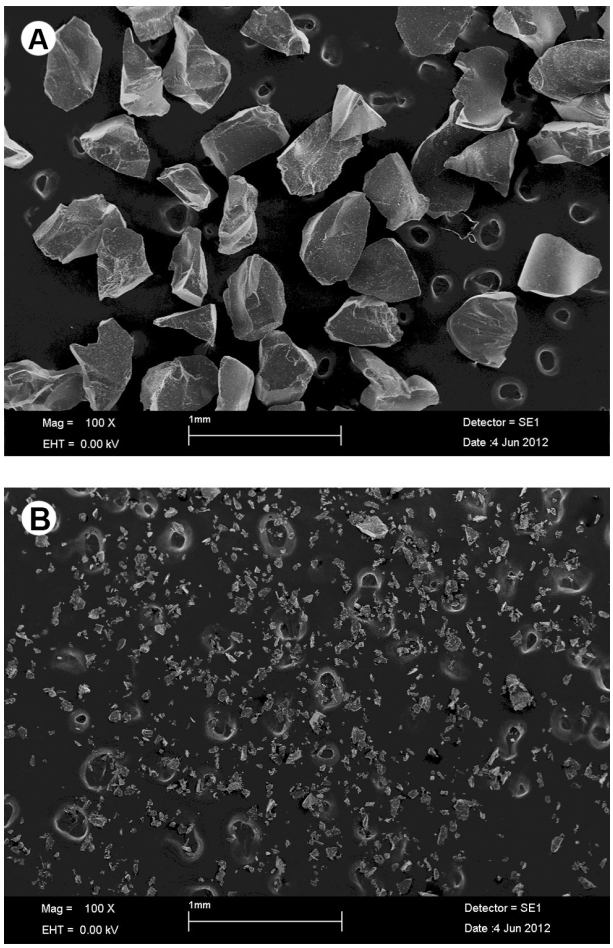

Figure 1. Particle size distribution of the glasses in SEM: A BG; B: NPBG.

Discussion

Among all the intracanal medications used in the study, CH showed the highest level of calcium ion release in the initial times of 10 min, 14, 21 and 30 days and the highest pH values in all the analyzed experimental times .

The fast alkaline effects of the calcium hydroxide medicaments after 10 min were due to the immediate contact of calcium hydroxide with the medium, resulting in an instantaneous release of hydroxyl ions, as previously reported (13). When calcium hydroxide was associated with chlorhexidine, from the 14th day on, the release of calcium ions was considerably reduced. This may be related to the pH neutralization observed in group CHX+CH from the 14th day. Duarte et al. (14) found similar results and suggested that the vehicle Natrosol may react with calcium, inhibiting its release in the initial periods. In the same way, it could be hypothesized that chlorhexidine inhibited the release of Ca^{2+} only after a longer period of action.

Some studies have focused on the effects of chlorhexidine on the pH of calcium hydroxide. Basrani et al. (15) observed that the presence of chlorhexidine did not alter the pH of calcium hydroxide, similar to the findings of Freire et al. (16). However, in another study, Calen associated with 0.4% chlorhexidine produced progressive increase in pH values starting at day 3, with higher pH values than Calen at 7 days, and no statistical difference in the subsequent periods until 60 days (17). The present study is in disagreement with these findings, because the pH of CH was significantly higher compared with the other groups for all the analyzed experimental time intervals. A possible explanation for that could be the different vehicles used and the concentration of chlorhexidine. In the present study calcium hydroxide was associated with propylene glycol 600 and chlorhexidine gel 2%, while the cited study used Calen paste containing calcium hydroxide, zinc oxide, propylene glycol 400 and glycerin associated with 0.4% chlorhexidine. Vehicles and other substances added to calcium hydroxide may affect its ability to release ions (18).

With regards to the studied bioactive glasses, it was observed that in the first 10 min, the pH of both of them was close to 9, and over the course of 7 days tended to neutralize, but from the 14th day on, the values began to rise again. Recently, bioactive glass was proposed as intracanal medicament and this biomaterial can be activated when in contact with tissue fluids, inducing an alkaline pH, similar to medications based on calcium hydroxide (19) and it is also capable of stimulating the proliferation of bone tissue, influencing the repair processes . This behavior depends on the chemical composition and the surface texture of the glasses (20). Although the antimicrobial effect of bioactive glasses is not completely understood, it may be related to an increase in pH in aqueous suspensions and the osmotic effects or Ca^{2+} concentration in the dentin environment (21). The release of Na^{+} and Ca^{2+} ions results in an environment with an alkaline pH, interfering in the microbial viability (22). Moreover, silica, calcium and

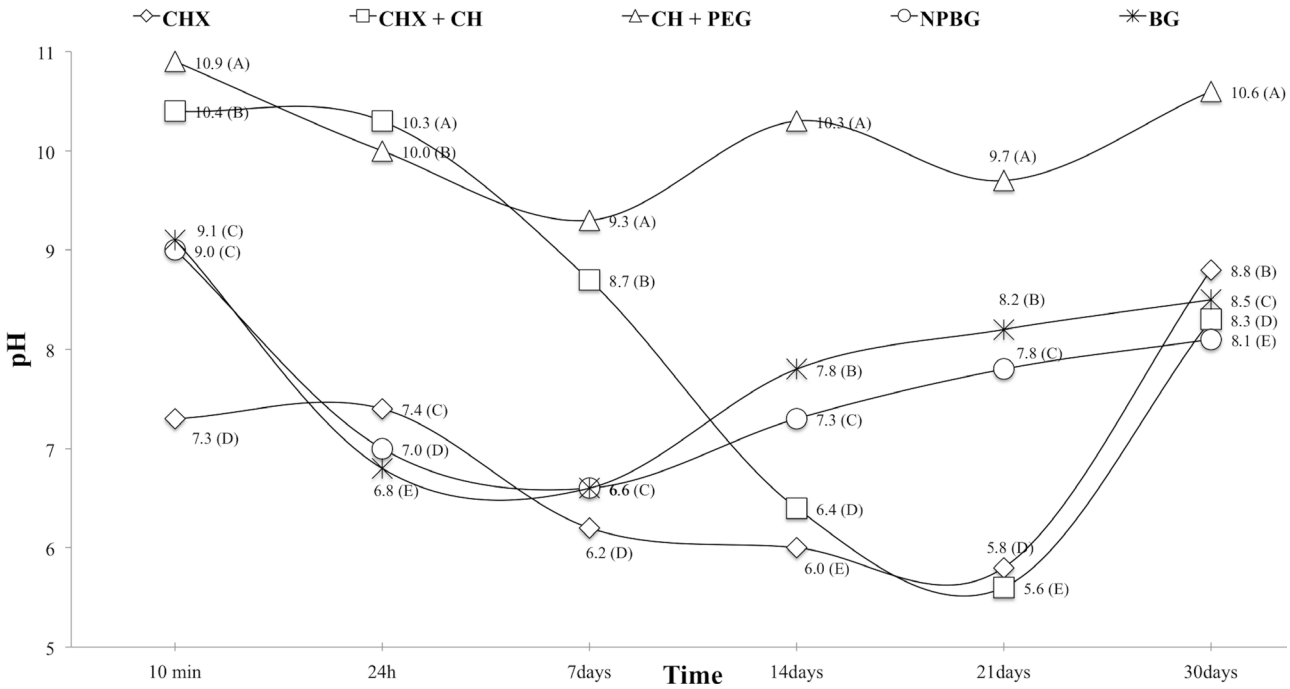

Figure 2. Mean pH of the medications at six times. Values followed by different superscript letters indicate statistically significant differences according to ANOVA ($p<0.05$) in the comparison among materials at same experimental times.

phosphor release appears to exert direct and indirect effects on the bacteria (7). It was shown recently that the experimental NPBG reduced biofilm formation and had the lowest live percentage of microbial cell biovolume at 14 days of incubation (23).

In the present study, the pH and release of different ions were analyzed directly from root dentin, closer to the clinical reality than the method that uses dentin powder. In spite of standardization of the chemomechanical preparation and foramen size, the anatomic variation and composition may differ from one tooth to another. Thus, a pre -(baseline) and post-medication analysis was performed, in order to achieve greater control of these variables. Furthermore, the teeth were placed in new flasks containing deionized water to detect at which time occurred the highest level of ion release, as well as to prevent saturation of the medium, as it was observed in another study (14). On the other hand, the used several endodontic medicaments had different solubility and vehicles and this could be a limitation of this study.

While chlorhexidine alone does not release calcium ions, the bioactive glasses released a moderate concentration, statistically lower than the groups that contained calcium hydroxide. In the first 10 min, Bioglass 45S5 (BG) released more calcium than the NPBG experimental glass, but from 24 h on, this release was equal and for Bioglass it ceased on the 14th day. In the experimental glass there was an interruption of release on the 14th day, but release began again on the 21st day and its levels were equal to the release at 7 days. A possible explanation for this is that the chemical stability of phosphate glasses increases when niobium is added (24,25), thus leading to a slightly slower release of ions.

A higher level of sodium ion release was observed for the groups CHX and GB, with a statistically higher immediate level of release for the glass than for all the other groups. CHX attained a statistically higher level of release on the 14th day. For NPBG moderate release was observed, equal to BG at some times. As for the phosphate ion, a similar release was observed for the studied glasses and for CHX, while for the other groups there was no release whatever. Chlorhexidine has high adsorption power, which is explained by electrostatic interaction. Due to the cationic character, chlorhexidine has a strong affinity for anions like the phosphate ions present in the dentin (26). Maybe chlorhexidine promotes a release of phosphate ions of the tooth. This effect did not occur in the association CHX+CH, due to inactivation and precipitation of chlorhexidine associated with calcium hydroxide.

The process of tissue repair is directly related to the alkaline potential of the used intracanal medications and their capacity to release ions into the periapical tissues (27). Different medications may present various levels of bioactivity and their composition has direct influence on the capacity to release ions. In spite of the BG glass presenting only 6.6% of P_2O_5 in its composition, while the NPBG glass had 35.2% of the same oxide (Table 1), both showed similar values of phosphate ion release, which may indicate a higher level of BG bioactivity.

The pH of chlorhexidine 2% gel was not in the

Table 2. Calcium ion release (mg/L) observed at the different times*

	10 min	24 h	7 days	14 days	21 days	30 days
CHX	94^{d}	823^{d}	695^{c}	0^{b}	0^{c}	0^{d}
CHX + CH	42.499^{b}	76.303^{a}	57.650^{a}	57.195^{a}	15.702^{b}	11.046^{b}
CH	89.706^{a}	54.412^{b}	29.025^{b}	50.165^{a}	37.116^{a}	29.101^{a}
NPBG	3.188^{d}	1.953^{cd}	2.465^{c}	0^{b}	210^{c}	2.086^{c}
BG	10.293^{c}	5.128^{c}	379^{c}	0^{b}	0^{c}	0^{d}

*Values followed by different superscript letters indicate statistically significant differences according to ANOVA ($p<0.05$) in the comparison among materials at the same experimental time.

Table 3. Sodium ion release (mg/L) observed at the different periods*

	10 min	24 h	7 days	14 days	21 days	30 days
CHX	2.706^{b}	19.208^{a}	34.144^{a}	104.490^{a}	19.521^{a}	9.325^{a}
CHX + CH	1.225^{b}	2.411^{c}	0^{c}	0^{c}	0^{b}	0^{b}
CH	1.172^{b}	1.559^{c}	0^{c}	1.615^{c}	2.284^{b}	0^{b}
NPBG	2.746^{b}	8.980^{b,c}	22.818^{b}	11.390^{b,c}	4.456^{a,b}	0^{b}
BG	6.719^{a}	15.024^{a,b}	29.484^{a}	27.482^{b}	15.436^{a}	7.856^{a}

*Values followed by different superscript letters indicate statistically significant differences according to ANOVA ($p<0.05$) in the comparison among materials at same experimental time.

Table 4. Phosphate ion release (mg/L) observed at the different periods*

	10 min	24 h	7 days	14 days	21 days	30 days
CHX	0	736^{a}	6.567^{a}	6.769^{a}	3.501^{a,b}	917^{a}
CHX + CH	0	0^{b}	0^{b}	0^{b}	0^{c}	0^{b}
CH	0	0^{b}	0^{b}	0^{b}	0^{c}	0^{b}
NPBG	0	0^{b}	4.969^{a}	2.061^{a}	1.885^{b}	859^{a}
BG	0	0^{b}	4.211^{a}	3.628^{a}	4.823^{a}	1.663^{a}

*Values followed by different superscript letters indicate statistically significant differences according to ANOVA ($p<0.05$) in the comparison among materials at same experimental times.

optimum range for its antimicrobial action, which is from 5 to 7 in all experimental periods. In this study, minimum pH was 5.8 and maximum 8.0. However, when calcium hydroxide was added to chlorhexidine, pH significantly increased during the 30 days of the study, reaching a minimum of 5.6 and a maximum of 10.4, much higher than the optimum for its action. When these medications are associated, high pH levels may cause precipitation of chlorhexidine, hence the usefulness of associating CHX+CH remains controversial. The pH of chlorhexidine 2% gel was significantly altered by the presence of dentin, raising it to values much higher than optimal for inducing antimicrobial activity (28). Barbin et al. (29) carried out the chemical analysis of 2% chlorhexidine by 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. Similar results were reported and observed that over 99% of the chlorhexidine mixed with calcium hydroxide precipitated. This finding suggests that either the high pH generated by calcium hydroxide or the dentin promoted the precipitation of chlorhexidine (30).

Even though the process of tissue repair is directly related to the alkaline potential of the used intracanal medications and their capacity to release ions, it is not known if the chlorhexidine could present some action in tissue repair in high pH. More important than tissue repair is the antimicrobial action, so if the pH of chlorhexidine is out of the 5-7 range, it may not be considered as beneficial.

Further combined studies of ion release, pH and antibacterial capacity of bioactive glasses in dentin are required to clarify the role of each ion in the antibacterial activity, before clinical recommendations be proposed.

Considering the evaluated time intervals, all the medications were capable of maintaining an alkaline pH at the end of 30 days. CH released a larger quantity of Ca^{2+}, while CHX released more Na^{+} and the bioactive glass released more PO_4^{3-} after 30 days.

Resumo

Este estudo avaliou o pH e a liberação de íons cálcio, sódio e fosfato de diferentes medicamentos em dentina humana. Cinquenta pré-molares foram preparados e divididos aleatoriamente em grupos: (CHX) - clorexidina gel 2%; (CHX + CH) - CHX + hidróxido de cálcio PA; (CH) - CH + propilenoglicol 600; (NPBG) - vidro experimental nióbio fosfato bioativo + água destilada; (BG) - vidro bioativo (Bio-Gran) + água destilada. Os espécimes foram submersos em água deionizada e as variações de pH foram mensuradas. A quantificação dos íons nas soluções foi feita por espectrometria de emissão atômica com plasma indutivamente acoplado (ICP - AES) nos tempos de 10 min, 24 h, 7, 14, 21 e 30 dias. Os resultados foram analisados por ANOVA e teste Tukey, com um nível de significância de 5%. Verificou-se que CH a teve a maior liberação íons de cálcio ao final de 30 dias, enquanto CHX e BG liberaram mais íons de sódio. BG, NPBG e CHX apresentaram a maior liberação de íons fosfato. O pH de CH foi significativamente maior em comparação com os outros grupos testados. O grupo CH aumentou o pH e a liberação de íons cálcio. Os vidros bioativos obtiveram uma maior liberação de íons sódio e fosfato e apresentaram pH alcalino imediato e ao final de 30 dias.

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