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Decalcifying effect of 15% EDTA, 15% citric acid, 5% phosphoric acid and 2.5% sodium hypochlorite on root canal dentine

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Abstract

Pérez-Heredia M, Ferrer-Luque CM, González-Rodríguez MP, Martín-Peinado FJ, González-López S. Decalcifying effect of 15% EDTA, 15% citric acid, 5% phosphoric acid and 2.5% sodium hypochlorite on root canal dentine. *International Endodontic Journal*, **41**, 418–423, 2008.

Aim To evaluate and compare *ex vivo* the decalcifying effect of 15% EDTA, 15% citric acid, 5% phosphoric acid and 2.5% sodium hypochlorite on root canal dentine.

Methodology Two 2-mm-thick slices were cut from the coronal third of the root of 10 human incisors. Each slice was sectioned into two equal parts. Specimens were assigned to one of four groups ($n = 10$) for immersion in 20 mL of either 15% EDTA, or 15% citric acid, 5% phosphoric acid or 2.5% NaOCl, for three time periods (5, 10 and 15 min). The concentration of Ca^{2+} extracted from the dentine was measured by atomic absorption spectrophometry. The amount of calcium extracted was analysed using the Kruskal–Wallis test for global comparisons and the Mann–Whitney *U*-test for pairwise comparisons.

Results In the three time periods, 15% EDTA and 15% citric acid extracted the largest amount of calcium, with no significant differences between them. The 2.5% NaOCl solution extracted insignificant amounts of calcium, whereas 15% EDTA extracted 86.72% of the calcium in the first 5 min, and 15% citric acid and 5% phosphoric acid had a similar pattern of calcium removal (77.03% and 67.08% in first 5 min, respectively).

Conclusions Solutions of 15% EDTA, 15% citric acid and 5% phosphoric acid decalcify root dentine, with most calcium extracted during the first 5 min of action. The efficacy of 15% citric acid and 15% EDTA solutions was significantly greater than that of 5% phosphoric acid solution at each time period (5, 10 and 15 min).

Keywords: citric acid, decalcification, EDTA, phosphoric acid, sodium hypochlorite, spectrophometry.

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Introduction

Many authors have concluded that the smear layer created during root canal preparation should be removed from the dentine surface of the canal wall. The following reasons have been cited: smear layer harbours bacteria and can be detrimental to effective disinfection of dentinal tubules by preventing sodium hypochlorite, calcium hydroxide and other intracanal medicaments from penetrating dentinal tubules (Clark-Holke *et al.* 2003, Shahravan *et al.* 2007); and its total removal improves the adaptation of filling materials to the root canal (Karagoz-Kucukay & Bayirli 1994, Sen *et al.* 1995), increases the bond strength of

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resin-based endodontic sealers to root dentine (Economides *et al.* 1999, Saleh *et al.* 2002, Gogos *et al.* 2003); and reduces apical and coronal microleakage with most sealers currently used for canal filling (Cobankara *et al.* 2002, Economides *et al.* 2004, Khayat & Jahanbin 2005).

Removal of the smear layer requires the use of irrigants that can dissolve both organic and inorganic components. Different irrigants have been recommended to remove the inorganic component of root dentine, e.g. EDTA solutions at a concentration of 15–17% and pH of 7–8 (Garberoglio & Becce 1994, Calt & Serper 2000, Di Lenarda *et al.* 2000, O'Connell *et al.* 2000), citric acid at a concentration of 5–50% (Ferrer Luque *et al.* 1993, Garberoglio & Becce 1994, Di Lenarda *et al.* 2000, Haznedaroglu 2003) and phosphoric acid at different concentrations and applied in different ways (Garberoglio & Becce 1994, Ayad 2001, Perez-Heredia *et al.* 2006). Sodium hypochlorite (NaOCl) solutions are used as the main irrigation agent for removing the organic component because of their bactericidal power and capacity to dissolve organic matter and necrotic tissue (Inaba *et al.* 1996, Zehnder *et al.* 2002). NaOCl is a halogenated compound used as a nonspecific proteolytic agent capable of removing magnesium and carbonate ions (Sakae *et al.* 1988). Baumgartner & Mader (1987) and Baumgartner & Cuenin (1992) suggested that its use may expose inorganic material, which would prevent greater dentine dissolution, or may leave a smear layer of mineralized tissue, which would increase the Ca/P ratio on the dentine surface.

Some reports (Hennequin & Douillard 1995, Doğan & Çalt 2001, Scelza *et al.* 2003, Ari & Erdemir 2005) have demonstrated that the mineral content of root dentine is modified by the use of EDTA and citric acid solutions to remove the inorganic component from instrumented canals alongside the use of sodium hypochlorite to remove the organic component.

Phosphoric acid, used daily in conservative dentistry, is a strong acid capable of removing the smear layer from root dentine. Ayad (2001) obtained partial smear layer removal with a 10% concentration of this acid and total removal with a 32% concentration. Garberoglio & Becce (1994) compared 17% EDTA, 3% EDTA and a combination of 24% phosphoric acid plus 10% citric acid for root canal cleaning and obtained similar results amongst the three solutions. A recent study (Perez-Heredia *et al.* 2006) alternated aqueous solutions of 2.5% sodium hypochlorite with demineralizing solutions of 15% citric acid, 15% EDTA or 5% phosphoric acid, reporting the efficacy of these agents to remove the smear layer during root canal preparation. However, there are no data on the decalcifying capacity of phosphoric acid in root dentine or on its efficacy in comparison with EDTA and citric acid solutions.

The hypothesis tested in this study was that there are no differences in decalcifying capacity of solutions of 15% EDTA, 15% citric acid, 5% phosphoric acid and that 2.5% sodium hypochlorite does not extract calcium from root canal dentine; in three immersion time periods.

The objective of this study was to assess, using atomic absorption spectrometry, the decalcifying capacity of solutions of 15% EDTA, 15% citric acid, 5% phosphoric acid and 2.5% sodium hypochlorite in three immersion time periods.

Materials and methods

Tooth selection

Ten maxillary central incisors, extracted for periodontal reasons from patients within an age range of 40–60 years, were stored in distilled water with thymol crystals until use. Patients were informed that the teeth would be used in this study applying the relevant ethical criteria: all patients consented.

Root canal preparation

Crowns were removed at the cemento-enamel junction level using an Accutom-50 diamond cutter (Accutom Hard Tissue Microtome, Struers, Ballerup, Denmark) under copious water cooling. Root cementum was removed from the root surface using a fine-grained diamond bur (Perio-Set, Intensive. Grancia, Switzerland) at low speed and under low water cooling.

Root canals were instrumented under constant water cooling with Peeso burs no. 4 to 6, (Dentsply Maillefer, Ballaigues, Switzerland) using a contra-angle handpiece. After each instrument change, root canals were irrigated with 5 mL of distilled water.

Two 2-mm-thick transverse sections were obtained from the coronal third of each root with an Accutom 50 automatic pre-programmed machine (Accutom Hard Tissue Microtome). Each slice was then divided in two equal halves, obtaining a total of four sections of each root (S1, S2, S3 and S4).

Sections were weighed using a HM 202 precision balance (A&D Engineering Inc., San Jose, CA, USA),

equalizing their weight with disks of 600-grit silicon-carbide paper (WS 18-B Struers, Ballerup, Denmark), which were always applied to the same central surface to avoid altering the geometry of the disks. Sections were then labelled and stored in flasks with distilled water at room temperature until use.

Sections of the same root (S1, S2, S3 and S4) had approximately the same weight, geometry and degree of calcification, allowing comparison of the decalcifying capacity of the four irrigation solutions by testing them on comparable specimens.

The 40 specimens obtained were divided into four experimental groups ($n = 10$) for treatment with different irrigation solutions – group 1 : 15% EDTA, pH 7; group 2 : 15% citric acid, pH 1.6; group 3 : 15% phosphoric acid, pH 1.02; group 4 : 2.5% sodium hypochlorite, pH 11.9. The pH of each solution was determined by using a pH meter equipped with Micro PH 2000 electrode (Crisol, Alella, Spain). The accuracy of the pH meter was ≤0.01.

The 2.5% sodium hypochlorite solution was prepared by diluting 10% hypochlorite solution (Panreac, Barcelona, Spain) four times in distilled water; the 15% citric acid solution by dissolving 30 g of monohydrated acid (Panreac) in distilled water to a volume of 200 mL; the 15% EDTA solution by dissolving 30 g of disodium EDTA (Panreac) in distilled water to a volume <200 mL, favouring dissolution with the addition of sodium hydroxide (NaOH) and adding 2 mol L^{-1} hydrochloric acid (HCl) to obtain a pH of 7; and the 5% phosphoric acid solution by dissolving 10 g of phosphoric acid (Panreac) in distilled water to a volume of 200 mL. All solutions were homogenized by constant stirring at 18–21°C using a magnetic multi-stirrer.

Initially, 20 mL of each solution was prepared as a blank to determine calcium levels without exposure to specimens. Each specimen was immersed in 20 mL of irrigant solution for three immersion times ($t_1 = 5$ min, $t_2 = 10$ min and $t_3 = 15$ min). Every 5 min, 5 mL of irrigant solution was extracted with a graduated pipette, which was then placed in hermetically sealed and labelled glass vessels.

Spectrometer examination

Three extracts were obtained from each sample and measured in a SpectrAA 220 FS atomic absorption spectrometer (Varian Iberica SL, Madrid, Spain) using an air/acetylene mixture as fuel for the flame. The spectrometer was calibrated using solutions of 2, 5 and 10 ppm of Ca^{2+} as reference pattern. The concentration of the original Ca^{2+} solution was 1000 ppm (Merck Inc., Whitehouse, NJ, USA). Values for extracts were expressed in mg L^{-1} (ppm).

The mg of Ca^{2+}/g (29) and the percentage mg Ca^{2+}/g extracted in each time period were calculated as follows:

$$\text{mg } Ca^{2+}/\text{g} = (\text{ppm } Ca^{2+}) \times (10^{-3}\text{L/mL}) \times \text{V/P (V, volume; P, weight of the specimen in mg)}$$

% mg Ca^{2+}/g = mg $Ca^{2+} \times 100$/total mg Ca^{2+}. This value expresses the % Ca increase in each time interval with respect to the total Ca^{2+} extracted.

Statistical analysis

First, a full-factorial regression model of repeated measures was used to assess the significance of the interaction between two factors (type of irrigation solution and immersion time in irrigation solution) for the extracted calcium data (mg Ca^{2+} and % mg Ca^{2+}) The Kolmogorov–Smirnov test was used to assess the distribution of the extracted calcium data. Because results for each group did not follow a normal distribution, variables were analysed using a nonparametric test. The amount of calcium extracted (mg Ca^{2+} and %mg Ca^{2+}) by different irrigating solutions and in different immersion times was analysed using the Mann–Whitney *U*-test (pairwise comparisons) and the Kruskal–Wallis test (global comparisons). The level of statistical significance was set at $P < 0.05$.

Results

Full-factorial regression analysis of the influence of the type of irrigation solution (15% EDTA, 15% citric acid, 5% phosphoric acid or 2.5% NaOCl) and of the time of immersion in solution (5, 10 or 15 min) revealed a statistically significant interaction between these two factors in the amount of calcium extracted ($P = 0.003$) and in the percentage of calcium extracted ($P < 0.001$). Table 1 shows the amount of calcium extracted (mg Ca2+) for each type of irrigant solution and immersion time. After a 5-min immersion in irrigant solution, 15% EDTA had extracted the greatest amount of Ca^{2+}, followed by 15% citric acid and 5% phosphoric acid, with a negligible amount of Ca^{2+} extracted in 2.5% sodium hypochlorite. The differences amongst solutions were significant ($P < 0.05$) except in the comparison between 15% EDTA and 15% citric

Table 1 Amount of calcium extracted (mg Ca^{2+}) as a function of irrigating solution and immersion time*

Irrigating solution ($\bar{x}$ ± SD	Immersion time		
	5 min	10 min	15 min
15% EDTA	0.085 ± 0.029^{b,1}	0.094 ± 0.028^{b,2}	0.098 ± 0.028^{b,3}
15% citric acid	0.075 ± 0.019^{b,1}	0.093 ± 0.024^{b,2}	0.099 ± 0.027^{b,3}
5% phosphoric acid	0.035 ± 0.015^{c,1}	0.046 ± 0.020^{c,2}	0.052 ± 0.023^{c,3}
2.5% NaOCl	0.009 ± 0.004^{a,1}	0.015 ± 0.004^{a,2}	0.019 ± 0.004^{a,3}

*In the full-factorial regression model, *P* values were <0.001 (for irrigating solution), <0.001 (for immersion time) and 0.003 (for irrigating solution × immersion time interaction).
Read vertically, the same letters indicate absence and different letters presence of significant differences.
Read horizontally, the same numbers indicate absence and different numbers presence of significant differences.

acid ($P = 0.820$). After a 10-min immersion, there was no significant difference ($P = 0.821$) between the amounts of Ca^{2+} extracted by 15% EDTA and 15% citric acid, but these were significantly greater than the amounts extracted by 2.5% NaOCl and 5% phosphoric acid. After a 15-min immersion, there was again no significant difference ($P = 0.623$) between the amounts of Ca^{2+} extracted by 15% EDTA and 15% citric acid but these were significantly greater than the amounts extracted by 2.5% NaOCl and 5% phosphoric acid. The global comparison amongst times shows statistically significant differences ($P < 0.001$) in the calcium extracted amongst the three immersion periods and amongst the four irrigation solutions. Table 2 shows the % calcium extracted during the three time periods. The most rapid decalcification rate was with 15% EDTA, which extracted 86.72 ± 7.49% of the calcium during the first 5 min, 10.02 ± 6.35% between 5 and 10 min, and 5.75 ± 4.19% between 10 and 15 min. A similar behaviour was shown by 5% citric acid and 5% phosphoric acid, which extracted 77.03 ± 11.98% and 67.08 ± 9.89% during the first 5 min of immersion. No significant differences were found ($P = 0.241$) in the % calcium extracted by 2.5% NaOCl between the 5-min and 10-min immersions.

Discussion

The efficacy of agents used to remove smear layer and demineralize and soften root dentine during root canal treatment has been examined by various means, including microhardness measurements, micro-radiographic assessments, spectrometry studies (Verdelis *et al.* 1999, Doğan & Çalt 2001, Scelza *et al.* 2003, Machado-Silveiro *et al.* 2004, Ari & Erdemir 2005, Gonzalez-Lopez *et al.* 2006) and, especially, electron microscopy studies (Ferrer Luque *et al.* 1993, Calt & Serper 2000, Di Lenarda *et al.* 2000, O'Connell *et al.* 2000, Ayad 2001, Haznedaroglu 2003, Perez-Heredia *et al.* 2006). The decalcifying efficacy of these acid and chelating agents depends on the root length, application time, diffusion in the dentine and, especially, the solution pH (Sen *et al.* 1995, Doğan & Çalt 2001, Serper & Calt 2002). The use of a neutral pH of around 7.3 is recommended for EDTA solutions (Serper & Calt 2002). Citric acid has shown to be effective at pH values of 0.8–1.9 (Hennequin & Douillard 1995, Haznedaroglu 2003). In the present study, the amount of extracted Ca^{2+} increased with time in all solutions and no significant differences were found between 15% EDTA and 15% citric acid. These findings are consistent

Table 2 Percentage of calcium extracted (mg Ca^{2+}) as a function of irrigating solution and immersion time*

Irrigating solution ($\bar{x}$ ± SD)	Immersion time		
	5 min	5–10 min	10–15 min
15% EDTA	86.72 ± 7.49^{b,1}	10.02 ± 6.35^{b,2}	3.08 ± 3.01^{b,3}
15% citric acid	77.03 ± 11.98^{b,c,1}	17.23 ± 9.46^{b,c,2}	5.75 ± 4.19^{b,3}
5% phosphoric acid	67.08 ± 9.89^{c,1}	22.30 ± 7.25^{c,2}	10.64 ± 4.29^{c,3}
2.5% NaOCl	43.43 ± 14.15^{a,1}	34.04 ± 7.99^{a,1}	22.56 ± 7.97^{a,2}

*In the full-factorial regression model, *P* values were 0.446 (for irrigating solution), <0.001 (for immersion time) and <0.001 (for irrigating solution × immersion time interaction).
Read vertically, the same letters indicate absence and different letters presence of significant differences.
Read horizontally, the same numbers indicate absence and different numbers presence of significant differences.

with previous results using 10% and 20% citric acid and 17% EDTA solutions (Scelza *et al.* 2003, Gonzalez-Lopez *et al.* 2006). However, in all three immersion times studied, the decalcifying capacity of 15% EDTA and 15% citric acid solutions was higher than that of the 5% phosphoric acid solution (Table 1). The reason for these differences may be that the concentration of phosphoric acid was lower than that of the EDTA and citric acid solutions. Thus, a higher extraction of Ca^{2+} ions could be expected if higher concentrations of phosphoric acid were used. Decalcification may also be higher at a specific pH, as in the case of citric acid solution at pH 1.1 (Hennequin & Douillard 1995). However, higher concentrations of phosphoric acid could cause reprecipitation of hydroxyapatite from the calcium phosphate solutions formed by the initial dissolution of root dentine. The formation of new calcium phosphate complexes would reduce the extraction of calcium ions from exposed root dentine (Marshall *et al.* 1993). An effective irrigation solution must also be able to remove the inorganic component from dentine, and a recent study (Perez-Heredia *et al.* 2006) demonstrated that the combined use of 5% phosphoric acid and 2.5% sodium hypochlorite solutions is adequate to remove the smear layer from the root canal. In this study, the % Ca^{2+} extracted was higher during the first 5 min of immersion in all solutions, with the highest percentage (86.72%) extracted with 15% EDTA solution. These results are in agreement with the report by Cergneux *et al.* (1987) of total removal of the smear layer after using a 15% EDTA solution for 4 min. Other studies demonstrated that the highest amount of Ca^{2+} ions is extracted during the first 3 min of immersion in a 17% EDTA solution (Scelza *et al.* 2003, Gonzalez-Lopez *et al.* 2006) and during the first 5 min in a 10% citric acid solution (Machado-Silveiro *et al.* 2004). After 5 min, the decalcification progressively reduced, and significant differences were found between the 10-min and 15-min immersion periods (Table 2). These results agree with those obtained by Gonzalez-Lopez *et al.* (2006) using the same methodology and could be explained in relation to the acid and chelating solutions studied, by an increase in the organic material exposed on root dentine surfaces after action of the demineralizing agents. The organic matrix of dentine may act as a limiting factor in the dissolution of the inorganic component, thus reducing the decalcifying action of chelating agents over time (Inaba *et al.* 1996, Verdelis *et al.* 1999, Doğan & Çalt 2001). In the present study, a 2.5% sodium hypochlorite solution had a small decalcifying effect. The extraction of Ca^{2+} ions was significantly lower than achieved with the acid and chelating solutions assessed. It has been reported that treatment with sodium hypochlorite causes mineral accumulation in human root dentine (Inaba *et al.* 1996). Sodium hypochlorite dissolves organic material and exposes inorganic material, thereby avoiding a greater dissolution of root dentine and it leaves a smear layer of mineralized tissue (Baumgartner & Mader 1987, Baumgartner & Cuenin 1992). It has been demonstrated that the use of 2.5% sodium hypochlorite as irrigation solution, either alone or combined with a 17% EDTA solution, significantly increases the Ca/P ratio of root dentine (Doğan & Çalt 2001). In this study, 2.5% sodium hypochlorite solution obtained a higher percentage of calcium ion extraction during the first 5 min followed by a slow decrease in the extraction rate, with no significant differences between the 5-min (43.43%) and 10-min (34.04%) immersion times. Changes in hydroxyapatite re-crystallization after sodium hypochlorite treatment (Perdigao *et al.* 2000) may be responsible for the decrease in calcium and phosphorus found in root dentine (Ari & Erdemir 2005). Furthermore, in removing the organic component from dentine, sodium hypochlorite also eliminates mineralization inhibitors and increases the porosity of residual dentine (Sakae *et al.* 1988, Inaba *et al.* 1996). Passage of Ca^{2+} ions into the irrigation solution would explain the decalcification results obtained with 2.5% sodium hypochlorite solution in the present study.

Conclusions

Within the limitations of the present study, it can be concluded that the use of solutions of 15% EDTA, 15% citric acid or 5% phosphoric acid produces root dentine decalcification, mainly during the first 5 min of action. The efficacy of 15% EDTA and 15% citric acid solutions was significantly higher than that of 5% phosphoric acid solution in all three immersion periods studied. It was also observed that 2.5% sodium hypochlorite solution is capable of extracting small amounts of calcium from root dentine.

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