Decalcification of root canal dentine by citric acid, EDTA and sodium citrate

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Abstract

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Aim To measure the demineralization capability of 1 and 10% citric acid, 10% sodium citrate and 17% EDTA during immersions of 5, 10 and 15 min on root canal dentine.

Methodology Crowns were sectioned from eight maxillary canines. The cementum was removed from the cervical third of the roots to expose the dentine. Canals were prepared using a handpiece-mounted Largo Peeso reamer. A 3-mm thick cross-sectional slice was obtained from the cervical third of each root. Each slice was sectioned into four equal parts. These specimens were assigned to one of four groups ($n = 8$) for the application of 1% citric acid, 10% citric acid, 10% sodium citrate or 17% EDTA. Each specimen underwent three successive 5-min immersions in each solution at room temperature. The solutions were not renewed between immersions. Two millimetres of solution were collected from the extracts and lanthanum oxide was added for the calcium reading by spectrophotometry. To compare the amounts of calcium removed by each solution, the Friedman test was used for the global comparison and the Wilcoxon test for paired comparisons. Differences between groups were evaluated using the Kruskal–Wallis test for the global comparison and Mann–Whitney test for paired comparisons.

Results Overall, 1 and 10% citric acid were more effective than EDTA or sodium citrate at the three immersion times ($P < 0.001$); 10% citric acid was more effective than 1% citric acid ($P < 0.001$). EDTA and 1 and 10% citric acid showed decreasing effectiveness with time, and the decrease was significant for citric acid at both concentrations ($P < 0.001$). Although sodium citrate removed little calcium during the three time periods, the small increase recorded was significant ($P < 0.01$).

Conclusions Citric acid at 10% was the most effective decalcifying agent, followed by 1% citric acid, 17% EDTA and 10% sodium citrate.

Keywords: decalcifying agents, root canal, smear layer.

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Introduction

Cavity preparation and root canal instrumentation leave a layer of debris that covers the walls of the cavity and root canal, known as the smear layer. The layer is composed of organic and inorganic components and, in cases of contamination, a bacterial component. The removal of smear layer requires organic and inorganic solvents (Scelza *et al.* 2003).

The smear layer was first described in endodontics by McComb & Smith (1975), who removed it with a chelating agent. Chelating agents induce changes in the structure of dental tissue and in calcium and phosphorus ion levels in the dentine (Rotstein *et al.* 1996).

The most widely used demineralizing agents are citric acid (at different concentrations) and EDTA

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trisodium salt (15–17% concentrations). The former has a maximum effectiveness at pH 1.2 (Hennequin *et al.* 1994) and the latter at pH 7.2 (Ravnik & Loe 1961). High concentrations (25–50%) of citric acid are more effective than EDTA in removing calcium ions from dentine (Pashley *et al.* 1981, Ferrer-Luque *et al.* 1996). However, although 1% citric acid is equally as effective as EDTA in removing dentine debris or tissue (Brancini *et al.* 1983), it has a low pH, which may have an irritant effect on periapical tissues (Garberoglio & Becce 1994).

Citric acid in the form of 10% sodium citrate has a pH close to neutral, which may make it more effective at decalcifying dentine, because dissolution is reduced markedly at low pH. In addition, a neutral pH is more biocompatible (Silveira 1990).

The present study used spectrophotometry to evaluate *in vitro* the demineralization capability of 1 and 10% citric acid, 10% sodium citrate and 17% EDTA after 5, 10 and 15 min.

Materials and methods

Specimen preparation

The crowns of eight recently extracted human maxillary canines, stored in distilled water until use, were sectioned at the cemento-enamel junction using a diamond disc water-irrigated. The cementum of the cervical third was then removed with a high-speed 8951KR diamond bur (Komet Dental, Gerbr., Brasseler, Lemgo, Germany) and abundant irrigation. The canal was widened with a large water-irrigated Peeso reamer (Dentsply Maillefer, Ballaigues, Switzerland) mounted on a handpiece with continuous water irrigation.

A 3-mm transverse section was obtained from the cervical third of each root using an irrigated diamond disc. Each section was divided equally into four with the same size and shape (S1, S2, S3 and S4), with each part constituting a sample specimen. After they were catalogued, the specimens were stored in distilled water.

Experimental method

The specimens were assigned to one of four experimental groups ($n = 8$) for treatment with various irrigants, as follows: group 1 [S1]: 1% citric acid, group 2 [S2]: 10% citric acid; group 3 [S3]: 10% sodium citrate; or group 4 [S4]: 17% EDTA.

Citric acid was prepared by dissolving monohydrated citric acid in distilled water to obtain concentrations of 1 and 10% (by volume); the pH of these concentrations was 2.2 and 1.8, respectively. The sodium citrate was obtained by dissolving its salt in distilled water to a concentration of 10% (pH 7.6). The EDTA was prepared by diluting the dehydrated salt of the acid to obtain a concentration of 17%; the pH was adjusted in a potentiometer with sodium hydroxide to a pH of 7.2.

Each specimen underwent three successive 5-min immersions (T5, T10 and T15) at room temperature (16–18 °C). The solution was not renewed between immersions. Each immersion was performed in a plastic beaker with 5 mL of the irrigating substance. Thus, three extracts were obtained for each sample for the reading of calcium levels.

All decalcification procedures were carried out on the same day and at the same room temperature.

Calcium reading

Two millilitres of solution were collected from all extracts. Two mL of 0.2% lanthanum oxide were added to the extracts from groups 1, 2 and 4 (1 and 10% citric acid and 17% EDTA). Two mL of 0.5% lanthanum oxide were added to the extracts from group 3 (sodium citrate), due to the sparse amount of calcium in these extracts. Lanthanum oxide is used as a buffer to avoid the false positives that may be produced in aqueous solutions that contain sodium, potassium and magnesium. These elements can compromise the accuracy of the calcium reading by forming other compounds in the flame.

An air/acetylene mixture was used as the oxidant and flame fuel for group 1, 2 and 4 extracts, and a nitrous oxide/acetylene mixture for group 3 extracts.

The spectrophotometer (model AA475; Varian, New York, NY, USA) was calibrated with standard reference solutions. Thus, for each substance under study, solutions were obtained with a known amount of calcium dissolved in the same substance. Because 1 and 10% citric acid extracts a large amount of calcium (verified in a preliminary study), their standard solutions were at concentrations of 0, 10, 20 and 50 mg L^{-1} of calcium. Because the preliminary analysis showed only a small amount of calcium for the sodium citrate, the standard solutions were established at concentrations of 0, 1.25, 2.5 and 5 mg L^{-1} of calcium. After the reading of each extract, the apparatus was recalibrated using the same standard solutions.

The extract readings were expressed in mg L^{-1}. The amount of calcium obtained in each extract was verified by converting the results into mg of calcium and adding together the data for the total time.

Statistical analysis

Because the results for each group did not follow a normal distribution, the variables were analysed using a nonparametric test. Amounts of calcium removed during the different time periods (T5, T10 and T15) were compared in the same group using the Friedman test for the global comparison and the Wilcoxon test for paired comparisons. Differences between the groups were studied by using the Kruskal–Wallis test for the global comparison and the Mann–Whitney test for paired comparisons.

Results

Table 1 contains the values obtained in the four study groups in the three time periods and globally. Table 2 shows the comparisons by time period and by group.

The effectiveness of EDTA and citric acid (at 1 and 10%) to remove calcium decreased over the three time periods, with statistically significant differences in the case of citric acid ($P < 0.001$). One per cent citric acid showed greatest effectiveness in the first 5-min immersion (0.1050 mg), after which its action was reduced and remained unchanged in the two subsequent time periods (0.0744 and 0.0763 mg, respectively). Ten per cent citric acid presented similar behaviour, although it removed over double the mg of calcium compared with 1% citric acid in each study period (T5, 0.2138 mg; T10, 0.1950 mg; T15, 0.1475 mg).

There were no significant differences in the decalcifying activity of EDTA between the three time periods (T5, 0.0563 mg; T10, 0.0544 mg; and T15, 0.0513 mg; $P = 0.381$).

The amount of calcium removed by sodium citrate was low in comparison with the other substances studied (T5, 0.0035 mg; T10, 0.0041 mg; T15, 0.0046 mg), although there was a small but statistically significant ($P < 0.01$) increase with longer immersion time.

Comparison between the substances showed statistically significant differences in the amount of calcium removed during the three time intervals and overall ($P < 0.001$). One per cent and 10% citric acid were more effective than EDTA or sodium citrate, and 10% citric acid was more effective than 1% citric acid.

Table 1 Calcium extract readings after the three immersion times in the four groups, expressed in mg of calcium

Group	Sample	5 min	10 min	15 min	Total
1% Citric acid	01	0.105	0.075	0.075	0.255
	02	0.105	0.080	0.085	0.270
	03	0.090	0.065	0.070	0.225
	04	0.100	0.070	0.065	0.235
	05	0.110	0.085	0.085	0.280
	06	0.105	0.070	0.080	0.255
	07	0.105	0.070	0.070	0.245
	08	0.120	0.080	0.080	0.280
10% Citric acid	01	0.195	0.170	0.120	0.485
	02	0.205	0.195	0.150	0.550
	03	0.185	0.170	0.135	0.490
	04	0.150	0.130	0.100	0.380
	05	0.245	0.255	0.185	0.685
	06	0.250	0.225	0.185	0.660
	07	0.205	0.175	0.125	0.505
	08	0.275	0.240	0.180	0.695
10% Sodium citrate	01	0.0045	0.0030	0.0035	0.0110
	02	0.0025	0.0030	0.0035	0.0090
	03	0.0025	0.0035	0.0035	0.0095
	04	0.0020	0.0030	0.0030	0.0080
	05	0.0030	0.0055	0.0060	0.0145
	06	0.0055	0.0060	0.0060	0.0175
	07	0.0040	0.0045	0.0065	0.0150
	08	0.0040	0.0045	0.0050	0.0135
17% EDTA	01	0.080	0.070	0.070	0.220
	02	0.040	0.040	0.035	0.115
	03	0.040	0.055	0.060	0.155
	04	0.040	0.045	0.050	0.135
	05	0.050	0.055	0.050	0.155
	06	0.065	0.060	0.050	0.175
	07	0.065	0.050	0.045	0.160
	08	0.070	0.060	0.050	0.180

Discussion

In the design of this study, the issue of biological variability among different teeth was addressed by comparing the effects of different solutions and immersion times among dentine sections from the same root slice. It was assumed that there was no biological variability among four sections of the same slice, which should behave in the same way because their calcium contents are likely to be identical.

All decalcification procedures were carried out on the same day at the same room temperature, because an increase in temperature accelerates the demineralization process.

Three immersion periods were studied, as has been done previously (Silveira 1990), although the periods were shorter in the present study. The solution was not renewed between immersions. Renewal of the solution increases the effectiveness of its action compared with a

Table 2 Comparison of means of calcium extractions by immersion time and group (mean ± SD, $n = 8$)

Group	1% Citric acid	10% Citric acid	10% Sodium citrate	17% EDTA	*P*
Time					
5 min	0.1050 ± 0.0085	0.2138 ± 0.0404	0.0035 ± 0.0012	0.0563 ± 0.0158	<0.001
10 min	0.0744 ± 0.0068	0.1950 ± 0.0421	0.0041 ± 0.0012	0.0544 ± 0.0094	<0.001
15 min	0.0763 ± 0.0074	0.1475 ± 0.0328	0.0046 ± 0.0014	0.0513 ± 0.0103	<0.001
P	0.001	0.001	0.01	0.381	
Total	0.2556 ± 0.0203	0.5563 ± 0.1133	0.0123 ± 0.0034	0.1619 ± 0.0314	<0.001

Values joined by vertical lines were not significantly different.

single continuous application over the same time period (Weinreb & Meier 1965) because it maintains the pH at neutral levels, thereby increasing its moisturizing and decalcifying capacity (Perez *et al.* 1989).

In the present study, the decalcifying action of EDTA was not dependent on immersion time. It was similar for the three time periods, with a slight tendency to a reduction with longer time. Scelza *et al.* (2003) also found that the action of EDTA was not time-dependent, although they reported a small nonsignificant increase in the decalcifying activity of 17% EDTA with time. The specific affinity of EDTA for the chelation of metal ions (Voguel 1981) may have influenced the speed of chemical reactions between EDTA and the substrate. The chelation energy of EDTA is more pronounced than its demineralizing activity. Time periods of less than 5 min are not recommended for EDTA (Holland *et al.* 1973), although chelating activity is observed at between 1 and 4 min (Cergneux *et al.* 1987, Çalt & Serper 2000).

The decalcifying action of 10% citric acid was double or more that of 1% citric acid but this depended on the duration of the application. The demineralizing action of 10% citric acid reduced during the three periods. Scelza *et al.* (2003) found that the demineralizing action of 10% citric acid significantly increased from 3 to 10 min, but was no longer time-dependent at 15 min. Another study (Sterrett *et al.* 1993) reported that the demineralizing action of 10% citric acid was not time-dependent during short immersion times (1, 2 and 3 min). In the present study, the activity of 1% citric acid was time-dependent for only the first 10 min, with no significant differences between the 10- and 15-min immersion periods.

Sodium citrate was the least effective agent studied, with a small amount of calcium removed during the three periods. Thus, the physicochemical characteristics of the original acid are not preserved in the salt. It can be assumed that the decalcifying action of citric acid, which needs an acid pH, is greater than its chelating action. It is possible that sodium citrate only has the chelation activity of the original acid, which is low. This may explain the lower decalcifying activity of sodium citrate compared with the other substances. It may be more effective over longer time periods, because a tendency for an increase in extracted calcium that reached significance between the second and third immersion periods was observed.

Some authors have recommended the combined use of EDTA and sodium hypochlorite as an effective method to remove the smear layer (Yamada *et al.* 1983, Baumgartner & Mader 1987). In the present study, decalcification was most effective in the first 5 min in the cases of 1 and 10% citric acid and EDTA. As the demineralization increases, so does the organic material content, which could reduce the action of these agents.

Mixture of an Acid (citric acid), Tetracycline isomer (doxycycline) and a Detergent (Tween-80)] (MTAD) was recently assessed for smear layer removal in root canals and proved more effective than 17% EDTA; moreover, when used in combination with low concentrations of NaOCl, it completely removed the smear layer without significantly changing the structure of the dental tubules (Torabinejad *et al.* 2003).

EDTA is usually applied at a 17% concentration (O'Connell *et al.* 2000). In contrast, citric acid is used at various concentrations (Takeda *et al.* 1999, Scelza *et al.* 2000, Haznedaroglu & Ersev 2001). Brancini *et al.* (1983) observed, using scanning electron microscopy, a similar action between EDTA and 1% citric acid in smear layer removal, and Sperandio (2000) found no difference in tissue reaction between 1% citric acid and EDTA in a histological study of the periapical tissues of dogs. In the present study, 1% citric acid proved to be a more effective calcifying agent compared with 17% EDTA, indicating that 1% citric acid is an effective option for the clinical removal of smear layer from dentine walls.

Conclusions

The most effective decalcifying substance was 10% citric acid, followed by 1% citric acid, 17% EDTA and 10% sodium citrate. A statistically significant difference between all four substances for each immersion time studied was found.

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