Decalcifying capability of irrigating solutions on root canal dentin mineral content

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Abstract

Background: Chelating agents are believed to aid root canal irrigation and to be able to remove the inorganic smear layer. **Aims:** The purpose of the present study was to evaluate and to compare the decalcifying capability of different irrigating solutions (Tubuliclean, Largal Ultra, ethylenediaminetetraacetic acid 17%, Tetraclean, Tetraclean NA). **Materials and Methods:** Sixty maxillary central incisors were used. Root canals were instrumented and irrigated. From each root, four comparable slices of cervical dentin were obtained. At three successive 5-min interval immersion times, the concentration of calcium extracted from root canal dentin was assessed with an inductively coupled plasma-atomic emission spectrometer. **Statistical Analysis Used:** Data were analyzed by means of Kruskal Wallis and Mann–Whitney tests. Significance was predetermined at $P < 0.05$. **Results and Conclusions:** For all irrigating solutions, the maximum amount of Ca^{2+} extracted from root canal dentin samples was reached after 10 min contact time except for citric acid-based agents (Tetraclean and Tetraclean NA) which induced a higher and still increasing calcium release even after 10 min contact time. In order to obtain an efficient decalcifying action on dentin and to facilitate the biomechanical procedures, citric acid-based irrigants can be applied.

Keywords: Ca^{2+}, ethylenediaminetetraacetic acid, irrigating solutions, root canal dentin, Tetraclean NA

Introduction

Biomechanical instrumentation of root canals creates an amorphous layer of organic and inorganic debris. In the case of contamination, a bacterial component is formed and deposited along the root canal walls.[1-4] The smear layer adheres weakly to root canals walls, reduces sealer adhesion and affects sealing negatively,[5] so it has to be removed before obturation to ensure a close contact of the sealer with the dentin surface. To eliminate the smear layer, irrigating solutions should be able to dissolve both organic and inorganic components.

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Chelating agents are believed to aid root canal irrigation and to be able to remove the inorganic smear layer.[5] The use of sodium hypochlorite could improve the efficacy of the chelating agents on dissolving the organic fraction of the smear layer.[2] In particular, the irrigating solutions have been chosen due to their direct action over calcium present in hydroxyapatite crystals of dentin. Any change in the calcium ratio can significantly alter the original proportion of organic and inorganic components, which can alter dentin permeability, microhardness, and solubility.[6] Although ethylenediaminetetraacetic acid (EDTA) present a long-standing history because it is the most frequently recommended for the removal of the smear layer in endodontics, its irritating potential has been highlighted.[7-10] Therefore, to remove the inorganic component of root dentin, other substances have been suggested. Citric acid at a concentration ranging from 5% to 50%, apple vinegar and phosphoric acid at different concentrations have been proposed with varying degrees of success.[11-20] MTAD and recently, Tetraclean has been investigated as new irrigating solutions based on a mixture of citric acid and antibiotic (tetracycline isomer): They are proposed as detergent for the final rinse of surfaces of instrumented root canals.[21-23] MTAD and Tetraclean differ in doxycycline concentration (150 mg/ 5 ml for MTAD and 50 mg/ 5 ml for Tetraclean) and in the type of the detergent (Tween 80 for MTAD, cetrimide and polypropylene glycol for Tetraclean). Recently Tetraclean NA has been developed: It is a new irrigating solution without antibiotics (citric acid + Cetrimide + Polypropylene Glycol).

Cetrimide (cetyltrimethylammonium bromide) is a quaternary ammonium salt and a cationic detergent added to many

products because its addiction improves efficacy of irrigating solutions: Cationic surfactants have been reported to have bactericidal and fungicidal properties.[24]

The aim of this study was to evaluate and to compare the decalcifying capability of different irrigating solutions at different contact times compatible with the endodontic treatment. In particular, the measurements performed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) of the concentration of calcium extracted from the root canal dentin have been evaluated at 5, 10, and 15 min extraction times.

Materials and Methods

Sixty maxillary central incisors, freshly extracted for periodontal reasons, were stored in 0.1% thymol solution until use. The crowns were removed at cemento-enamel junction using an Accutom-50 diamond cutter (Accutom Hard Tissue Microtome, Struers, Ballerup, Denmark) under water-cooling. The root surface was treated with a low-speed fine-grain diamond bur (Perio-Set, Intensiv, Grancia, Switzerland) under abundant irrigation to remove the root cementum. Root canals were instrumented under abundant irrigation with Peeso burs n° 4–6 (Dentsply Maillefer, Ballaigues, Switzerland) using a contra-angle handpiece. After each instrument, the root canal was irrigated with 5 ml of distilled water. Two transversal sections of 2-mm thickness were obtained from the cervical third of each root [Figure 1] using a preprogrammed automatic Accutom-50 diamond cutter (Accutom Hard Tissue Microtome, Struers, Ballerup, Denmark). Each slice was then sectioned into four equal sections, obtaining a total of four (S1, S2, S3, S4) samples from each root [Figure 2]. To yield each sample with same calcification, geometry, and weight, the samples were weighted on a HM 202 precision balance (A and D Engineering Inc., San Jose, CA, USA) and when necessary, equalized their weight with disks of 600-grit silicon-carbide paper, always removing from the central part of the section to avoid modification of the geometry.

Figure 1: Transversal slice of 2-mm thickness obtained from the cervical third of each root using a preprogrammed automatic Accutom-50 diamond cutter (Accutom Hard Tissue Microtome, Struers, Ballerup, Denmark)

This method allows testing irrigating solution decalcifying capacity on comparable specimens. Samples were then catalogued and stored into distilled water at room temperature. The specimens were assigned to one of the six experimental groups ($n = 6$) for treatment with different irrigating solutions, as follows:

- Group 1: "Tubuliclean" (EDTA 10%), Ogna Laboratori Farmaceutici, Muggiò, Italy
- Group 2: "Largal Ultra" (EDTA 15% + Cetrimide 0.75%), Septodont, Saint-Maur-des-fossés, France
- Group 3: "EDTA 17%" (EDTA 17%), Ogna Laboratori Farmaceutici, Muggiò, Italy
- Group 4: "Tetraclean" (citric acid 10.5% + Cetrimide 0.2% +1% Doxycycline Hyclate + Polypropylene Glycol), Patent n. KR2004A000001
- Group 5: "Tetraclean NA" (citric acid+ Cetrimide + Polypropylene Glycol), Patent n. KR2012A000001
- Group 6: Saline solution as control.

The pH of each solution was measured by a PHM 84 Research pH meter (Radiometer, Copenhagen, Denmark) and a combined ORION glass electrode (Thermo Electron Corp, Waltham, Mass). The accuracy of the pH meter was ±0.01.

The initial calcium concentration in each irrigating agent was analyzed by ICP-AES (blank). Each specimen was initially immersed in 20 ml of the correspondent irrigating solution and kept under constant stirring using a magnetic stirrer. At three successive 5-min interval immersion times ($t_1 = 5$ min; $t_2 = 10$ min; $t_3 = 15$ min), 5 ml of irrigant was sampled with a graduated pipette and placed in labeled tubes. An (ICP-AES Perkin Elmer, Monza, Italy) was used for calcium determination in each solution, by external standard calibration: Linearity

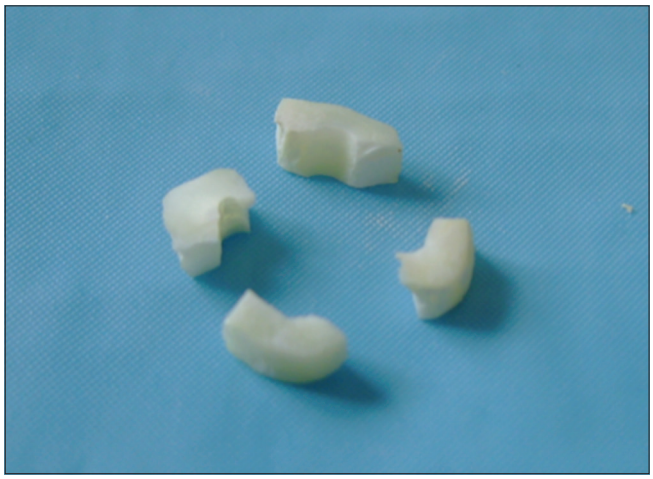

Figure 2: Slice sectioned in four equal sections, obtaining a total of four (S1, S2, S3, S4) samples

range between 0.5 and 100 mg/L; limit of detection 0.1 mg/L, limit of quantification 0.5 mg/L.

Data analysis was performed by Stata 12 (StataCorp, Stata Statistical Software: Release12, College Station, TX, USA). The assessment of normality was developed with Shapiro–Wilk test ($P < 0.05$) while comparison tests between groups were conducted with Mann–Whitney U-test with a level of significance of $P = 0.05$.

Results

Descriptive statistics of calcium values released from root dentin for each irrigating solution were calculated and reported in Table 1. The null hypothesis that the distributions are modeled as a normal were not confirmed with Shapiro–Wilk test at a level of significance of $P = 0.05$. Mann–Whitney U-test was conducted to investigate possible differences in calcium release from root canal dentin after the application of the different irrigating solutions for 5 and 10 min and for 10 and 15 min. The results reported in Table 1 show that the irrigating solutions tested promote higher calcium release values from root canal dentin at 10 min exposition time than at 5 min; instead calcium release is not significantly different between 10 and 15 min, except for citric acid-based agents (Tetraclean NA) which induced a higher and still increasing calcium release even after 10 min contact time ($P < 0.05$). Moreover both the citric acid-based agents (Tetraclean and Tetraclean NA) promote the highest calcium release at the three different contact times.

Figure 3 reports the amounts of calcium extracted (mg/L Ca) by the different irrigating solutions at different immersion times.

Discussion

A significantly higher release of Ca^{2+} was observed in samples submitted to citric acid-based agents. The amount

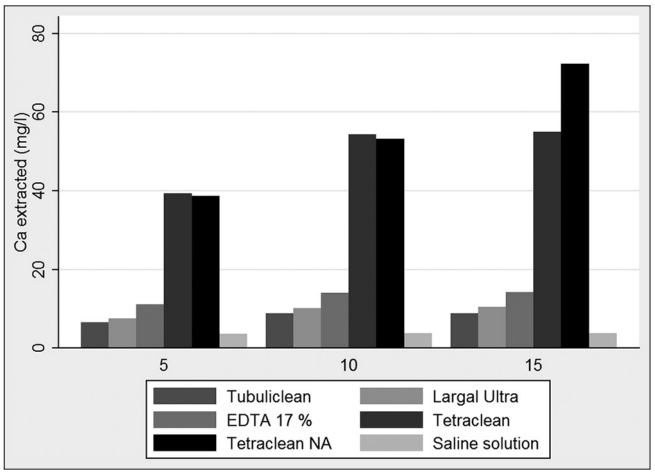

Figure 3: Amount of calcium extracted (mg/L) with five irrigating solutions (and saline solution) at different immersion times

Table 1: Comparison of calcium released (mg/L) from the root canal dentin between 5 and 10 min treatments with tested solutions and between 10 and 15 min treatments: results of the Mann-Whitney test

Immersion time	Irrigating solution	Mean	SD	Median	Mann-Whitney test (at 0.05 level) probability value (*P*)	
					5 versus 10	10 versus 15
5	Tubuliclean	6.6	0.6	6.7	0.0000<0.05	0.7192>0.05
10		8.9	0.4	8.9		
15		8.8	0.3	8.9		
5	Largal Ultra	7.6	0.7	7.5	0.0000<0.05	0.1555>0.05
10		10.2	0.7	10.1		
15		10.5	0.7	10.4		
5	EDTA 17%	11.1	1.2	10.9	0.0000<0.05	0.2179>0.05
10		14.1	0.4	14.1		
15		14.3	0.5	14.4		
5	Tetraclean	39.4	0.9	39.4	0.0000<0.05	0.1464>0.05
10		54.4	1.6	54.7		
15		55	1.3	55.4		
5	Tetraclean NA	38.7	1.1	38.5	0.0000<0.05	0.0000<0.05
10		53.2	2	53.2		
15		72.3	1.8	72.5		
5	Saline solution	3.6	0.1	3.6	0.0003<0.05	0.6398>0.05
10		3.8	0.1	3.8		
15		3.8	0.1	3.8		

SD: Standard deviation, NA: Not available, EDTA: Ethylenediaminetetraacetic acid

of Ca^{2+} extracted from root dentin samples for all irrigating solutions at 10 min did not show significant differences when compared to the values reported at 15 min. Therefore, in the present investigation, an application of 10 min is sufficient to reach the maximum release of Ca^{2+} for all irrigating solutions tested. Results are in agreement with those obtained by other Authors that compared the decalcifying effect of 15% EDTA and 15% citric acid on root canal dentine: Pérez-Heredia *et al*. analyzed the amount of calcium extracted from dentin samples by atomic absorption spectrophotometry and obtained similar results at three immersion times, with no significant differences between EDTA and citric acid.[17] They observed no significant differences between 10-min and 15-min immersion. This could be explained, in relation to the acid and chelating solution studied, by an increase in the organic material exposed on root dentin surface 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.[17] Higher release in this study can be due to the lower pH of the citric acid solutions (pH < 2), thus increasing the removal of major inorganic elements like calcium present in the hydroxyapatite crystals. This is in agreement with previous results.[6] Moreover, another investigation reported that the pH of citric acid solution is a more important factor than concentration in demineralization test.[25] Authors reported that this could be due to a balance between the decrease in pH and the increase in viscosity of the solution caused by the increase in the constituent concentration. In fact at high citrate concentrations, the quantity of Ca^{2+} released is dramatically reduced. The addition of 1% cetrimide did not affect the extraction properties of the EDTA and citric acid solutions because the values of concentration of Ca^{2+} released in the two solutions did not significantly differ.[26] Higher amount of Ca^{2+} extracted from root dentin in the present study is due at a greatest concentration of citric acid and surfactants of Tetraclean NA than Tetraclean as confirmed in the previous study.[26] It indicates that to obtain an efficient smear layer removal and to facilitate the biomechanical procedures, citric acid-based agents can be applied and cetrimide can help in improving efficacy of the irrigating solutions, moreover cetrimide does not affect the ability of demineralization of citric acid and EDTA solutions. Cationic surfactants are potent antimicrobial agents that have also been shown to act on biofilm components, but they have no decalcifying effects on root canal dentin.[25] EDTA and citric acid solutions are not effective against the biofilms at any concentration or time-tested,[27] but the antimicrobial activity of chelating agents in combination with cetrimide is greater than the use of chelating agents alone[28] and the combination of 0.2% cetrimide with either 15% EDTA or 15% citric acid gave 100% bacterial kill after 1-min of contact with the biofilms.[29] To improve their efficacy, root canal irrigants must be in contact with the dentin walls and debris.[30] The intimacy of this contact depends on the wettability of the irrigant on a solid dentin, and this property of the liquid is strictly correlated to its surface tension.[30] The surface tension is defined as "the force between molecules that produces a tendency for the surface area of a liquid to decrease."[31] This force tends to limit the ability of the liquid to penetrate a capillary tube. Endodontic irrigating solutions should have very low surface tension. The wettability of the solution governs the capability of its penetration both into the main and lateral canals, and into the dentinal tubules.[32] By lowering the surface tension of irrigating solutions, their wettability improves and solutions modified with surfactants immediately spread on the dentin surface, yielding a zero-degree contact angle.[33] As Tetraclean is an antibiotic-irrigation solution based on a tetracycline isomer, there may be problems with staining, resistance, and sensitivity.[34] To use a solution without antibiotic as Tetraclean NA could be a viable alternative. Preliminary *ex vivo* studies showed that Tetraclean NA has a low surface tension value similar to Tetraclean (29.9 mJ/m^2 vs. 29.1 mJ/m^2) and antimicrobial activity on 60 days *Enterococcus faecalis* mature biofilm developed in 45 human maxillary central incisors analyzed at 20 min and 72 h after the irrigation of 45 root canals used in this study by turbidity of the culture medium under ultraviolet spectrophotometry (data not published). Results of this preliminary antimicrobial test showed that Tetraclean Na without antibiotic was able to kill 49,08% and 82,03% of *E. faecalis* biofilm at 20 min and 72 h after the irrigation, respectively. This is not a surprise: Citric acid as cetrimide has antibacterial effectiveness, and testing revealed that citric acid does demonstrate antimicrobial properties against anaerobic bacteria of the infected root canals, especially against cocci.[35] The results here presented indicate that to obtain an efficient removal of the smear layer and to facilitate the biomechanical procedures, citric acid-based agents can be applied and cetrimide can help in improving efficacy of irrigating solutions. Cetrimide does not affect the ability of demineralization of citric acid solutions. Further clinical and *in vitro* studies are needed to verify if a higher amount of Ca^{2+} extracted from root dentin can be useful or harmful. Citric acid values of demineralization are considerably higher than EDTA values, in presence or absence of cetrimide. Under the experimental conditions and restricting to the irrigating solutions considered in this investigation, to obtain an efficient decalcifying action on dentin (and subsequently the smear layer removal) and to facilitate the biomechanical procedures, citric acid-based irrigants can be applied. Moreover, the presence of cetrimide in the irrigating solutions does not prevent the extraction of Ca^{2+} from root dentine, and it could be considered useful to improve the antibacterial activity of irrigating solutions.

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