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Effect of cetrimide in decalcifying efficacy of irrigating solutions

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Abstract
The aim of the study is to evaluate and compare the efficacy of cetrimide on decalcifying capability of different irrigating solutions. Thirty maxillary central incisor teeth have been collected. The specimens were randomly divided into 6 experimental groups (n=10) according to tested irrigating agents. Irrigating agents consisted in different composition of EDTA and citric acid solutions, addicted or not with cetrimide. Each specimen was submitted to three successive 5-min immersions in each solution. After exposures, the concentration of Ca^{2+} extracted was measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Data were analysed by means of Kruskal Wallis and Mann Whitney tests. Significance was predetermined at $p < 0.05$. For all irrigants, the amounts of Ca2+ extracted from root canal dentin samples at 10 minutes were not significantly different from values reported after 15 minutes respectively. Therefore, for all irrigants tested, 10 minutes of application are sufficient to obtain maximum Ca2+ release. Moreover citric acid based agents observed a higher release of Ca2+. The addition of cetrimide did not affect the decalcifying capability of the EDTA and citric acid solutions.

Keywords: Chelating Agents; Dentin; Dentistry.

Introduction
During biomechanical instrumentation of root canals an amorphous layer (smear layer) of organic and inorganic debris is formed. This layer can be deposited along the canal walls, [1, 2] thus reducing sealer adhesion and negatively affecting the sealing [3, 4]. Therefore it should be removed before obturation, to ensure a close contact of the sealer with the dentin surface. To eliminate the smear layer, irrigants able to dissolve both organic and inorganic components could be applied. Chelating agents are believed to aid root canal irrigation and to be able to remove the inorganic smear layer; [5] the addition of sodium hypochlorite (NaOCl) could improve the efficacy of the chelating agent on dissolving the organic fraction of the smear layer [4]. In particular, the selected irrigating solutions showed direct action over calcium present in hydroxyapatite crystals of dentin. Any change in the calcium ratio can significantly modify the original proportion of organic and inorganic components. This can alter dentin permeability, microhardness and solubility [6].
Although EDTA presents a long-standing history because it is the most frequently recommended solution for the removal of the smear layer in endodontics, previous studies demonstrated its irritating potential [7, 8, 9, 10] Citric acid at concentration ranging from 5% to 50%, apple vinegar and phosphoric acid at different concentrations [11, 12, 13, 14] has been proposed with different percentages of success [14]. MTAD and Tetraclean [15, 16] have been investigated as new irrigating solutions based on a mixture of citric acid and antibiotics (tetracycline isomer). Cetrimide is a quaternary ammonium compound and a cationic detergent that is effective against many Gram-positive and Gram-negative bacteria. Many products contain cetrimide, because its addiction improves efficacy of irrigating solutions: cationic surfactants have been reported to have bactericidal and fungicidal properties [17, 18]. Many studies reported the ability of chelating solutions to reduce or eliminate the smear layer, but no reports evaluated concentration of calcium ions removed from teeth with different chelators [19].
Accordingly, the objective of the present study was to evaluate and compare the efficacy of cetrimide on decalcifying capability of different irrigating solutions.

Methodology

Thirty maxillary central incisor, recently extracted for peridontal reasons, were stored in 0,1% tymol solution until use. The crowns were removed at cemento-enamel junction using an Accutom-50 diamond cutter under water- cooling. The root surface was treated with a low speed fine-grain diamond bur under abundant irrigation in order to remove the root cementum. 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 using a pre-programmed automatic Accutom-50 diamond cutter. Each slice was then sectioned in four equal sections, obtaining a total of four (s1, s2, s3, s4) samples from each root. To yield each sample with same calcification, geometry and weight the samples were weighted on a HM 202 precision balance 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.

This method allows testing irrigating solution decalcifying capacity on comparable specimens The specimens were then assigned to one of 6 experimental groups (n = 10) for treatment with different irrigating solutions, as follows: group 1: EDTA 15%, group 2: EDTA 15% + cetrimide 1%, group 3: EDTA 17%, group 4: EDTA 17% + cetrimide 1%, group 5: citric acid 10.5%, group 6: citric acid 10.5% + cetrimide 1%.

The solutions were prepared in laboratory using analytical pure grade reagents. In particular EDTA solutions were prepared by dissolving the opportune quantity of EDTA disodium salt in ultrapure water, favouring dissolution with the addition of sodium hydroxide 40% and adding 1 M hydrochloric acid to obtain a final pH around 7.

Citric acid solutions were obtained by dissolving the opportune quantity of citric acid (Sigma- Aldrich, Milan, Italy) in ultrapure water, favouring dissolution with the addition of sodium hydroxide 40% and adding 1 M hydrochloric acid to obtain a final pH around 7. Citric acid solutions were obtained by dissolving the opportune quantity of citric acid in ultrapure water. Cetrimide 1% (CH3(CH2)15N(Br)(CH3)3 / was added to the EDTA or citric acid solutions.

The accuracy of the pH meter was ± 0.01. In order to determinate the initial calcium concentration in each irrigating agent, before the exposure to specimen, 5 ml of solution was analysed and the calcium content was taken as blank. Each specimen was immersed in 20 ml of the correspondent irrigant solution and kept under constant stirring using a magnetic stirrer. At three successive 5-min immersion times (t1=5 min; t2=10 min; t3=15 min), without renewing the solution, 5 ml of irrigant was separated with a graduated pipette, and then placed in hermetically sealed and labelled tubes. An inductively coupled plasma atomic emission spectrometer was used for calcium determination in each solution. The linearity of the instruments is verified by analysing standard solutions in a concentration range between 0 and 100 mg/l. Descriptive statistics that included the mean, standard deviation, confidence limits for the mean and median, were calculated for each of the groups tested. The amount of calcium extracted (mg/l Ca2+) by different irrigating solutions and in different immersion times was analyzed using Kruskal–Wallis test and Mann–Whitney tests (rank based non parametric tests used to determine differences between two or more variables on continuous dependent variable). The level of statistical significance was predetermined at $p < 0.05$.

Results

The efficacy of several irrigating solutions used to remove calcium from root dentin has been tested. Dentine specimens were immersed in each agent for 5, 10 and 15 minutes. For each test solution, descriptive statistics of the values of Ca2+ released from root dentin at different times. For each irrigating solution lowest amount of Ca2+ released was recorded after 5 minutes of application ($p < 0.05$). No significant difference in amount of Ca2+ released was showed when comparing 10 and 15 minutes groups of each irrigating solution ($p > 0.05$). Moreover no significant differences were reported among groups 1, 2, 3 and 4 ($p > 0.05$), that all showed significantly lower ($p < 0.001$) amount of calcium extracted than groups 5 and 6, that showed no significant difference between them ($p > 0.05$).

The amount of calcium extracted (mg/l Ca2+ -mean value of 5 replicates) with five irrigating solutions at different immersion times is reported in Figure. The error bars represent the SD.

Discussion

The null hypothesis of the study has been rejected. A significantly higher release of Ca2+ was observed in samples submitted to citric acid based agents. The amount of Ca2+ extracted from root dentin samples for all irrigating solutions at 10 minutes did not show signifi- cant differences when compared to the values reported at 15 minutes. Therefore in the present investigation an application of 10 minutes is sufficient to reach the maximum release of Ca2+ 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. They analysed the amount of calcium extracted from dentin samples by atomic absorption spectrophotometer and obtained similar results at three immersion times, with no significant differences between EDTA and citric acid. 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 demineralising 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 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 such as calcium present in the hydroxyapatite crystals. This is in agreement with previous results. Moreover; another investigation [20] reported that the pH of citric acid solution is a more important factor than concentration in demineralisation test. 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 Ca2+ 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 Ca2+ released in the two solutions did not significantly differ.

The results here presented indicate that, to obtain an efficient removing 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. This is probably due to its bactericidal and fungicidal properties: cetrimide does not affect the ability of demineralization of citric acid and EDTA solutions. Is important to establish that if a high demineralization of root canal dentin is required, EDTA is considered the gold standard in endodontics. 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.

Conclusion

Under the experimental conditions and restricting to the irrigants 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 improve the extraction of Ca^{2+} from root dentine and it could be considered useful to complete antibacterial activity of irrigating solutions.

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