Original Article

Chitosan: A Natural Substitute of EDTA Solution for Final Irrigation in Endodontics Treatment

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Abstract

Background: The purpose of this study was to assess the quantity of the chelated calcium ions and the smear layer removal efficiency after root canal final irrigation with three different solutions. **Materials and Methods:** Forty-five teeth were instrumented with rotary-files, then randomly divided into 3 equal groups (n = 15) depending on the final irrigation solution; group I: 17% ethylenediaminetetraacetic acid (EDTA), group II: 0.2% chitosan, and group III: 10% trisodium citrate. According to the time of application, every group was divided into 3 subgroups (1 min, 5 min, and 24 h). The quantification analysis of chelated calcium ions was performed by flame atomic absorption spectrometry (FAAS). Then, the presence of smear layer was examined by splitting the samples longitudinally and using scanning electron microscopy (SEM) to examine coronal, middle, and apical root canal levels. One-way analysis of variance (ANOVA) test was used for the evaluation of treatment effect. Kruskal–Wallis test was executed to detect a significant difference between groups, while Mann–Whitney U test has determined the difference between each two groups for smear layer. **Results:** Both 17% EDTA and 0.2% chitosan had not been statistically significant difference for smear layer removal efficiency and observed calcium ion concentrations. Although, they were more efficient of 10% trisodium citrate with a significant difference ($P < 0.05$). **Conclusion:** The application time of the chelators' solutions must not exceed 5 min to completely remove smear layer, and 0.2% chitosan is a natural substitute for 17% EDTA with a safety application for 24 h.

Keywords: *Calcium ion, chelator, chitosan, scanning electron microscopy, smear layer*

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Introduction

The contemporary endodontic instrumentation faces a big challenge to prepare all root canal's surfaces. The techniques, using nickel-titanium files, leave more than 35% of the root canal's surfaces uninstrumented.[1]

The profusely irrigation of root canal is important to kill microorganisms and remove debris, and both the organic and inorganic portions of the smear layer from the root canal system.[2] There are several methods are applied to remove the smear layer including ultrasonic,[3] chemical, and laser techniques. All of them have limited efficacy.[4]

In the chemical technique, the irrigants are used to remove the smear layer. The gold standard of the irrigants is sodium hypochlorite (NaOCl) due to its special qualities as an antiseptic and its tissue dissolving effects.[5] Even though, it is not an ideal irrigant due to some disadvantages such as its toxic effects to periapical tissues, however, some studies have mentioned that it degrades micromechanical characteristics of dentine.[6] Furthermore, it has no effect on the inorganic part of the smear layer,[7] so it should be used with decalcifying agent.

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Ethylenediaminetetraacetic acid (EDTA) is an artificial amino acid, biocompatible with pH 7 that is used as a root canal irrigant. One of the main characters of EDTA solution is its capability to chelate with metallic ions needed for growth microbes, which can kill them, even though it has no antibacterial effect.[8] EDTA at concentrations of 15–17% eliminates calcium from dentine at approximate depths of 20–30 μm within 5 min.[9] EDTA erodes the dentine depending on two factors, its concentration and application time, and leaving an organic matrix without any fatal effect to periapical tissues.[10] As mentioned, EDTA is an artificial component, does not exist in nature and possesses harmful effect on periapical tissues.[11] Furthermore, EDTA is the most widely used as a chelating agent in clinical application by dentists.[12] Hence, with the quest for more biocompatible solutions, EDTA is still going on.

Citric acid has been verified as a less harmful irrigant than EDTA to vital tissue.[13] The concentration of citric acid is an effective factor on limited antibacterial properties which reacts rapidly with calcium ion.[14] For that reason, citric acid alone cannot be sufficient to provide both a good antibacterial and good chelating effects at the same time.

Citric acid in the form of 10% sodium citrate has almost a neutral pH, that gives sodium citrate greater biocompatibility and more efficiency in decalcifying dentine, since dissolution is reduced clearly at a low pH.[15] Moreover, there are not so many researches studied it.

Chitosan as a natural glucosamine has many properties like biocompatibility, biodegradability, bioadhesion, antimicrobial activity,[16,17] and it is used in many fields as food, cosmetics, biomedical, and pharmaceutical applications.[18,19] Additionally, lack of toxicity with high chelating capacity for various metal ions in acidic conditions,[14,20] makes chitosan a very exciting irrigant in the field of dental research. According to these properties, chitosan has been used in the treatment of dentinal tubule infection, in cases of direct pulp capping,[21] and in tissue regeneration in pulp wounds.[22]

However, studies on the effectiveness of chitosan as a chelating agent for calcium ions from dentin and its ability to remove the smear layer are so limited in the medical literature. The antifungal effect of a 2% chitosan gel containing 0.1% chlorhexidine against Candida albicans has been demonstrated,[23] and its addition to calcium hydroxide paste as an intracanal medication has promoted the prolongation of calcium ion release.[24]

Chitosan is considered as a natural copolymer obtained from chitin of crustaceans and shrimps shells. The deacetylation of chitin by alkaline substances yields in the formation of this cationic aminopolysaccharide copolymer "chitosan".[25]

However, chitosan chelating properties have not been fully investigated on canal walls. Thus, the possibility to apply chitosan in root canal treatment remains a question to be assessed. Based on the above evidence, the present study aimed to assess the smear layer removal ability of 17% EDTA, 0.2% chitosan, and 10% trisodium citrate solutions using scanning electron microscope (SEM). SEM has many benefits including the assessment of the ability of chelating agents in removing the smear layer, the opening of dentin canals, and the presence or absence of an erosion on peritubular and intratubular dentine, on the coronal, middle, and apical thirds of instrumented root canals.[26] This method used in many research studies. Moreover, the concentration of calcium in the obtained solution of irrigated root canals was determined by flame atomic absorption spectrometry (FAAS).[12,27]

Materials and Methods

Sample selection and preparation of root canals

This study was approved in 2019 by the institution's Research Ethics Committee (reference no. HU-FD-18/19-1078).

Forty-five orthodontally or periodontally freshly extracted human teeth were used, with specific characteristics including intact, anterior, and mature with straight single root canals. The selected teeth have specific relative dimensions and similar morphology with absence of any cracks, caries or defects within root portions.

All the teeth were placed in a 2.5% NaOCl solution for 15 min. The tissue and debris remnant on root surface were removed and stored in a normal saline solution at 37°C until use within 2 months after extraction.

By using spherical diamond-tipped drills (SybronEndo Corporation, Orange, CA, USA) connected to a high-speed motor, the access to the pulp chamber was accomplished under water cooling.

A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was passively introduced into each canal until its tip was just visible at the apex, and the working length was established by subtracting 1 mm from this length. Nickel-titanium instruments "rotary ProTaper" (Protaper, Dentsply, Switzerland) activated by X-Smart electric motor (Dentsply Maillefer) were used for canal preparation according to a crown-down technique up to F2 file (size 25/0.08 apical third taper). Throughout preparation, the canals were irrigated with 5 mL of saline at each change of instrument. The

syringe has connected to a plastic capillary tip, which introduced half the working length. The crowns were removed before 1 mm of the cementum–enamel junction by carborundum discs (Brasseler, USA) attached to a slow-speed motor under water cooling. Before final irrigation for smear layer removal, the canals were dried using absorbent paper points (Dentsply Maillefer, Ballaigues, Switzerland).

Samples classification

The teeth were randomly divided depending on chelator solution into three groups 17% EDTA (pH = 7.3), 10% trisodium citrate (pH = 7.6), and 0.2% chitosan (pH = 3.2). Chitosan solution was prepared by dissolving 0.2 g of chitosan (Acros Organics, 90% degree of deacetylation "Panvo Organics, Chennai, India") in 100 mL of 1% acetic acid. A magnetic stirrer for 2 h was used to overcome the difficulty of chitosan dissolving and obtain homogenous clear solution. The solutions were prepared and directly applied on the teeth. Each group distributed into three equal subsets depending on the application time (1 min, 5 min, and 24 h).

The application time (24 h) has been performed to investigate the duration of effectiveness and negative effects on the structure of dentin. This long treatment time (24 h) is important in different cases such as using a dressing for calcified and narrow canals, and insufficient washing of the canals after the completion of the chelating agent application.

The respective chelating solution (3 mL) was delivered into the root canal using a sterile 36-gauge nickel-titanium needle (NanoFil, Hamilton Co, Reno, Nevada, USA). The calcium ions absorbance of light was obtained by FAAS (Perkin Elmer LLC, Norwalk, CT, USA). The longitudinal sectioning of the specimens was performed by carborundum discs attached to a slow-speed motor under water-cooling. Then, a bi-bevel chisel was used to split the teeth in half lengthwise. The selected side was the hemisected with fewer defect, which best represented the total root canal length. Each specimen was divided by lead pencil into three sections: cervical, middle, and apical at 10–11 mm, 6–7 mm, and 1–3 mm, to apex respectively. The smear layer was scanned using SEM (JSM5410, JEOL, Tokyo, Japan) at two magnifications (×1000 and × 2000).

SEM analysis

In this study, the rating system for completing a qualitative evaluation of the canal cleanliness was depended on Torabinejad *et al.*[28] method as the following:

Score 0 = smear layer and debris totally removed with opened dentinal tubules.

Score 1 = smear layer exists only in the apertures of the dentinal tubules.

Score 2 = the root canal surface and dentinal tubular apertures covered with thin smear layer.

Data collection and statistical analysis

The data were tabulated for statistical analysis using SPSS 19.0 computer software. The frequency of every score for each tested group was counted to give the descriptive analysis. Inferential statistical analysis was done using one-way analysis of variance (ANOVA) test for analysis of calcium loss. Kruskal–Wallis test was performed to detect a significant difference between groups, while Mann–Whitney *U* test was implemented to test for the difference between each two groups for analysis of remaining smear layer.

A significance level of 5% was adopted.

Results

Calcium loss

By comparing the three solutions, 17% EDTA and 0.2% chitosan have associated with the highest chelated calcium ion concentrations followed by 10% trisodium citrate in the three-time periods. In connection, one-way (ANOVA) test revealed that 17% EDTA and 0.2% chitosan have analogous effect ($P < 0.05$) and significantly different from 10% trisodium citrate application ($P < 0.05$). Tables 1–3 present the mean and

Table 1: Mean (μ) and standard deviation (σ) of Ca^{2+}measurement in the solution, expressed as mg L^{-1} in 1 min

Groups	μ±σ
17% EDTA	44. 8±10.2
0.2% chitosan	43.7±4.9
10% trisodium citrate	37.9±8.9

Table 2: Mean (μ) and standard deviation (σ) of Ca^{2+}measurement in the solution, expressed as mg L^{-1} in 5 min

Groups	μ±σ
17% EDTA	117.6±27.5
0.2% chitosan	101.3±14.9
10% trisodium citrate	67.4±6.9

Table 3: Mean (μ) and standard deviation (σ) of Ca^{2+}measurement in the solution, expressed as mg L^{-1} in 24 h

Groups	μ±σ
17% EDTA	180.6±8.5
0.2% chitosan	179.0±9.8
10% trisodium citrate	107.7±4.4

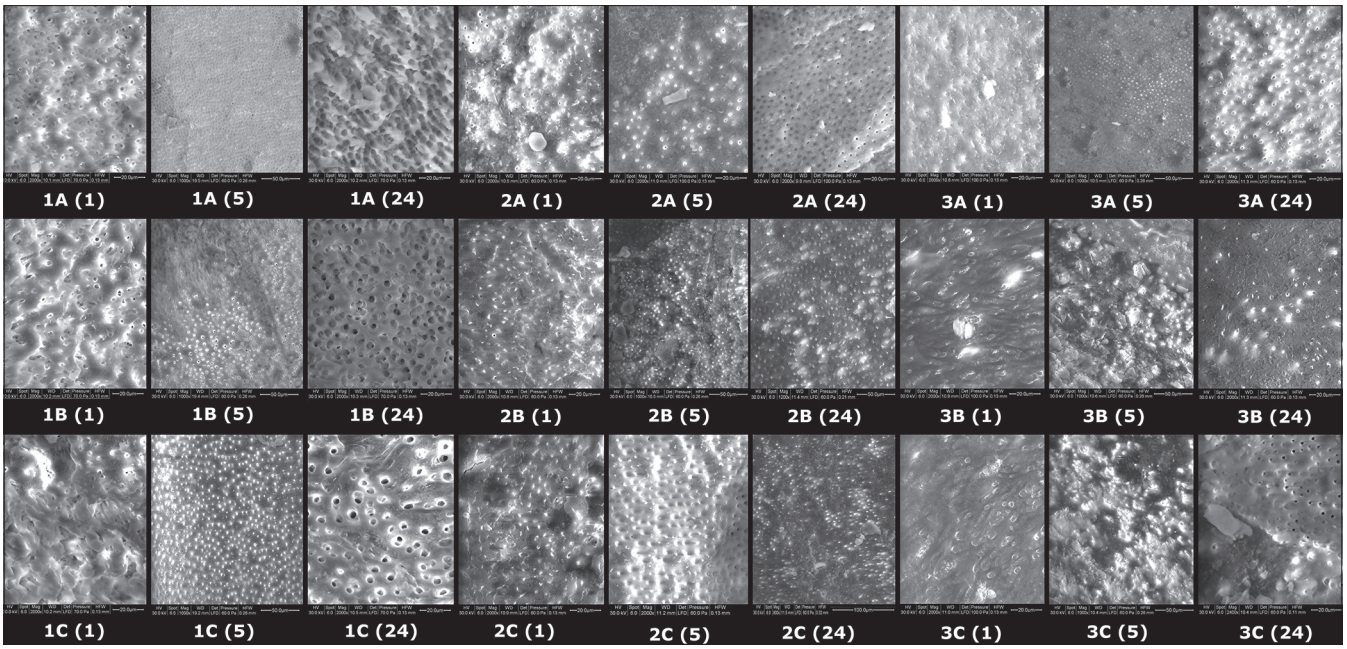

Figure 1: The SEM images (1000X, 2000X – 30KV) of the root canal: 17% EDTA [1], 0.2% chitosan [2], and 10% trisodium citrate [3]. Cervical [A], middle [B], and apical [C]. 1 min [(1)], 5 min [(5)], and 24 h [(24)]

standard deviation of calcium ion concentration for each chelating solution.

Smear layer observations

SEM analysis revealed that 10% trisodium citrate has a minimum effect on eliminating smear layer in the three-time periods at three levels of the root canal ($P < 0.05$). Furthermore, 17% EDTA caused a moderate erosion and severe erosion on peritubular and intratubular dentine when it is applied for 5 min and 24 h, respectively. Whereas, 0.2% chitosan gave a slight erosion of dentin for 24 h ($P < 0.05$). The apical third was less affected than the two thirds coronal in terms of removal the smear layer when used the three solutions in the three-time periods ($P < 0.05$). The time of treatment (24 h) was more efficient in removing the smear layer when applied the three solutions at three levels of the root canal ($P < 0.05$) [Figure 1].

Discussion

Replacing the chelating agent's protons (H^+) with dentin calcium ions results in a reduction in the pH of the medium. The releasing of H^+ reduces the efficiency of some chelating agents like EDTA with time.[29,30] On the other hand, the interaction of H^+ with hydroxyapatite negatively affects the solubility of the dentin.[31,32] We can recognize two simultaneous reactions, the first is a complex formation and the second is the protonation, which can be expressed as in the followed reactions (1) and (2).[33]

$$EDTAH^{3-} + Ca^{2+} \rightarrow EDTACa^{2-} + H^+ \quad (1)$$

$$EDTAH^{3-} + H^+ \rightarrow EDTAH^{2-} \quad (2)$$

Since most of the chelating agents have almost neutral pH, the bond between calcium ions and hydroxyapatite will be broken.[31,32] As a result, the available calcium ions for reaction with the chelating agent will augment. That reaction will continue until all chelating agents in the solution have been complexed with Ca^{+2} as follow:[34]

$$Ca_{10}(Po_4)_6(OH)_2 \Leftrightarrow 10Ca^{+2} + 6PO_4^{-3} + 2OH^-$$

(Dissociation of hydroxyapatite)

+

$$C_{10}H_{13}N_2Na_3O_8(EDTANa_3)$$

↓

$$EDTANa\text{-}Ca + 2Na^+ + 9Ca^{+2} + 6PO_4^{-3} + 2OH^-$$

Evaluation of calcium loss

The current results exposed that time of application of chelator agent for root canal dentin has a great impact on the chelated calcium ion concentrations which meet with Machado-Silveiro *et al.*[27] and Kamakshi *et al.*[35] outcomes that showed a consistent harmony between time of 17% EDTA and 10% trisodium citrate and 17% EDTA applications, respectively with chelated Ca^{2+} content in the root canal dentin. Even that consistency in the application and results (as the higher rate in 1 min), with the passage of time continued chelation reaction slow in rate. To the best of our knowledge, there is no study has investigated the time of 0.2% chitosan chelation to the calcium ions content in the root canal. Therefore, the current results have

presented that the maximum effect is reached in the first minute of the application of this solution and then with the passage of time, the chelation reaction rate has degraded. In addition, no differences in the three-time periods between the applications of 17% EDTA and 0.2% chitosan solutions for chelating calcium ions have been registered. The later notification totally agrees with Silva *et al.*[14] despite they performed 3 min as an application time of EDTA solution. In another study, the impacting effect of the chelating agent appears at 5 min and decreases dramatically after 24 h, the phenomenon that satisfies the current findings.[36]

In this sequence, there were clear differences attained between the Ca^{2+} chelation efficiencies of the 17% EDTA and 10% trisodium citrate solutions under the same working conditions (5 min and 24 h application times) and that sounds compatible with Machado-Silveiro *et al.*[27] despite the difference of the time of application, which did not exceed 15 min. The higher chelating efficiency of EDTA compared to 10% trisodium citrate met partly with Spanó *et al.*[11] who only practiced 15% EDTA for 5 min.

Based on the studies carried by Pimenta *et al.*[25] and Silva *et al.*,[14] the EDTA and chitosan showed similar chelating efficiency. In the meantime, EDTA solution overcomes the trisodium citrate solution efficiency which was confirmed by Machado-Silveiro *et al.*[27] and Spanó *et al.*[11] Therefore, the chitosan solution overpasses the trisodium citrate solution efficiency, and this meets the results of our study. We need to compare these findings with new supportive research.

Evaluation of the smear layer

The results of Spanó *et al.*[11] were consistent with the results of the current study that the 17% EDTA solution is better than 10% trisodium citrate solution in removing the smear layer, despite using the 15% EDTA solution with 5 min application time on middle third of the root canal.

The results agreed with Pimenta *et al.*,[25] Silva *et al.*,[14] and Madhusudhana *et al.*[26] that the capabilities of EDTA and chitosan solutions are similar in removing the smear layer when 15% EDTA is applied for 3 min,[14,25] and 17% EDTA for 1 min,[26] respectively. Furthermore, the available researches have compared the effects of 10% trisodium citrate with 15% EDTA, but no other study has ever considered the varying effects of 10% trisodium citrate and 0.2% chitosan solutions. It is concluded that the 0.2% chitosan solution has more efficiency than 10% trisodium citrate solution, and that matched with our results.

The results agreed with Darrag[37] that the use of 0.2% chitosan solution for 3 min has obtained better fallouts than the application of 17% EDTA for 1 min at the three levels of the root.

Further, the outcomes approved with Silva *et al.*[19] that with longer application time, 0.2% chitosan increases the efficiency of removing the smear layer. Even though, the previous research,[19] showed that an application of 0.2% chitosan solution for 5 min caused expansion of the diameter of the dentinal tubules and heavy erosion with deterioration of dentin surface, our research disagrees with this. Perhaps, the accelerated erosion of dentinal tubules was caused by using 1% NaOCl in irrigation at each change of instrument, and for the same reason there was a disagreement with investigational results of Silva *et al.*[14]

Our findings coincided with the upshots of Çalt and Serper[10] and Kamble *et al.*[38] which confirmed the efficiency of a good cleaning after application of 1–5 min and increasing application time until 10 min causes erosion on peritubular and intratubular dentine. However, in this research the erosion dentinal tubules has occurred in 24 h, this is due to the participation of NaOCl in irrigation and this was confirmed by Niu *et al.*[39] which showed that the application of EDTA alone did not cause corrosion.

It was noticed by studying the images of SEM a decreasing of open tubules dentin numbers in the direction of the apical, and this is in line with Scelza *et al.*,[40] and therefore be larger effective in the coronal and middle thirds of the root.[41,42] This is what came up with our research.

The maximum effect for all solutions tested was in the cervical and middle thirds root canal in the three-time periods, and this is in accordance with Teixeira *et al.*[43]

Future studies must evaluate the chitosan solution and gel in clinical treatment. Ideally, any intracanal medicament should be studied to evaluate antibacterial properties, effects on periapical tissues, sealer penetration,[44] and restorative materials.

Conclusion

Under the experimental conditions and within the limitations of this investigation, the time of application of the chelators' solutions must not exceed 5 min as a maximum time for a completely removal of smear layer, and 0.2% chitosan solution can be the promising endodontic irrigation solution in future. Since, this was an *in vitro* study; results have to be correlated with *in vivo* results. Thus, irrigation techniques strive to maintain a critical balance between cleaning efficacy and patient safety.[45]

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Conflicts of interest

There are no conflicts of interest.

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