An in vitro study comparing the effects of novel irrigating solutions on the elimination of the smear layer and the chelation of calcium ions from the root canal

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ABSTRACT:

Background information: During biomechanical preparation, the action of endodontic tools causes the creation of a smear layer. Smear layer reduction not only improves dentinal tubule sanitation but also the three-dimensional sealing of the root canal system.

Goal: Using a scanning electron microscope (SEM), determine how well different final irrigation solutions remove smear layers from root canal walls, and measure the concentration of calcium ions in these solutions following irrigation using atomic absorption spectrophotometry with flame.

Materials and Procedures To measure the concentration of calcium ions released with 0.2% chitosan, apple cider vinegar, and 15% ethylenediaminetetraacetic acid (EDTA), 40 human maxillary canines were chosen, prepared, and the final irrigation was carried out. The calcium ions were then composed and analysed using atomic absorption spectrometry. SEM was used to assess the elimination of the smear layer from the middle and apical thirds of the root canal.

Results: In terms of removing the smear layer, there was a statistically significant difference between 0.2% chitosan and the other treatments. Apple cider vinegar produced the greatest levels of calcium ions, followed by 0.2% chitosan and 15% EDTA.

As compared to apple cider vinegar, which promoted the release of the largest amounts of calcium ions compared to the other solutions evaluated, about 0.2% chitosan demonstrated higher smear layer removal.

Keywords: ethylenediaminetetraacetic acid, calcium, chitosan, apple cider vinegar, scanning electron microscopy, and smear layer

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Introduction

Comprehensive cleaning and contouring of the root canal system are essential for a successful endodontic procedure. With the aid of cutting-edge technologies like nickel titanium files, more than 35% of the root canal surface can be left unprepared after the root canal procedure. The standard root canal procedure must thus include an irrigation system or other intervention. The primary goal of irrigation is to cleanse in a way that biomechanical preparation cannot. [2] The majority of endodontic treatments advise accurate debridement of the root canals. [3] Various procedures, including chemical, ultrasonic, and laser treatments, have been developed recently to remove the smear layer, however neither of them has gained widespread acceptance nor shown to be more effective. [4] According to a study of the literature on current endodontic procedures and irrigating therapies, the capacity of irrigants to dissolve wide range antibiotics and necrotic pulp makes them most likely to be advised. [5] These irrigants also aid in the dissolution of inactive endotoxins, which are important for endodontic therapy and are also known to totally dissolve or suppress the smear layer. [3] Chitosan is a naturally occurring polysaccharide that has become well-known in the world of dentistry due to its qualities (biodegradability, biocompatibility, bioadhesion, and no toxicityetc). [6] Its strong chelating activity for diverse metal ions is a result of its acidic pH, which supports its employment in a variety of sectors. Its usage is more environmentally benign because it is the second most prevalent material in nature (after cellulose). [8] Under acidic circumstances, chitosan has a strong ability to chelate a variety of metal ions, including the ions of zinc, cobalt, iron, magnesium, and copper (Zn2, Co2, Fe2, Mg2, and Cu2, respectively). [9] These characteristics led to the use of chitosan in a number of dental procedures, including direct pulp capping, the treatment of dentinal tubule infection,[10] and tissue regeneration in pulp wounds. [11] Applications for this chemical have mostly been observed in the fields of biotechnology, environment, agriculture, cosmetics, and food. [12] In dentistry, 2% chitosan gel with 0.1% chlorhexidine has been proven to have antifungal effects against Candida albicans, and its use to calcium hydroxide paste as an intracanal medicine has been shown to facilitate sustained calcium ion release. [14]

The most popular irrigant for removing the smear layer during root canal preparation is ethylenediaminetetraacetic acid (EDTA), which works by acting on an inorganic substance.

[15],[16],[17] Dentine begins to decalcify at around 20–30 m depths within 5 minutes as a result of its interaction with the calcium ions present in the dentine, which causes calcium chelation. [18] EDTA in conjunction with various sodium hypochlorite concentrations was the suggested irrigant for completely removing the smear layer (NaOCl). [17] Only a few studies have shown that EDTA can injure periapical tissues as its frequency of use increases. [5] Depending on how long it is applied for and at what concentration, EDTA can also erode dentin. [19] Therefore, efforts to find a chemical that is more biocompatible than EDTA continue. According to Sen et al., using 1%, 5%, 10%, and 15% of EDTA for 1 minute resulted in root dentin erosion. [20] Another problem is that EDTA is regarded as a contaminant because it was not created by nature. In addition to being biocompatible, this chemical [17] is a weak acid with chelating activity and concurrent protein denaturing that increases dental permeability and facilitates the action of intracanal medicine [22] and the link between dentin and endodontic cements [23]. [24]

Using chelating agents other than EDTA that would be more effective and biocompatible with organic structures was the goal of certain investigations. Apple vinegar [17] and citric acid [25, 26] have both been researched as a result. In addition to eliminating the smear

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layer and reducing dentinal microhardness, apple cider vinegar has been shown to have antibacterial properties. It lowers the surface tension of the solution by combining acetic, citric, formic, lactic, succinic (succinate), and tartaric acids with a little quantity of alcohol. However, acetic (5%) and malic (0.35%) acids make up the majority of the vinegar's acid content. [28] Due to the favourable outcomes obtained when compared to EDTA and NaOCl, the use of apple vinegar as a secondary solution in the preparation of root canals has also been investigated and merits consideration. [29]

This study's objectives were to determine the effectiveness of different final irrigants for removing smear layers, such as 0.2% chitosan, apple cider vinegar, and 15% EDTA, using scanning electron microscopy (SEM), and to count the calcium ion concentration using atomic absorption spectrophotometry with flame (AASF) after root canal irrigation.

Materials and methods

Forty removed human maxillary canines were used in the investigation. The teeth were immersed in a 2.5% NaOCl solution for 15 minutes. The leftover tissue and debris that had been removed from the root surface were then kept in 0.9% saline solution that had been combined with thymol. The chosen teeth were free of cavities, fractures, fissures, and prior restorations or endodontic treatments. After then, the samples were divided into four groups.

Getting ready for the root canal

At the intersection of the cement and enamel, the sound teeth were decoronated. A K-file #10 (Dentsply Maillefer) was used to measure the working length; it was inserted into each tooth's root canal until it was visible at the apical foramen and then 1 mm of length was deducted from it. Using rotary nickel-titanium ProTaper Universal (DentsplyMaillefer) devices and the manufacturer's recommended crown-down approach, the canals were widened. Each sample group was irrigated in turn using various irrigants placed in between the files. 15% EDTA was utilised in Group I (n = 10) and 10% in Group II (n = 10) For the 0.2% chitosan solution, 0.2 g of the chitosan material was diluted in 100 ml of 1% acetic acid, and the sample was then agitated with a magnetic stirrer for 2 hours. Apple cider vinegar was utilised in Group III (n = 10).

Preparation of samples

Each tooth was put in a 15-ml falcon tube with a cover that was perforated to allow the tooth to be inserted with the root within the tube and the crown outside. Then, 5 ml of the appropriate chelating solution were introduced into the root canal over the course of three minutes using a needle measuring 0.45 mm by 13 mm, travelling through the whole root canal and leaving through the patent foramen into the collecting tube. Top Dam Blue ® light-cured glue (FGM/Dentscare, Joinvile, SC, Brazil), which sealed the canal entry to prevent solution flow back, was used to hold the needle in place. Each group's teeth underwent these operations once again. The total volume of the solution that collected in the tubes was utilised by AASF to calculate the concentration of calcium ions.

Flame analysis with absorption spectrophotometry

The teeth were taken out of the tubes and separated after the solutions had been collected. New lids were applied to the tubes, and they were tagged and sent on their way so that the calcium ion concentration in the liquid could be determined by spectrometry. There were ten tubes in each group (one for each tooth). After obtaining individual results, a mean value was computed for each group. The instrument of choice was an Analyst 400 atomic absorption spectrometer with integrated flame furnace. The next set of criteria was used: For measuring absorbance, hollow calcium cathode lamps and an acetylene gas were employed, and a standard calcium solution from Ultra Scientific with a

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concentration of 100 mg/L was utilised to modify the curve calibration for calcium ions. To avoid chitosan's polymeric matrix interfering with the measurement of calcium ions, the EDTA and apple vinegar solutions were both diluted in deionized water before analysis, while the chitosan solution was diluted in a 0.1% lanthanum solution (by mass/volume).

Analysis with a scanning electron microscope

Using a diamond disc, horizontal grooves were created on the buccal and lingual sides of the root without entering the canal. The roots were then split in half with the aid of a chisel. The half of each root that had the longest canal and the most visible section of the apex was coded and selected. For the purpose of defining the root thirds, each specimen's length was measured using a digital calliper from the apex to the cementoenamel junction. To determine the middle and apical thirds, respectively, the locations corresponding to 12 and 1/6 of the root length were separated starting from the apex. The SEM study used these regions. Three endodontic professionals used a scanning electron microscope to examine SEM micrographs with a magnification of 1.5Kx [Figure 1] in order to determine how much smear layer was still present on the dentinal walls. Following a modified version of Takeda et alscoring .'s methodology, scores of 1 to 5 were assigned: [30] I the whole surface is covered in smear layer; (ii) a small portion of the surface and a few visible tubules are partially coated in smear layer; (iii) the remaining portion of the surface is covered with smear layer; (iv) there is no smear layer visible on the surface.

Statistic evaluation

The Tukey-Kramer test and one-way analysis of variance were applied to the study of calcium ion concentration. The residual smear layer was analysed using the Kruskal-Wallis test. A 5% threshold of significance was used.

Results

The current investigation found that chitosan (0.079 mg/L), EDTA (0.018 mg/L), and apple vinegar (0.0110 mg/L) had the greatest calcium ion concentrations. The averages and standard deviations of the calcium ion concentration for each chelating solution are shown in [Table 1].

Table 1: Means and standard deviations of the calcium ion concentration of the solutions (mg/L)

Group	***n***	Mean±SD.	**SE**	**Minimum**	**Maximum**
EDTA	**10**	**0.01±0.01**	0.003	**0.02**	**0.02**
Chitosan	**10**	**0.07±0.07**	0.018	**0.01**	**0.10**
Apple cider	**10**	**0.1±0.02**	0.022	**0.02**	**0.60**
Total	**30**	**0.60±0.08**	**0.01**	**0.03**	**0.30**

Tukey's test revealed that 0.2% chitosan, apple cider vinegar and 15% EDTA were significantly different from each other (P< 0.05) [Table 2].

Table 2: Comparison of means of calcium ion concentration of three groups by one-way ANOVA

Calcium ion			**ANOVA**		
concentration	**Sum of squares**	**df**	**Mean square**	***F***	***P***
Between groups	**0.02**	**1**	0.01	**6.22**	**0.00***
Within groups	**0.70**	**17**	0.04		
Total	**0.20**	**34**			

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Results from Group I (EDTA), Group II (apple cider vinegar), and Group III were comparable according to SEM analysis (Chitosan). The group with the maximum smear layer elimination was Group III (0.2% chitosan). The distribution of the mean and standard deviation for the three groups' middle and apical smear layer eradication scores is shown in [Table 2].

Kruskal–Wallis test

At the middle and apical root canal levels, the dentinal tubules covered by the smear layer were compared. Following apple cider vinegar and 15% EDTA in terms of smear layer elimination in the middle and apical third was 0.2% chitosan, according to SEM analysis. Therefore, according to statistics, the P value in the middle third is statistically significant (P 0.05), however it is not statistically significant (P > 0.05) in the apical third.

For the comparison of individual specimens within groups, the Kruskal-Wallis test revealed statistically significant differences between the middle and apical thirds.

Discussion

After root canal instrumentation, the calcium ion concentrations in the chelating solutions used for final irrigation were analysed. The results showed that apple vinegar and 0.2% chitosan had higher values than 15% EDTA. When compared to 15% EDTA and apple cider vinegar, SEM examination revealed that 0.2% chitosan, even at such a low concentration, was able to eliminate the smear layer. In this investigation, the middle third (P 0.05) removed the smear layer more effectively than the apical third (P > 0.05). According to the majority of investigations, the apical area did not exhibit considerable efficacy. [31],[32],[33]

Chelating agent application is influenced by the solution's pH, concentration, and volume. [21] Additionally, it was shown that highly concentrated solutions applied for an extended length of time result in roughness of the dentin surface, suggesting that there must be a relationship between the concentration of the chelating solution and the application duration. [34] The final irrigants' application time and concentration, however, were inconsistent in this investigation. These were selected in accordance with the manufacturer's recommendations and earlier studies' findings. [4],[34],[35],[36] The combined use of AASF and SEM findings was confirmed based on the investigations by Marques et al. [15] and Spanó et al. [17].

Due to its chelating ability, this study found that apple cider vinegar's calcium ion concentration was more effective than 0.2% chitosan, 15% EDTA. In a study by Silva et al.,[37] the evaluation of calcium ion concentrations in chelating solutions (comprising 15% EDTA, 0.2% chitosan, 10% citric acid, and 1% acetic acid) used for final irrigation after root canal instrumentation revealed higher calcium ion concentration values in 15% EDTA and 0.2% chitosan than in 10% citric acid, which also showed higher calcium ion concentration values than in 1% ace.

Malic acid gives apple cider its biocompatibility properties. In this process, certain bacteria are present while the generated ethyl alcohol is transformed and oxidised into acetic acid. Acetification is the process in question. [28] The presence of H+ ions contributes to the overall quantity of calcium ions observed in the apple vinegar solution. The acid would attack more effectively the higher the quantity of H+ ions. [38] Furthermore, because it contains a wealth of minerals including potassium, phosphorus, and magnesium, apple cider vinegar has the potential to be therapeutic. It is thought that adsorption, ionic exchange, and chelation are responsible for the removal of dentin calcium ions despite not completely understanding their mechanisms of action. [39]

When compared to all of the evaluated chelating agents, 0.2% chitosan performed

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the best. A study found that using 0.2% chitosan solution for 3-5 minutes is the most practical method for usage on the root dentin. [40] According to a study, chitosan's characteristics are superior and have improved cleaning and chelating. [42] Based on the method utilised in chelating with chitosan, researchers have proposed two possibilities. The foundation of the bridge concept is the method through which two or more amino groups on the chitosan chain bind the same metal ion. [42] According to the second hypothesis, the metal ion is only linked to one of the amino groups on the chitosan chain. [43] Ion exchange, chelation, and adsorption are the causes of chitosan and metal ion complexes, according to further investigation. [41] The kind of contact, the solution's pH, and the structure of the chitosan all affect how chelating occurs. [44] Chitosan is reported to have conditioning effects on radicular dentin based on a recent study.

Researchers have discovered that 0.2% chitosan at 3.2% pH possesses dentin-reducing effects.

[45] It may be inferred from this that chitosan citrate is a perfect conditioner for radicular dentin as well. [45] The calcium ion concentration of 0.2% chitosan in this investigation, as determined by atomic AASF analysis, was found to be 0.079 mg/L, which is significantly different from the calcium ion concentrations of apple vinegar and 15% EDTA [Graph 1]. Chitosan-irrigated groups outperformed EDTA and apple vinegar in terms of smear removal (see Graphs 2 and 3). If both solutions have a comparable chelation effect, then the less concentrated solution should be preferred when there are financial considerations. The precursor of chitosan, chitin polysaccharides, is the second most prevalent material in nature to be taken into account after cellulose.

Silva et al. suggested that using EDTA to remove the smear layer is beneficial.[37] Spanó et al. demonstrated the highest calcium ion concentration using SEM and atomic absorption spectroscopy. [17] Gu et al. discovered that EDTA was more efficient than NaCl and NaOCl at opening the dentinal tubule and removing the smear layer. [46] A high hydrogen ion concentration is not necessary for EDTA to be effective at neutral pH levels in decalcification. The pH also drops as a result of hydrogen and calcium exchanging roles in the dentin. Over time, the effectiveness of EDTA decreases as a result of this pH reduction. In comparison to other solutions with minor erosion, 0.2% chitosan was the most effective at removing the smear layer and smear plug when applied for 3 min. 0.2% chitosan can be substituted with EDTA. [21]

All treatments were effective in removing calcium ions from the root canal walls, according to an atomic AASF study. It is crucial to stress that decalcification of the smear layer's inorganic structure does not result in calcium being present in the irrigation solution. Chelating and demineralizing solutions affect the dentine's hydroxyapatite calcium matrix, exposing collagen and lowering microhardness in the process. [47] According to an analysis of the data from the two experiments, there appears to be a clear connection between the capacity to remove smear layers and the volume of calcium ions extracted from root canals. Marques et al.[15] discovered that 17% cyclohexane diamine tetraacetic acid and 17% cethylenediaminetetraacetic acid, in addition to encouraging more effective cleaning than 17% ethylene glycol-bis(-aminoethyl) ether tetraacetic acid, also had the largest quantities of calcium ions.

The 0.2% chitosan solution was shown to be the most successful in the current investigation for eliminating the smear layer and smear plug with the least amount of erosive impact, while apple vinegar performed best in terms of calcium ion concentration.

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Conclusion

The removal of the smear layer from the centre and less in the apical thirds of root canals was achieved using 0.2% chitosan, apple cider vinegar, and 15% EDTA under the experimental settings and within the parameters of this inquiry. Additionally, 0.2% chitosan, apple cider vinegar, and 15% EDTA were shown to have the highest impact on the root dentine demineralization.

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