Comparative Evaluation of the Effect of Different Chelating Agents on Mineral Content and Erosion of Radicular Dentine: A FESEM-EDS Analysis

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ABSTRACT

Objective: Irrigating solutions play an important role in the debridement and disinfection of the root canal space, and thus, it is crucial to comprehend their effects on the composition and surface structure of radicular dentine. This study evaluated and compared the effects of 17% ethylenediaminetetraacetic acid (EDTA), 9% 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) and 0.2% chitosan on the mineral content and erosion of radicular dentine when used as a final rinse.

Methods: Sixty extracted human mandibular premolar teeth were decoronated and instrumented to ProTaper size F2. After final instrumentation, the samples were randomly divided into 4 groups (n=15) according to the type of final irrigant used: Normal saline (control), 17% EDTA, 9% HEBP and 0.2% chitosan. Field emission scanning electron microscopy was used to assess the erosion of radicular dentine, and energy dispersive X-ray spectroscopy was used to quantify the radicular dentine mineral composition at the coronal, middle and apical levels of all the prepared samples after final irrigation. The one-way analysis of variance was used for intra-group and inter-group comparisons of means, the Kruskal Wallis test for intra-group and inter-group comparisons of medians and Tukey's post hoc test for pairwise comparisons.

Results: There was no significant difference in the levels of calcium (Ca), phosphorus (P) and Ca/P ratio after final rinse with 17% EDTA, 9% HEBP and 0.2% chitosan at all three root levels ($p > 0.05$); except at the coronal level, where 0.2% chitosan caused significantly less alteration in Ca levels and Ca/P ratio than 17% EDTA and 9% HEBP respectively ($p < 0.05$). 17% EDTA, 9% HEBP and 0.2% chitosan caused no erosion at the middle and apical levels. Meanwhile, 17% EDTA and 9% HEBP caused moderate erosion at the coronal level.

Conclusion: Alternatives to 17% EDTA during final irrigation can be 9% HEBP and 0.2% chitosan.

Keywords: Chelating agents, chitosan, ethylenediaminetetraacetic acid, etidronate

HIGHLIGHTS

- 17% EDTA, 9% HEBP and 0.2% chitosan altered the inorganic elements of the radicular dentine.
- 17% EDTA and 9% HEBP moderately eroded the radicular dentine at the coronal level, whereas none of the chelating agents eroded the radicular dentine at the middle and apical levels.
- 9% HEBP and 0.2% chitosan can be used as alternatives to 17% EDTA during the final irrigation of the root canal.

INTRODUCTION

The successful outcome of root canal treatment relies primarily on meticulous shaping and cleaning of the root canal complex followed by three-dimensional obturation to achieve a hermetic seal, thus preventing the failure of root canal therapy. Irrigating solutions and mechanical instrumentation are essential for shap-

ing, cleaning and disinfecting the pulp space. However, the complexity of the endodontic space due to the presence of isthmuses, anastomoses, fins, ramifications, deltas and lateral canals poses a challenge for thorough debridement using mechanical instrumentation alone (1). These complexities can be accessed with irrigating solutions and filled three-dimensionally with traditional and novel obturation techniques using bioceramic sealer (1). The primary objective for the success of root canal treatment is the elimination of necrotic remnants, microorganisms, bacteria and their by-products, and smear layer formed during mechanical instrumentation (2, 3).

The smear layer is a granular, amorphous and uneven structure composed of organic (blood cells, nerve fibres, necrotic or vital pulp tissue, bacteria and their by-products, saliva, collagen, odontoblastic remnants, coagulated proteins) and inorganic portion (hydroxyapatite containing dentine debris) (4). Whether to eliminate or retain the smear layer was debatable; however, a systematic review and meta-analysis concluded that the smear layer should be eliminated to enhance the root canal system's fluid-tight seal (4). Ideally, an irrigating solution should be able to eliminate the smear layer from the root canal space while being biocompatible with the radicular dentine. However, there is a lack of such an irrigating solution that can simultaneously eliminate both organic and inorganic contents.

The most popular irrigation solution is sodium hypochlorite (NaOCl) in the concentration range of 0.5% to 5.25% because of its capacity to dissolve organic material and its antibacterial properties. However, the drawback of NaOCl is that the inorganic components of the smear layer cannot be efficiently removed (5). Thus, a chelating agent is recommended to eliminate inorganic components (5).

In 1957, Nygaard-Østby introduced chelating agents in endodontics for negotiating the root canals that were calcified and narrow (6). The most used chelating agent is ethylenediaminetetraacetic acid (EDTA), which complexes with calcium ions and has a nearly neutral pH (6). However, the microstructure of dentine may be severely altered by EDTA. It causes the sequestration of calcium ions, leading to the decalcification of dentine at approximate depths of 20-30 μm (7). When used for a prolonged duration, it causes inadvertent erosion of the peritubular and intertubular dentine (8). Also, its interaction with NaOCl decreases free available chlorine, thus decreasing its antimicrobial effect (5). Therefore, the search for alternatives to EDTA is receiving attention to overcome the drawbacks of EDTA.

Etidronate (1-hydroxyethylidene-1,1-bisphosphonate; HEBP) or etidronic acid is a mild chelating agent that is biocompatible and can be combined with NaOCl without affecting either solution's inherent properties (9). As a result, a combined solution of sodium hypochlorite and etidronic acid could be used as a single irrigant during the shaping and cleaning process and after instrumentation, thus eliminating the plausible formation of a smear layer (9). Also, etidronate minimally interferes with dentine's microhardness and roughness (10). Although the chelating capacity of etidronate is relatively weak, some researchers have suggested that it has the potential to be used as an alternative to EDTA (11).

A solution that contained purely 9% HEBP was used in this study, unlike other researchers who had advocated mixing 18% HEBP and 5% NaOCl in a 1:1 ratio and using it as a single solution (12, 13). The objective behind not mixing HEBP and NaOCl in the present study was to test the chelating ability of HEBP in particular. Unlike EDTA, HEBP doesn't hinder NaOCl's ability to dissolve organic tissues, and thus, a mixture of NaOCl and HEBP used as a single irrigant can help eliminate both organic and inorganic portions simultaneously (14).

Chitin, a natural polymer found in crab and shrimp shells, is deacetylated to form chitosan. Chitosan is non-toxic to human cells, biocompatible, biodegradable, and bioadhesive (15). Chitosan can remove the smear layer and unblock dentinal tubules without promoting significant erosion of the root dentine (15). Also, the antibacterial and antifungal effects of chitosan against Enterococcus faecalis and Candida albicans are found to be similar to NaOCl and chlorhexidine (CHX) (16). Additionally, when mixed with NaOCl, the free available chlorine remained unchanged, and the pH of the mixture remained at 11.05, showing that NaOCl's ability to dissolve tissues predominated (17). Due to these added advantages, studies have proposed chitosan as a natural substitute for EDTA (18). The exact mechanism of action of chitosan is not known; however, the development of complexes between chitosan and metal ions is most likely caused by adsorption, chelation and exchange of ions (19, 20). The root canal space can be debrided and disinfected efficiently with irrigation. Although complete smear layer removal is essential for the successful outcome of root canal treatment, numerous chelators used for the same have been reported to alter the structure and composition of the radicular dentine (21, 22).

Dentine is composed of inorganic components (70%), organic components (20%) and water (10%). Calcium found in hydroxyapatite [$Ca_3(PO_4)_5OH$] crystals is the main inorganic component of dentine. The original proportion of these organic and inorganic components can be significantly altered if there is any alteration in the calcium ratio (23). During smear layer removal, the mineral content of dentine and the proportion of calcium (Ca) to phosphorus (P) in the hydroxyapatite can both be altered by irrigating fluids at the same, which may result in a decrease in the microhardness and erosion of dentine (24). Exposing the root dentine to irrigating solutions may remove excessive organic and inorganic content, damaging the root structure and leading to complications such as vertical root fracture (25). Literature comparing the effect of EDTA, HEBP and chitosan on radicular dentine's mineral content and erosion is scarce.

Thus, this study aimed to evaluate and compare the effect of 17% EDTA, 9% HEBP and 0.2% chitosan on the mineral content and erosion of radicular dentine. The null hypothesis was that there is no significant difference in the mineral content as well as erosion of radicular dentine after final irrigation with 17% EDTA, 9% HEBP and 0.2% chitosan.

MATERIALS AND METHODS
Ethical clearance for the study protocol was granted by the Institutional Ethics Committee (No. RDC/29/2011/2049) of Srimanta Sankaradeva University of Health Sciences, Assam, India. The principles of the Declaration of Helsinki were followed in this study.

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Sample Selection

Human non-carious single-rooted mandibular premolars extracted preferably due to periodontal or orthodontic cause, having single canal and closed apices were chosen. These extracted teeth samples were collected from patients within the age range of 16 to 45 years. Teeth with previous endodontic treatment or restorations, root caries, dilacerations, anatomical or morphological deformities, resorptions, cervical abrasions, calcifications, cracks or fractures and immature apices were excluded. The statistician determined a total sample size of 60 teeth at a 95% confidence level.

The following formula was used to determine the sample size for the study:

$n = \{(Za/2*s)/E\}2$, where $Za/2 = 1.96$ (at 95% confidence level), s is the estimated population standard deviation (estimated from previous studies), and E is the desired error of the estimated population mean.

Preparation of 9% HEBP Solution

A 9% HEBP solution was prepared and used within 120 minutes to irrigate the respective samples in the 9% HEBP group. 10 mL of normal saline was taken in a plastic beaker, then two capsules of Twin Kleen (Maarc Dental, Maharashtra, India) containing HEBP powder (0.45 gm + 0.45 gm) were added. The mixture was then stirred using an agate spatula for approximately 45 seconds, thus forming the desired 9% HEBP solution, which was allowed to rest for 30 seconds.

Sample preparation

The samples were stored in distilled water after extraction till further use. Soft tissue tags and bony fragments were removed with a scalpel blade, and stains or calculus, if present, were cleaned with an ultrasonic scaler (DTE D5, Guilin Woodpecker, Guangxi, China). Teeth were decoronated using a diamond disc to standardise the root length to 15 mm. The working length was calculated by deducting 1 mm from the recorded distance of an ISO size #10K file (Dentsply Maillefer, Ballaigues, Switzerland) placed into the root canal until its tip was just visible at the apical foramen. Modelling wax was used to seal the root apex to prevent extrusion of the irrigant. ProTaper Universal rotary file system (Dentsply Maillefer, Ballaigues, Switzerland) was then used to instrument the root canals in a sequential crown-down manner from SX to size F2. A 30 gauge side vented needle (Denmax, Tamil Nadu, India) connected to a 2.5 mL disposable syringe was used to irrigate the canals with 2 mL of 3% NaOCl (Prime Dental Products Pvt. ltd., Maharashtra, India) after each instrument change.

After final instrumentation, the samples were randomly divided into four groups (n=15) according to the final irrigant used: Normal saline (control), 17% EDTA, 9% HEBP, and 0.2% chitosan. In each group, the respective irrigant was used for final irrigation as follows: 1 mL per minute for 5 mins using a 30 gauge side vented needle penetrating 1–2 mm short of the working length. All samples were irrigated with 5 mL of deionised water after the final irrigation to eliminate any precipitate that may have formed. Then, absorbent paper points were used to dry the canals. A di-

amond disc was used to prepare longitudinal grooves externally on the buccal and lingual surfaces of the samples, taking care not to penetrate the canal space. Splitting of the grooved samples was achieved with a double-ended chisel. One-half of each sample was selected and later stored in a lab incubator (CLE-102, Coslab, Haryana, India) at 37°C until further analysis.

Analysis of Mineral Content

Elemental characterisation of the radicular dentine was performed with energy-dispersive X-ray spectroscopy (EDS). The selected halves of the samples from each of the four groups were serially dehydrated with ethanol at increasing concentrations (25%, 50%, 75%, and 100%), mounted on an aluminium holder, sputter-coated with gold, and then analysed using EDS (Sigma 300, Zeiss, Oberkochen, Germany). Levels of Potassium (K), Magnesium (Mg), Calcium (Ca), Phosphorus (P) and Ca/P ratio of the radicular dentine surface were measured in weight percentage (wt%) at the coronal (10–12 mm from apex), middle (6–7 mm from apex) and apical (1–2 mm from apex) level of each sample at a voltage of 15 kV.

Analysis of Radicular Dentine Erosion

Field emission scanning electron microscopy (FESEM) was used to examine the radicular dentine erosion. The gold-coated samples used for EDS analysis were then examined with FESEM. The radicular dentine surface was examined with FESEM (Sigma 300, Zeiss, Oberkochen, Germany), and photomicrographs (×3,000) were obtained at the coronal, middle and apical levels of each sample at a voltage of 3 kV. The degree of erosion of radicular dentine was scored according to the criteria prescribed by Torabinejad et al. (26), which are as follows:

- Score 1=No erosion. All tubules looked normal in appearance.
- Score 2=Moderate erosion. The peritubular dentine was eroded.
- Score 3=Severe erosion. The intertubular dentine was destroyed, and the tubules were connected.

Statistical Analysis

The data was statistically analysed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corporation, Armonk, New York, USA). The one-way Analysis of variance was used for intra-group and inter-group comparisons of means, Kruskal Wallis test for intra-group and inter-group comparisons of medians and Tukey's post hoc test for pairwise comparisons. The tests were conducted at a 5% level of significance.

RESULTS

Analysis of Mineral Content

EDS analysis of the different elements detected on the radicular dentine surface at three different root levels in control and experimental groups is presented in Table 1.

Analysis of Radicular Dentine Erosion

The median erosion scores in different experimental groups at three different root levels are presented in Table 2. None of the

TABLE 1. Comparison of different elemental values (wt%) of radicular dentine

Element	Group	Coronal Mean±SD	Middle Mean±SD	Apical Mean±SD
K	Normal Saline	0.13±0.08[aA]	0.21±0.25[aA]	0.39±0.52[aA]
	17% EDTA	1.66±1.74[bA]	1.12±1.20[bA]	0.98±0.68[bA]
	9% HEBP	0.30±0.19[aA]	0.28±0.18[aA]	0.26±0.14[aA]
	0.2% Chitosan	0.21±0.08[aA]	0.20±0.09[aA]	0.24±0.12[aA]
Mg	Normal Saline	2.39±0.49[aA]	2.31±0.46[aA]	2.03±0.39[aA]
	17% EDTA	4.69±1.23[bA]	3.87±0.9[bAB]	3.64±1.06[bB]
	9% HEBP	3.00±0.38[aA]	2.77±0.33[aA]	2.81±0.52[cA]
	0.2% Chitosan	2.45±0.41[aA]	2.63±0.71[aA]	2.64±0.67[acA]
Ca	Normal Saline	62.95±2.94[aA]	64.36±3.71[aAB]	67.30±6.04[aB]
	17% EDTA	58.52±5.33[bA]	61.47±1.70[bAB]	62.23±2.29[bB]
	9% HEBP	60.01±0.71[abA]	61.53±0.34[bcB]	62.36±1.40[bC]
	0.2% Chitosan	62.90±1.20[aA]	63.24±1.43[abcA]	63.50±1.67[bA]
P	Normal Saline	34.54±2.6[aA]	33.10±3.50[aAB]	30.29±6.42[aB]
	17% EDTA	35.13±2.98[aA]	33.54±1.94[abAB]	33.15±1.24[bB]
	9% HEBP	36.69±0.61[aA]	35.55±0.34[bB]	34.76±1.19[bC]
	0.2% Chitosan	32.28±8.97[aA]	33.93±0.91[abA]	33.62±1.04[bA]
Ca/P	Normal Saline	1.84±0.23[aA]	1.98±0.34[aAB]	2.40±0.90[aB]
	17% EDTA	1.68±0.27[abA]	1.84±0.14[abAB]	1.88±0.13[bB]
	9% HEBP	1.64±0.05[bA]	1.73±0.03[bB]	1.79±0.11[bC]
	0.2% Chitosan	1.82±0.08[aA]	1.87±0.09[abA]	1.89±0.11[bA]

Different lowercase letters in the same column of each element indicate statistically significant differences in inter-group comparisons. Different capital letters in the same row of each element indicate statistically significant differences in intra-group comparisons. SD: standard deviation, K: potassium, Mg: magnesium, Ca: calcium, P: phosphorus, EDTA: Ethylenediaminetetraacetic acid, HEBP: 1-hydroxyethylidene-1,1-bisphosphonate

samples in the saline group could be scored for erosion as the radicular dentine surfaces were completely covered by a smear layer, making it impossible to visualise the dentinal tubules (Fig. 1). Thus, the saline group was excluded from the comparison of erosion scores. Final irrigation with 17% EDTA resulted in moderate erosion of the radicular dentine at the coronal level, whereas the middle and apical levels showed no erosion (Fig. 2). Similar findings were noted on final irrigation with 9% HEBP (Fig. 3). However, 0.2% chitosan did not erode the radicular dentine at any of the three root levels when used as a final irrigant (Fig. 4).

DISCUSSION

Intra-group comparison of elemental values showed that the mean Ca values in all the experimental groups were higher in the apical level than the coronal and middle levels after final irrigation with the respective chelating agents. The higher Ca values could be due to the diminished activity of the chelating agents at the apical level. These findings were in accordance with the study performed by Verdelis et al. (27), who concluded that non-collagenous proteins are found in lower concentrations in the apical portion. As the chelating activity of EDTA is also based on the removal of calcium from organic constituents of dentine, such as water-soluble non-collagenous proteins, the extent of EDTA decalcification is found to be reduced in the apical region (27). Another reason could be radicular dentine sclerosis in the apical root region, which might have affected the chelating ability of the agents (21). Additionally, the apical vapour lock caused by the closed root canal complex and the narrowing of the canal space in the apical level might have prevented the chelating agents from acting effectively in the apical region.

TABLE 2. Comparison of median erosion scores

Group	Root level		
	Coronal	Middle	Apical
17% EDTA	2[aA]	1[aB]	1[aB]
9% HEBP	2[aA]	1[aB]	1[aB]
0.2% Chitosan	1[bA]	1[aA]	1[aA]

Median values are shown in the table. Different lowercase letters in the same column indicate statistically significant differences in inter-group comparisons. Different capital letters in the same row indicate statistically significant differences in intra-group comparisons

Inter-group comparison of elemental values showed that the Ca levels were lower in all three experimental groups as opposed to the control group at the coronal, middle and apical levels. These findings showed that apart from 17% EDTA, which is known to be a strong Ca chelator (22), both 9% HEBP and 0.2% chitosan also altered the Ca levels. The ability of HEBP and chitosan to alter Ca levels authenticates their chelating action, as confirmed by earlier studies (18, 19, 28). The P levels were higher in the 9% HEBP group at all three root levels. These findings were similar to the results reported by Rath et al. (12) and Cobankara et al. (28). The explanation for such findings could be that HEBP is a bisphosphonate, and all bisphosphonates have a P-C-P structure, which is made up of two phosphonate groups bonded to a single carbon atom (29). These phosphonate groups present in HEBP could have been a source of phosphorus ions that led to ions adhering on the radicular dentine surface during irrigation, thus resulting in increased P levels in the 9% HEBP
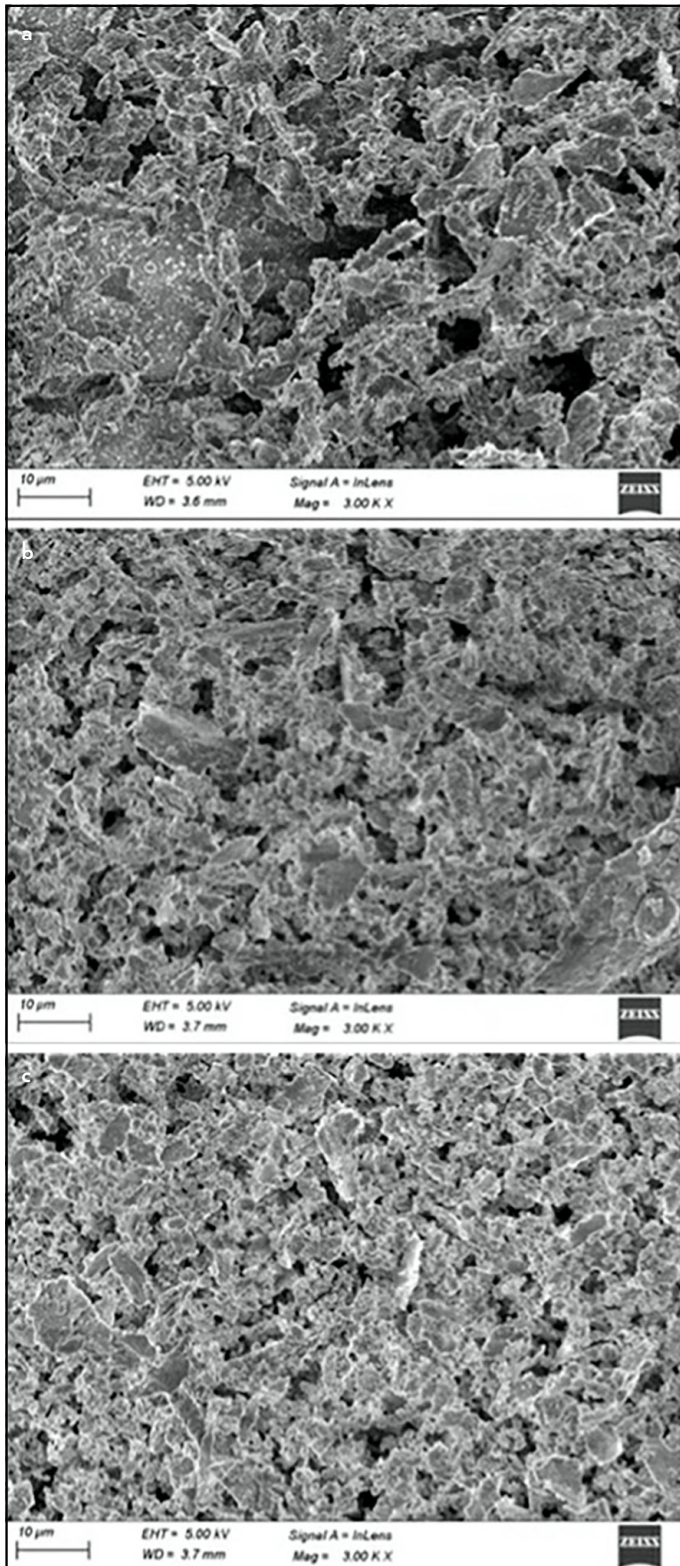
Figure 1. Representative FESEM photomicrographs (×3,000) of the radicular dentine wall after final irrigation with normal saline (control) at (a) coronal, (b) middle and (c) apical levels

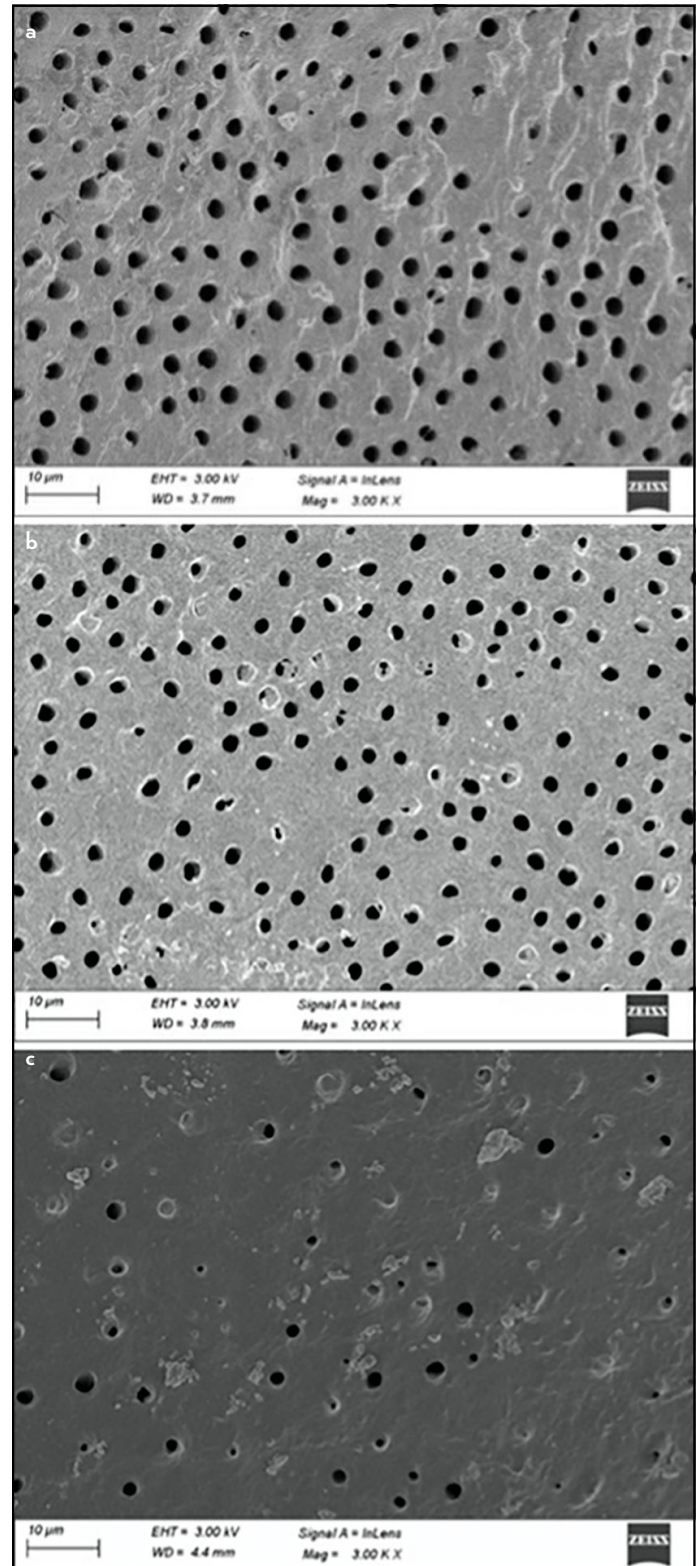FESEM: Field emission scanning electron microscopy

Figure 2. Representative FESEM photomicrographs (×3,000) of the radicular dentine wall after final irrigation with 17% EDTA at (a) coronal, (b) middle and (c) apical levels

EDTA: Ethylenediaminetetraacetic acid

group during EDX analysis. The decrease in the Ca/P ratio in all the experimental groups at the different root levels was due to the decreased Ca levels after final irrigation with the respective chelating agents.

Apart from Ca and P, K and Mg were also noted on the radicular dentine surface in traces during EDX analysis. Mg appears to impact the mineralisation process, and its absence causes a decrease in the number of odontoblasts, which prevents or
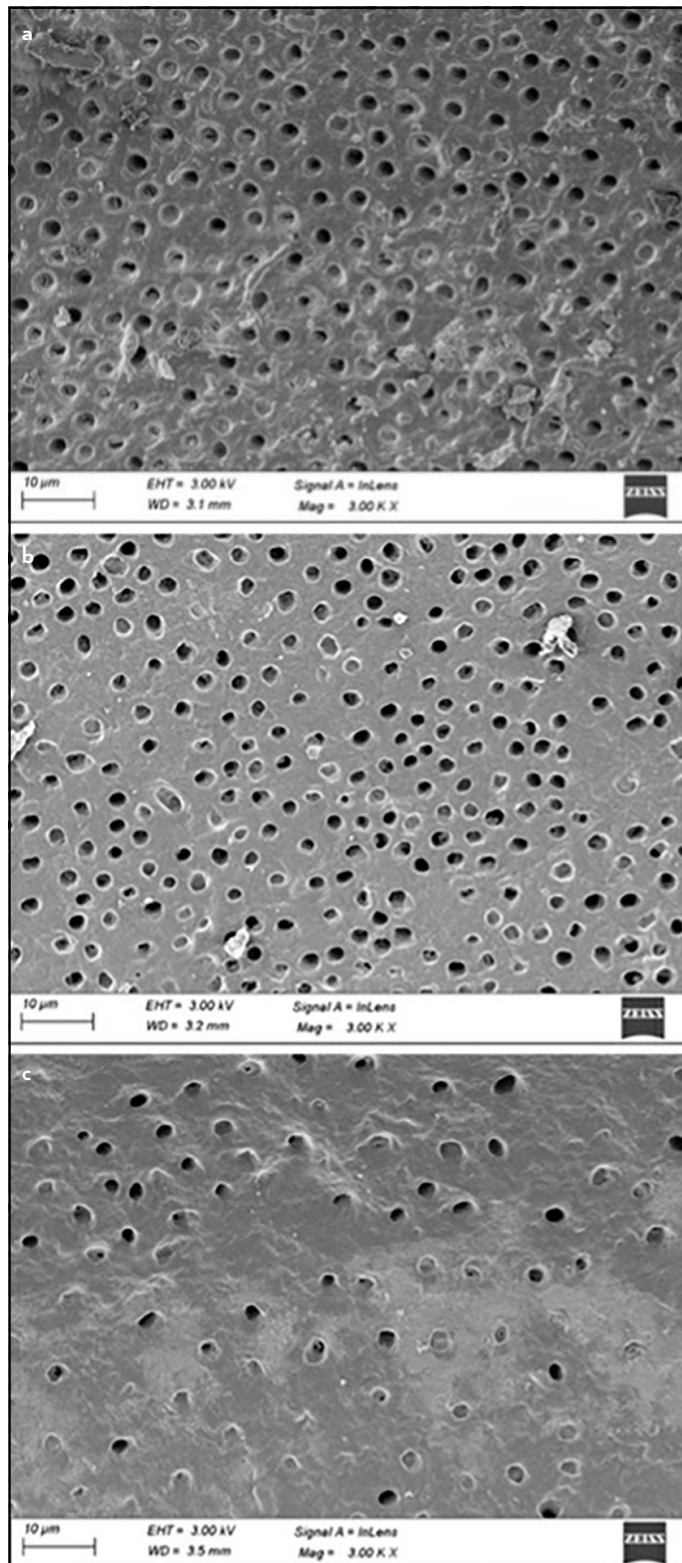
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Figure 3. Representative FESEM photomicrographs (×3,000) of the radicular dentine wall after final irrigation with 9% HEBP at (a) coronal, (b) middle and (c) apical levels

HEBP: 1-hydroxyethylidene-1,1-bisphosphonate

Figure 4. Representative FESEM photomicrographs (×3,000) of the radicular dentine wall after final irrigation with 0.2% chitosan at (a) coronal, (b) middle and (c) apical levels

slows dentine production (30). Currently, there is little knowledge about the function of K, which is found intracellularly (31). In the present study, levels of K and Mg were found to be significa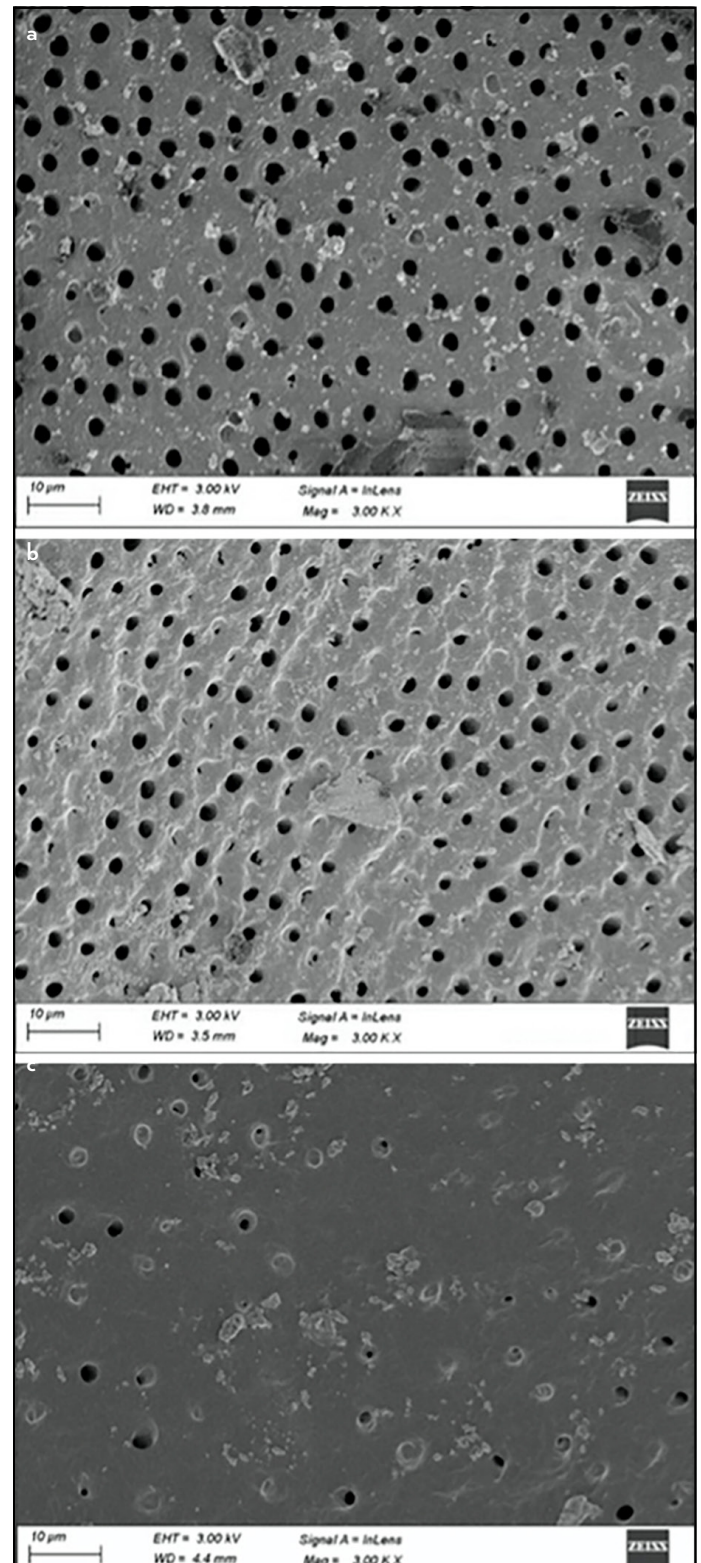ntly elevated in the 17% EDTA group in contrast to all the other groups at the coronal, middle and apical levels. Although these findings were like that of Cobankara et al. (28), the plausible reason for such occurrence is still unclear.

Pairwise comparison between 17% EDTA and 9% HEBP groups showed that Ca, P levels and Ca/P ratio after final irrigation with

the respective chelating agents were similar with no significant differences. These observations were similar to the results of Cobankara et al. (28) but contradictory to the results of Lottanti et al. (21) and Zehnder et al. (11), which showed that EDTA caused significantly higher loss of Ca ions than HEBP. In the current study, 17% EDTA and 0.2% chitosan also had comparable effects in terms of alteration in Ca, P levels and Ca/P ratio when used as a final rinse, except at the coronal level, where 0.2% chitosan caused significantly less alteration in the Ca levels. The studies conducted by Silva et al. (19) and Sarkees et al. (18) also showed that chitosan and EDTA did not differ significantly in the ability to remove calcium ions. However, Mathew et al. (22) found that 0.2% chitosan caused significantly less alteration in the Ca/P ratio than 17% EDTA during final root canal irrigation.

As per our knowledge, no study has compared the effects of 9% HEBP and 0.2% chitosan on the mineral content of the radicular dentine in the published literature. The current study found that 9% HEBP and 0.2% chitosan had similar effects on the mineral content with no significant differences when used as a final rinse. However, the Ca/P ratio was significantly reduced after final irrigation with 9% HEBP than 0.2% chitosan at the coronal third. These findings could have been due to the increased phosphorus levels in the 9% HEBP group, as discussed earlier. Most of the comparisons in this study showed no significant differences in the elemental values between experimental groups. Thus, the null hypothesis was partially accepted.

FESEM analysis revealed that normal saline as a final irrigant failed to eliminate the smear layer, which was also reported by earlier studies (32). Thus, the current study also validates that the irrigation regime should include a chelating agent in combination with NaOCl, which is supported by earlier studies in the literature (33). Although this study was based on evaluating the effect of chelating agents, the rationale behind using NaOCl after each instrument change was to mimic more precise and comparable clinical settings.

As the degree of erosion was scored in whole numbers, median values were calculated for statistical comparisons. The final rinse with 17% EDTA and 9% HEBP caused moderate erosion of the radicular dentine with significant differences from 0.2% chitosan at the coronal level. In contrast, none of the three chelating agents eroded the root dentine at the middle and apical levels. Thus, the null hypothesis was partially accepted. Lima Nogueira et al. (34) observed that the degree of erosion did not differ significantly between 17% EDTA and 9% HE, which was in accordance with the current study's findings. Silva et al. (15) found that 0.2% chitosan used for 5 mins caused severe erosion of the radicular dentine, which was in contrast with the results of the current study. An additional rinse with NaOCl after irrigation with chitosan might have resulted in severe erosion.

The duration for which the irrigating solution is kept in contact with the radicular dentine surface is critical. However, the ideal contact period is debatable, varying from 1 to 15 mins (8, 35). In the current study, all the irrigating solutions were used for 5 mins, similar to earlier studies (26, 32). A limitation of this in vitro study was its small sample size, as considering a larger sample size allows the researcher to increase the significance level of

the findings. The mineral content of the respective samples was not assessed before treatment. It is unlikely that samples in all the groups were equal before treatment because the teeth were taken randomly from patients, and the mineral content was likely varied for each tooth. This could be a limitation of this study. The irrigant delivery and activation method employed during root canal irrigation should also be considered. As found by earlier studies, the syringe-needle irrigation method is debatable regarding root canal cleanliness (36). Using methods employing a negative irrigation system or activating the irrigant with sonic and ultrasonic systems could have potentiated the effects of chelating agents, as reported by earlier studies (13, 37–39). This could be another limitation of the current study. Moreover, the concept of coronal reconstruction before endodontic treatment should also be considered as it may also affect the smear layer removal efficacy of the irrigating solution, with the pre-endodontic restoration serving as a reservoir to maintain the volume of the irrigating solution in the coronal access cavity (40).

The observations of the current study designate both 9% HEBP and 0.2% chitosan as promising chelating agents when used as a final rinse, with the potential to be used as alternatives to conventionally used 17% EDTA. However, further studies with sizeable specimens are required to assess and compare the effects of 9% HEBP and 0.2% chitosan on varying parameters such as dentine microhardness, surface roughness, smear layer removal, fracture resistance, sealer penetration depth and push-out bond strength before their intended clinical use, to confirm their benefits as effective root canal chelating agents.

CONCLUSION

Within the constraints of this in vitro investigation, it can be concluded that 17% EDTA, 9% HEBP and 0.2% chitosan had specific effects on the mineral content of radicular dentine when used as final irrigating solutions. 17% EDTA and 9% HEBP caused moderate erosion of the radicular dentine with significant differences from 0.2% chitosan at the coronal level. In contrast, none of the chelating agents eroded the radicular dentine surface at the middle and apical levels. Thus, 9% HEBP and 0.2% chitosan can be used as alternatives to 17% EDTA during the final irrigation of the root canal.

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