Original Article

Comparative evaluation of continuous and sequential chelation on the dentinal tubule penetration of bioceramic-based sealer – A confocal laser scanning microscopic study

Komal Gupta, Soumya S. Shetty, Anita B. Tandale, Manisha Srikurmam[1], Harsha Nihalani

Department of Conservative Dentistry and Endodontics, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, Maharashtra, [1]Private Practitioner, Conservative Dentistry and Endodontics, Secunderabad, Hyderabad, India

Abstract

Context: Effective endodontic treatment relies heavily on proper instrumentation, thorough disinfection, and precise three-dimensional obturation of the root canal system.

Aims: This study aims to evaluate and compare continuous chelation (etidronic acid and sodium hypochlorite [NaOCl]) and sequential chelation (citric acid and NaOCl) on the dentinal tubule penetration of bioceramic-based sealer (Bio-C)-A confocal laser scanning microscopic study.

Settings and Design: *In vitro* experimental study.

Subjects and Methods: Sixty extracted permanent single-rooted teeth were selected and decoronated below cementoenamel junction to get a standardized length of 12 mm across all samples followed by enlargement of root canals till rotary ProTaper F3. The samples were randomly divided into three groups: Group I (control): Canals were irrigated with 3 mL 17% ethylenediaminetetraacetic acid/3 mL 3% NaOCl; Group II: Canals were irrigated with 10 mL etidronic acid + NaOCl solution; and Group III: Canals were irrigated with 3 mL 10% citric acid followed by 3 mL 3% NaOCl. 0.1% rhodamine B dye was mixed with Bio-C sealer followed by obturation. All the samples were subjected to confocal laser scanning microscopy evaluation.

Statistical Analysis Used: One-way analysis of variance with the Bonferroni *post hoc* test was used for the statistical analysis. The level of significance was set at $P < 0.05$.

Results: The pairwise comparison of depth of penetration showed statistically significant results in all the coronal, middle, and apical thirds. Group II showed better depth of penetration than Groups I and III.

Conclusions: Continuous chelation protocol using etidronic acid and NaOCl showed greater and statistically significant sealer penetration depth when compared to the sequential chelation protocol, i.e., citric acid at all three levels.

Keywords: Bioceramic sealer; continuous chelation; dentinal tubule penetration; irrigation; sequential chelation

Address for correspondence:
Dr. Anita B. Tandale,
Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, Maharashtra, India.
E-mail: anita.tandale@dpu.edu.in

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INTRODUCTION

Effective endodontic treatment relies heavily on proper instrumentation, thorough disinfection, and precise three-dimensional obturation of the root canal system.

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The smear layer, created as a result of instrumentation of the canal system, forms a layer of inorganic and organic debris that may harbor bacteria and their by-products.

Despite the contentious discourse regarding the retention or removal of the smear layer, its complete removal is deemed critical. A bonded interface between the core restorative material and root dentin is crucial for successful endodontic treatment as it produces a better adaptation of the sealer to the dentinal walls and enhances the dislocation resistance of the root-filling material.[1] An optimal endodontic irrigation solution should be antimicrobially effective, remove the smear layer, disintegrate necrotic tissue, and have little systemic toxicity. Sodium hypochlorite (NaOCl) alone is ineffective in eradicating the inorganic portion of the smear layer. Thus, for optimal smear layer removal, NaOCl must be used in conjunction with chelating agents such as ethylenediaminetetraacetic acid (EDTA) and citric acid.[2]

The most widely used irrigation procedure in root canal therapy, i.e., sequential chelation protocol (NaOCl followed by EDTA), causes dentinal tubular aperture expansion and intertubular tunneling owing to dentin degradation and decrease in flexural strength.

It enables thorough decalcification of the intertubular dentin surface from 1 to 5 μm, while also extending up to 20 μm in the dentinal tubular walls.

To address the issues related to the use of NaOCl/EDTA, a novel root canal irrigation strategy, continuous chelation was established. Here, NaOCl is incorporated with the salt of a weak chelator, etidronate or, 1 hydroxyethylidene-1, 1-bisphosphonate (HEBP), because the tetrasodium HEBP salt is highly attuned with NaOCl. Since HEBP cannot be used merely as a final rinse due to its weak chelating effect, it was combined with NaOCl as it does not affect its proteolytic or antimicrobial properties.

Twin Kleen™ (Maarc Dental Innovations Endo, India), a commercially available product of etidronic acid, consists of 9% HEDP.[3] It has been indicated for continuous soft chelation of the root canals and is safe to use with NaOCl.[4]

Another example of a mild chelating agent, i.e., citric acid is shown to have good chemical stability and can effectively combat both facultative and obligatory anaerobes.[5] Its use as a root canal irrigating solution was suggested due to its properties such as removal of the inorganic component of smear layer and dentin decalcification. Various researchers have used citric acid in different concentrations ranging from 1% to 50%.[6]

Tricalcium silicate-based sealers have piqued interest further in terms of enhancing filling quality because of their excellent biocompatibility, low cytotoxicity, and viscosity. They demonstrate significant physicochemical qualities and bactericidal activity which enhance the chemical bonding and mechanical retention of the dentinal walls followed by the formation of a physical barrier to fluids and nutrients.[7] The adoption of calcium silicate-based sealers has risen because of their superior physicochemical qualities, bactericidal activity, small particle size, and biocompatibility, as demonstrated by the encouraging results acquired in recent years.[8] Bio-C® sealer is an injectable, nonresinous, alkaline sealer with high radiopacity due to the presence of zirconium.

The ability of sealers to penetrate the dentinal tubules plays a critical role in minimizing the surface area interaction between gutta-percha and root dentin. The sealer's capacity to conform to the root canal wall reduces the danger of microleakage, hence increasing the antimicrobial effect. Furthermore, deep sealer penetration improves root canal fracture resistance.

The objective of this study was to evaluate the dentinal tubule penetration of a Bioceramic sealer (Bio-C) using different irrigation protocols, i.e., continuous chelation using etidronic acid and NaOCl and sequential chelation using citric acid followed by NaOCl. The null hypothesis is that there is no difference in the dentinal tubule penetration between EDTA, etidronic acid, and citric acid.

SUBJECTS AND METHODS

Sample preparation

A total of 60 freshly extracted, intact single-rooted teeth with single canal and closed apices were included in the study. Multirooted teeth, teeth with root caries, cracks, endodontically treated teeth, internal resorption, or calcification were excluded. All the samples were cleaned of debris, autoclaved, and stored in 0.1% thymol solution until use. All teeth were decoronated with diamond discs at low speed below the level of cementoenamel junction to have a standardized length of 12 mm across all samples. Working length was determined with a #15 K file (Mani Inc., Japan). Root canals were enlarged using the ProTaper Gold rotary system (Dentsply Sirona, USA) up to size F3 at 0.5–1 mm from the apical foramen.

Randomization sequence generation

Each sample was given a unique number. Each group's sample allocation was done using a computer-generated random sequence table. This was done using sequentially numbered, opaque, sealed envelope technique, and concealed. The samples were then randomly divided into three groups with 20 teeth each based on the irrigation protocol ($n = 20$):

- Group I (control) ($n = 20$) - samples were irrigated with 3 mL 17% EDTA (Prime Dental Products, Mumbai, India) followed by 3 mL 3% NaOCl (Prime Dental Products, Mumbai, India) with intermittent flushes of saline
- Group II ($n = 20$) - 0.9 g etidronic acid + NaOCl solution. According to the manufacturer's instructions, 10 mL of 3% NaOCl was mixed with two capsules of Twin Kleen each of which contains 0.45 g of HEBP
- Group III ($n = 20$) - Irrigation was done using 3 mL of 10% citric acid (Prime Dental Products, Mumbai, India) followed by 3 mL of 3% NaOCl with intermittent flushes of saline.

After final irrigation, all the canals were irrigated with 5 mL of distilled water and dried using absorbent paper points (Dentsply Sirona, USA).

The Bioceramic sealer (Bio-C sealer, Angelus, Brazil) was dispensed on the glass slab and labeled with Rhodamine B (HiMedia, Mumbai) at an approximate concentration of 0.1% to allow analysis under the confocal laser scanning microscopy (CLSM). Root canal walls were then coated with the Bio-C sealer followed by obturation using the lateral condensation technique and temporization using Cavit G (3M ESPE, Germany). To allow the sealer to set, all the samples were kept at 100% humidity and 37°C for 7 days.

To avoid frictional heat, the roots were sectioned horizontally at 3 mm (apical), 4 mm (middle), and 5 mm (coronal) using a carborundum disc under continuous water cooling [Figure 1]. The thickness of all sections was maintained at 1 mm. Samples were dried and examined under CLSM (Zeiss LSM 700) at ×10. The assessor of the outcome was blinded after assignment to intervention. The maximum depth of penetration was measured from the root canal wall to the deepest point of irrigant penetration [Figure 2].

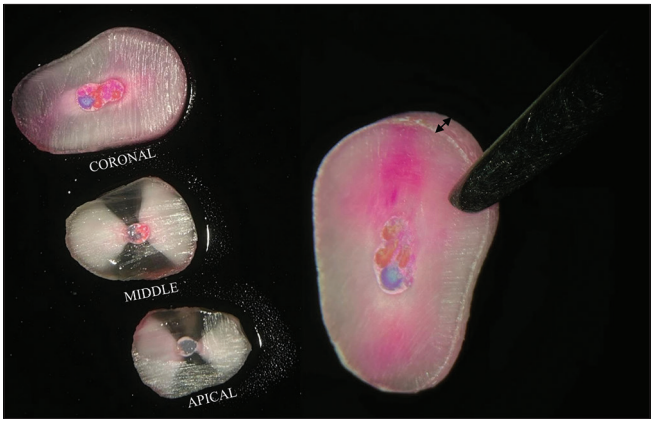


Figure 1: Transverse sectioning of the specimens at coronal, middle, and apical third using a carborundum disc. Thickness of the cut sections was maintained at one mm

RESULTS

For evaluating the difference in mean penetration depth between the groups' analysis of variance with the Bonferroni *post hoc* test was applied. All the statistical tests were performed keeping the confidence interval at 95% and ($P < 0.05$) was considered statistically significant.

The mean penetration depth in the coronal, middle, and apical regions was the highest in Group II followed by Group III and Group I. This difference in mean depth was statistically significant with respect to all the groups ($P < 0.05$) [Graph 1].

In the coronal region, when pairwise comparison was done between the groups, there were statistically significant differences in mean depth between Group I and Group II ($P < 0.05$), Group I and Group III ($P < 0.05$), and between Group II and Group III ($P < 0.002$). In the middle region, when pairwise comparison was done between the groups, there were statistically significant differences in mean depth between Group I and Group II ($P < 0.05$), Group I and Group III ($P < 0.05$), and between Group II and Group III ($P = 0.003$). In the apical region, when pairwise comparison was done between the groups, there were statistically significant differences in mean depth between Group I and Group II ($P < 0.05$), Group I and Group III ($P < 0.05$), and between Group II and Group III ($P < 0.05$) [Table 1].

Group II, i.e., continuous chelation using 9% etidronic acid exhibited significantly higher mean penetration depth in the coronal, middle, and apical third than continuous chelation.

DISCUSSION

Tubular penetration of sealers is a crucial property as it minimizes the surface area of interaction between root dentin and gutta-percha. This improves the mechanical interlocking[9] and the fracture resistance of the canal walls. Hence, our study aimed to evaluate the dentinal tubule penetration of the sealer using different irrigants/chelating protocols. This study utilized the ProTaper Gold Rotary system to shape all specimens to ProTaper F3 to increase the volume exchange of irrigants at the working length and to assist in achieving a satisfactory cleaning and permeation of irrigants into the tubules. This was carried out in accordance with past studies that demonstrated that larger apical preparations allow for a significant reduction in remaining bacteria and help in more effective elimination of the smear layer compared to smaller preparations.[10]

The interaction between NaOCl and EDTA can lead to the loss of free accessible chlorine for NaOCl, which in turn

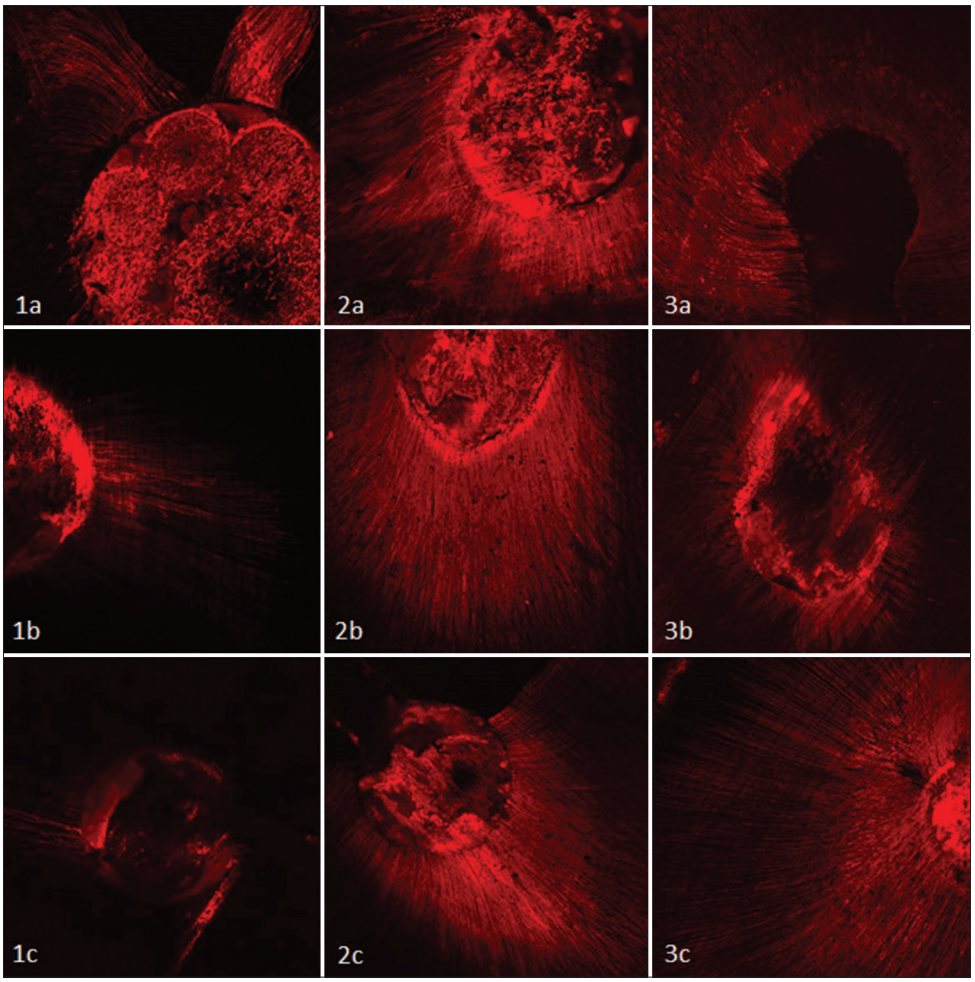


Figure 2: Confocal laser scanning microscopy images of depth of sealer penetration at ×10 in Group I - 1a: Coronal, 1b: Middle, 1c: Apical, Group II - 2a: Coronal, 2b: Middle, 2c: Apical, Group III - 3a: Coronal, 3b: Middle, 3c: Apical

Table 1: Pairwise comparison of penetration depth

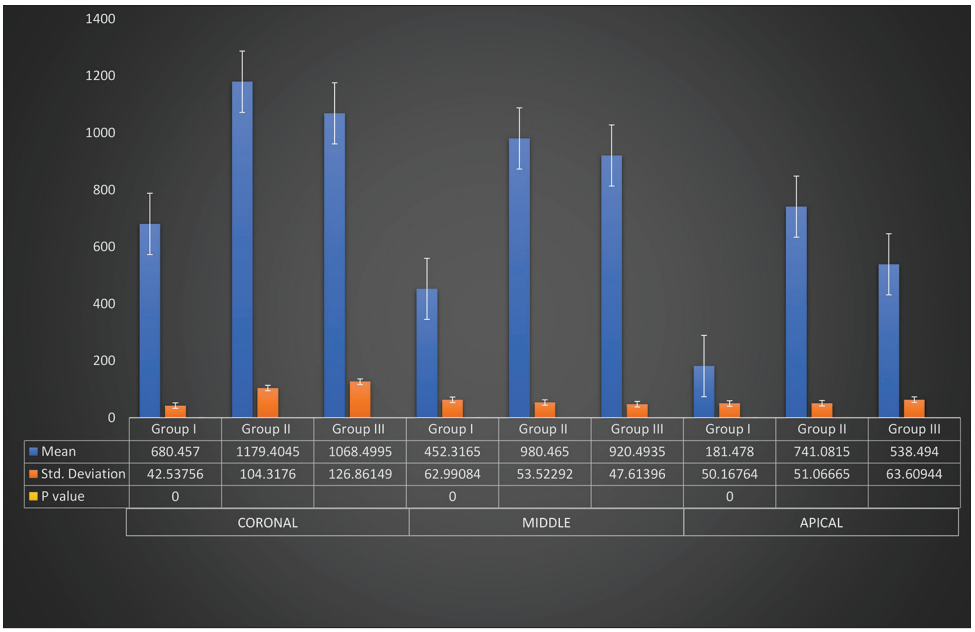
Dependent variable	(I) group	(J) group	Mean difference (I−J)	*P*
Coronal third - penetration depth	Group I (3 mL 17% EDTA/3 mL 3% NaOCl)	Group II (etidronic acid + 10 mL 3% NaOCl)	−498.94750*	0.000
	Group I (3 mL 17% EDTA/3 mL 3% NaOCl)	Group III (3 mL 10% citric acid/3 mL 3% NaOCl)	−388.04250*	0.000
	Group II (etidronic acid + 10 mL 3% NaOCl)	Group III (3 mL 10% citric acid/3 mL 3% NaOCl)	110.90500*	0.002
Middle third - penetration depth	Group I (3 mL 17% EDTA/3 mL 3% NaOCl)	Group II (etidronic acid + 10 mL 3% NaOCl)	−528.14850*	0.000
	Group I (3 mL 17% EDTA/3 mL 3% NaOCl)	Group III (3 mL 10% citric acid/3 mL 3% NaOCl)	−468.17700*	0.000
	Group II (etidronic acid + 10 mL 3% NaOCl)	Group III (3 mL 10% citric acid/3 mL 3% NaOCl)	59.97150*	0.003
Apical third - penetration depth	Group I (3 mL 17% EDTA/3 mL 3% NaOCl)	Group II (etidronic acid + 10 mL 3% NaOCl)	−559.60350*	0.000
	Group I (3 mL 17% EDTA/3 mL 3% NaOCl)	Group III (3 mL 10% citric acid/3 mL 3% NaOCl)	−357.01600*	0.000
	Group II (etidronic acid + 10 mL 3% NaOCl)	Group III (3 mL 10% citric acid/3 mL 3% NaOCl)	202.58750*	0.000

*Significance at $P<0.05$. NaOCl: Sodium hypochlorite, EDTA: Ethylenediaminetetraacetic acid

reduces the antibacterial activity, tissue dissolving capacity, and dentin structural integrity.[11] It has been reported that rinsing with distilled water or saline could mitigate the interaction between two endodontic irrigating solutions.[12] This protocol was followed through all the samples in Group I.

Crumpton *et al*.[13] conducted a study that showed the efficacy of a final rinse comprising 1 mL of 17% EDTA and 3 mL of 5.25% NaOCl in the removal of the smear layer. However, our study found that Group I had the lowest sealer penetration in all thirds: coronal, middle, and apical. This could be linked to the fact that EDTA can collapse the dentinal matrix, preventing the sealant from infiltrating even after the smear layer has been removed.[14]

Further studies conducted by Ballal *et al*.[15] and Mancini *et al*.[16] demonstrated that EDTA is not as effective in removing the smear layer from the apical third as compared to the coronal and middle thirds.



	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
Mean	680.457	1179.4045	1068.4995	452.3165	980.465	920.4935	181.478	741.0815	538.494
Std. Deviation	42.53756	104.3176	126.86149	62.99084	53.52292	47.61396	50.16764	51.06665	63.60944
P value	0			0			0		
	CORONAL			MIDDLE			APICAL		

Graph 1: Mean depth of sealer penetration of all three groups at coronal, middle, and apical third

Baumgartner and Mader[17] conducted a study that revealed that the combination of EDTA and NaOCl can lead to the dissolution of peritubular and intertubular dentin.

Several studies have also demonstrated that the sequential use of NaOCl and EDTA can lead to dentinal erosion, which can adversely affect dentin flexural strength and microhardness.[18]

Citric acid has been proposed as an irrigating solution for root canals, owing to its efficacy in removing the inorganic component of the smear layer and its ability to decalcify dentin. Studies conducted by Banode *et al*.[19] revealed that the elimination of the smear layer from the canal space was more successfully achieved using citric acid as the final irrigation method than with EDTA. These results were consistent with our study in which citric acid resulted in better tubular penetration as compared to EDTA in all thirds. The reason behind the enhanced adhesion of the sealer to root canal irregularities is the increased roughness of root dentin caused by citric acid.[9] This leads to a stronger micromechanical bonding between the sealers and the root canal walls. Moreover, some research discovered that varying amounts of citric acid cleared the canal walls and left the dentinal tubules accessible.[20]

Thus, a 10% citric acid solution was utilized in our investigation due to its biocompatibility and capacity to eradicate microbes, contaminated tissue, and inorganic smear layer found within the root canal dentin.

A novel irrigation protocol, i.e., continuous chelation was introduced to overcome the disadvantages of EDTA. This protocol allows the simultaneous use of etidronic acid along with NaOCl without affecting the properties of NaOCl. Etidronic acid, a weak chelator, when used in combination with NaOCl, does not compromise the ability of NaOCl to break down the tissues or inhibit biofilm formation.

Maximum penetration was seen with Group II, i.e., continuous chelation in all the thirds as compared to Group I, i.e., sequential chelation and Group III and these results were statistically significant. Hence, the null hypothesis was rejected. These results were in accordance with previous studies conducted by Zehnder *et al*.,[21] who demonstrated that the continuous presence of etidronic acid prevents the development of a smear layer along the walls of dentin. Similar results were seen in a previous study conducted by Kamin *et al*.,[4] who demonstrated that the continuous presence of HEDP prevents the development of a smear layer along the walls of dentin. The observed phenomenon is likely attributable to the enhanced removal of the smear layer, which results in the exposure of a greater number of open dentinal tubules.[22] Consequently, better removal of the smear layer can occur as NaOCl can act directly on dentinal tubules and lateral canals in the apical third. When a combination of NaOCl and HEDP was used to irrigate the root canals instead of 2.5% NaOCl alone, Paqué *et al*.[23] reported that the deposition of hard-tissue debris was significantly reduced. However, these results were contradictory to a study by Sunanda *et al*. in which a greater depth of penetration was seen when chitosan was combined with NaOCl as compared to etidronic acid combined with NaOCl.[24]

The results of our study showed that the maximum penetration of the Bio-C sealer was seen at the coronal third, followed by the middle-third, and least in the apical third in all experimental

groups, regardless of the irrigation protocol used. The data presented by McMichael *et al*.,[25] who discovered that tubular penetration was greater at the level of 5 mm from the apex as compared to the sealer penetration at 1 mm, are likewise consistent with this conclusion. This could be because the tubular density of dentin is generally less due to which the delivery of the irrigant is decreased.[26] The smaller diameter of the tubules impedes circulation and impairs the action of the irrigating solutions in the apical third.[26]

The Bio-C sealer can adhere to the walls in the presence of moisture due to its hydrophilic nature. As our study was done in vitro, the exact *in vivo* conditions could not be simulated. Hence, thermocycling of all the samples was done to simulate the oral environment.

Sealer-dentin interface can be examined/determined using various microscopic techniques, such as transmission electron microscopy, scanning electron microscopy, and stereomicroscope. CLSM using fluorescent organic dyes has become an established standard for assessing tubular penetration.[27] It excludes the requirement for sectioning of samples, dehydration, and polishing artifacts[28] that might obstruct dye penetration. However, CLSM has a key drawback in that it cannot directly show nonfluorescent materials. As a result, the sealer employed in our investigation was incorporated with a fluorescent dye. According to recent literature, rhodamine B does not affect the setting of sealers. It has a smaller particle size and more surface-active molecules as compared to methylene blue. According to the American Dental Association (ADA) specifications, it was determined that the sealer coated with 0.1% rhodamine did not exhibit any variations in the flow. Hence, in our study, a 0.1% concentration of rhodamine B dye was used.

This study was conducted in the laboratory conditions (*in vitro* study) in a controlled setting. Further *in vivo* studies with diverse parameters are recommended to evaluate continuous chelation protocol in dynamic oral environmental conditions in teeth with more complex anatomical features.

CONCLUSIONS

Within the limitations of this *in vitro* study, it can be concluded that continuous chelation protocol using etidronic acid and NaOCl showed greater and statistically significant sealer penetration depth when compared to the sequential chelation protocol, i.e., citric acid at all three levels. Tubular penetration was seen maximum at the coronal third, followed by middle and apical third.

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Conflicts of interest
There are no conflicts of interest.

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