ORIGINAL RESEARCH

Push-out Bond Strength of Two Bioceramic Sealers after Using Various Final Irrigation Solutions: An *In Vitro* Study

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Abstract

Aim: This study assessed how different final irrigation solutions impact the push-out bond strength (PBS) of EndoSequence Bioceramic (BC) and cerafill BC sealers.

Methods: Eighty single-root lower premolars were selected and decoronated. The root canals were then cleaned and shaped chemo-mechanically using ProTaper files up to F5, with 5.25% sodium hypochlorite applied between each file for rinsing. Subsequently, the teeth were categorized into four primary groups of 20 each, distinguished by their final irrigation solution. Group A utilized 17% glycolic acid (GA), group B used a 5% apple vinegar solution, group C employed 17% ethylenediamine tetraacetic acid (EDTA), and group D used saline. Each group was further divided into two subgroups of 10 teeth each, based on the type of BC sealer applied—Cerafill BC sealer for subgroup a, and EndoSequence BC sealer for subgroup b. Two dentin disks were then obtained from the middle third of each root ($n = 20$) and subjected to the PBS test. Upon completion of this test, the dentin disks were scrutinized under a stereomicroscope to determine the mode of failure for each disk.

Results: The use of EndoSequence BC sealer in obturation led to the apple vinegar final irrigation group exhibiting significantly higher PBS than the GA and saline solution groups. Furthermore, when implemented with cerafill BC sealer, the EDTA final irrigation solution group displayed the greatest PBS, showing a significant difference compared with the GA and saline solution groups.

Conclusion: Apple vinegar solution could be a potential alternative to EDTA as a chelating agent during root canal treatment, though further research is required.

Clinical significance: The EDTA and apple vinegar are effective at removing smear layers, which improves sealer penetration and prevents obturation materials dislodgment.

Keywords: Apple vinegar, Chelating agents, Glycolic acid, Push-out, Root canal.

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Introduction

The primary aim of root canal treatment is to chemo-mechanically eradicate microorganisms and provide a firm seal that prevents canal reinfection.[1] While mechanical instrumentation is crucial for root canal cleaning, its capacity to disinfect and cleanse is restricted. Previously, substantial unmodified dentin surfaces have been noticed in these spaces after mechanical instrumentation.[2] Also, the complex root canal anatomy often includes oval-shaped canals, lateral canals, and isthmus.[3–5] As a result, it is difficult to effectively clean and disinfect these inaccessible areas. Thus, chemical irrigation is required to disinfect and remove debris from these still-touched dentin surfaces and hard-to-reach spots. Sodium hypochlorite (NaOCl) is frequently the preferred endodontic irrigation method because of its low viscosity, broad antimicrobial activity, and ability to dissolve organic tissues.[6] However, to remove the smear layer, a chelating or decalcifying agent after NaOCl is recommended.[7,8] Ethylenediamine tetraacetic acid (EDTA) is a common follow-up to NaOCl. Currently, using NaOCl and EDTA together is considered the optimal irrigation protocol.[9] Despite being effective at removing the smear layer, EDTA can be toxic to the periapical tissue and can potentially weaken and reduce the radical dentin's microhardness.[10–12] Lately, variety of acids have been found to effectively remove the smear layer; among those acids are glycolic acid (GA) and apple vinegar.[13–16]

Glycolic acid ($HOCH_2CO_2H$), also known as hydroxyacetic acid, is a colorless, odorless and highly water-soluble material.[17,18] Recently, it has been proposed as a substitute for phosphoric acid as an etchant for enamel and dentin in restorative procedures.[19] It has shown promising results as a final irrigant in endodontic treatments, demonstrating comparable efficacy to EDTA in removing the smear layer after root canal cleaning and shaping.[13] Notably, GA also exhibited lower cytotoxicity than EDTA.[13]

Another acidic agent that has been shown to effectively remove the smear layer is apple vinegar.[15,16] Its high biocompatibility is mainly due to its rich malic acid content.[20] Furthermore, apple vinegar has demonstrated antimicrobial properties against common endodontic microbiota.[21]

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The removal of the smear layer from the canal wall enhances the sealer's contact with the dentin and enables the sealer to mechanically interlock with the root dentin tags.[22] As a result, root canal preparation followed by smear layer removal results in increased push-out bond strength (PBS) of the filling.[23]

The gutta-percha cone remains the preferred solid filler for root canal obturation. Nonetheless, because gutta-percha does not stick to root canal walls, an endodontic sealer must be applied to thoroughly seal the spaces between the dentin canal walls and the gutta-percha.[24] Endodontic sealers, primarily classified by their chemical structure, are varied. Sealers based on calcium silicate are typical. These hydraulic polymers set when they come into contact with water or moisture. Their alkaline pH and capacity to release calcium ions, leading to the deposition of an apatite layer, might explain their bioactivity and biocompatibility. The EndoSequence BC sealer (Brasseler, Savannah, GA, USA), a popular bioceramic sealer, has been available since 2009.[25] As stated by the manufacturer, the EndoSequence BC sealer is a premixed, ready-to-use, aluminum-free bioceramic sealer.[26] The EndoSequence BC sealer, as indicated by the manufacturers, consists of zirconium oxide, tricalcium silicate, dicalcium silicate, calcium hydroxide, and some undisclosed fillers.[26] Many researchers have studied it, finding it to be nontoxic, biocompatible,[27] bioactive, and with antibacterial properties.[25,28–30] Moreover, it reportedly has a strong sealing ability and good bond strength to dentin.[31,32]

A new, premixed, calcium silicate-based sealer, Cerafill (Prevest DenPro, USA), has been released on the market. The Cerafill BC sealer, as the manufacturer stated, is composed of calcium silicates, calcium phosphates, zirconium oxide, bioactive glasses, calcium sulfate, and calcium oxide, along with unspecified fillers, accelerators, and thickening agents.[33] The manufacturer describes it as a premixed bioceramic sealer that does not contain alumina and boasts superior physical characteristics. They report that upon setting, the sealer forms hydroxyapatite crystals and chemically bonds to root dentin.

Studies evaluating the effect of different irrigation protocols targeted at eliminating the smear layer on the PBS of calcium silica-based sealers to dentin are limited. The purpose of this study was to assess the impact of varying final irrigation solutions on the PBS of EndoSequence BC and Cerafill BC sealers. The modes of failure were also examined.

Materials and Methods

Sample Size Determination

We determined the sample size based on an extensive literature review, which typically recommends using 10–30 teeth per group in similar studies.[34–37] Thus, we selected a sample size of 20 disks per group.

Teeth Selection

Ethical approval was obtained from King Abdulaziz University Ethical Committee (#065-03-23). The study was conducted over a period of 1 year (2022–2023).

A total of 80 single-rooted lower premolars were collected. The teeth were extracted due to periodontal, orthodontic, or prosthetic causes. Radiographs of the teeth were obtained, and teeth with internal or external resorption, root canal treatment, open apex, cracks, extra canals, and severe curvatures were excluded. The teeth were autoclaved and stored in 1% thymol solution until used.[38]

Specimen Preparation

The teeth were decoronated with a diamond disk. Size 10 hand files were inserted into the canals and visualized at the apical foramen. The working length of the root canals was set to 1 mm less than the apical foramen. The ProTaper Gold system (Dentsply, Ballaigues, Switzerland) was used to clean and shape the teeth up to size F5, with 2 mL of 5.2% NaOCl being used to irrigate each canal between each file.

The roots were then randomly divided into four groups of 20 roots, each corresponding to a different final irrigation protocol: group A used 5 mL of 17% GA (CDH, India) for 1 minute; group B used 5 mL of 5% apple vinegar for 1 minute; group C used 5 mL of 17% EDTA for 1 minute; and group D used 5 mL of normal saline solution for 1 minute.[13,39] A 30-G side-vented endodontic irrigation needle was utilized to irrigate all roots with their respective solutions, followed by a final rinse with 5 mL of normal saline solution. The roots were then dried with #F5 paper points.

Following the final irrigation, the roots were obturated using a single cone technique with gutta-percha and sealer. Each group was then divided into two subgroups, each containing 10 roots, based on the type of endodontic sealer used: group a utilized a gutta-percha cone and Cerafill BC sealer, while group b employed a gutta-percha cone and EndoSequence BC sealer. The flow of the methods performed is presented in the flow diagram (Fig. 1).

Evaluation of PBS

The roots were coronally sealed with zinc eugenol temporary restoration (Cavit G), and then kept in an incubator at 37°C and 100% humidity for 1 week to ensure the sealer was completely set. Afterward, the roots were embedded in transparent, self-curing acrylic resin blocks. Using a precision cutting machine (Buehler, USA), each root was cut perpendicular to its long axis to produce dentin disks 1 mm thick at approximately 10 and 7 mm from the root apex. This process yielded two disks, each representing the middle third of the root and totaling 20 disks per group ($n = 20$). A digital caliper was used to verify the thickness of each disk, ensuring the uniformity of the specimens' dimensions.

The PBS test was carried out using a universal testing instrument (Instron, model no. 5944, USA). This involved deploying a 0.5-mm plugger at a crosshead speed of 1 mm/minute until the canal filling shifted place. The test proceeded from the apical to the coronal side of the specimen to avoid canal taper influence. The maximum failure force necessitated shifting the filling, recorded in Newtons, was represented by sudden decrease in load. The PBS for each specimen was calculated by dividing the maximum failure force by the total bonding area (mm^2) of the specimen, and was expressed in megapascals (MPa). To calculate the total bonding area, the formula $\pi(r1 + r2)h$ is used where $r1$ and $r2$ represent the apical and coronal radii of the root canal, respectively, h is the thickness of the dentin disk specimen, and π equals 3.14.

The final formula was as follows:

$$\text{Bond strength (MPa)} = \frac{\text{Maximum failure bond } (N)}{\pi(r1 + r2)h}$$

Mode of Failure Analysis

After completing the push-out test, all dentin disk specimens and dislodged gutta-percha were examined under a stereomicroscope with 35× magnification. The failure mode was recorded and categorized based on a previous study: Adhesive failure at the

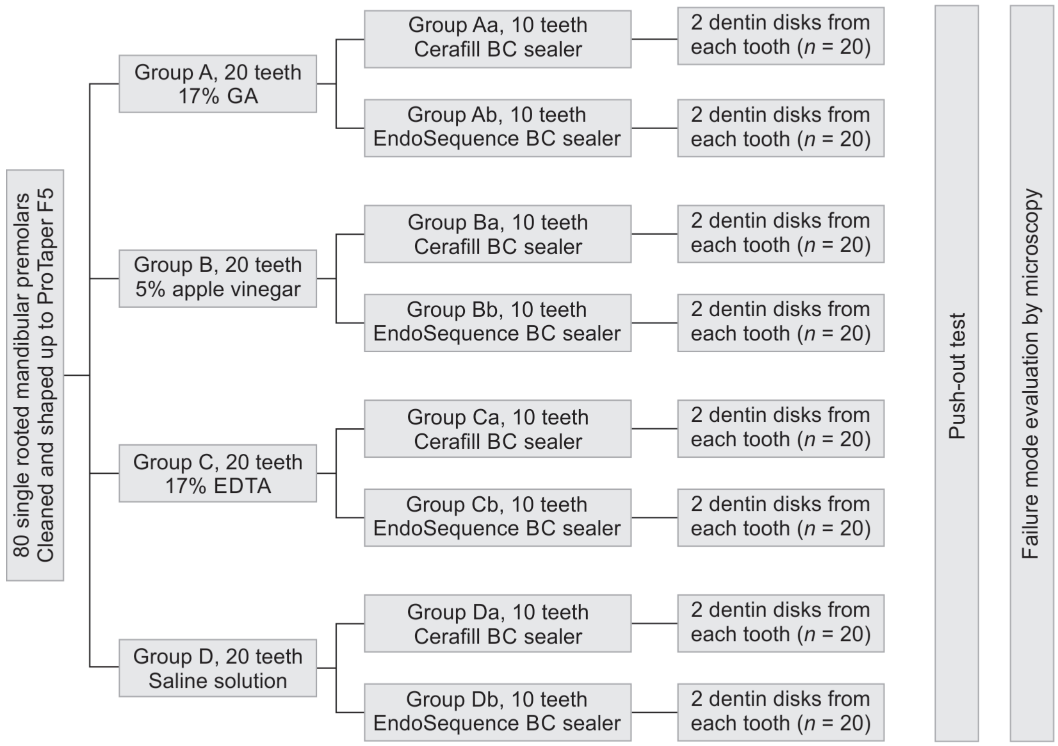


Fig. 1: Flow diagram of the methods performed in the current study

dentin/sealer or sealer/gutta-percha interface, cohesive failure within the sealer, and mixed failure that includes both adhesive and cohesive failures.[31]

Statistical Analysis

The data was expressed using descriptive statistics, such as mean and standard deviation. The Shapiro–Wilk test confirmed that the data was not normally distributed. To examine the effect of different sealer and irrigation types on the mean PBS, we conducted a two-way analysis of variance (ANOVA) with bootstrapping. This method accounted for the non-normal data distribution. Further analyses involved pairwise multiple comparisons to ascertain differences between each sealer under different irrigation circumstances. The Chi-square test compared failure modes between irrigation types in each sealer group, with a significance level set at $p < 0.05$. We performed statistical analyses using Statistical Package for the Social Sciences, version 28.

The manuscript was prepared in compliance with the 2021 Preferred Reporting Items for Laboratory Studies in Endodontology.[40]

Results

Table 1 displays the average PBS values (MPa) and standard deviations for all the groups. Intragroup variability was assessed using standard deviations (SD) to determine consistency within each group. For the EndoSequence sealer, the saline group exhibited the lowest intragroup variability, with an SD of 0.81 MPa, indicating consistent results. In contrast, the apple vinegar group had the highest SD of 3.14 MPa, reflecting greater variability within this group. Similarly, for the Cerafill sealer, the GA group showed the lowest intragroup variability, with an SD of 0.61 MPa, while the EDTA group had the highest variability, with an SD of 3.66 MPa.

Table 1: The means and standard deviations (SD) of the PBS values of the experimental groups (MPa)

Sealer	*Irrigation solution*	*Sample size (n)*	*Mean*	*SD*
EndoSequence	Glycolic acid	20	1.06	0.87
	Saline	20	1.03	0.81
	Apple vinegar	20	3.44	3.14
	EDTA	20	1.24	0.80
Cerafill	Glycolic acid	20	0.52	0.61
	Saline	20	1.29	1.36
	Apple vinegar	20	1.85	1.45
	EDTA	20	5.29	3.66

Table 2: Two-way ANOVA test results

Variable (source)	*Sum of squares*	*df*	*Mean square*	*F*	*p-value*
Sealer	11.950	1	11.950	3.225	0.08
Irrigation solution	167.160	3	55.720	15.038	<0.001*
Sealer X irrigation solution	181.074	3	60.358	16.289	<0.001*
Error	563.216	152	3.705		
Total	1,541.818	160			

*Statistically significant at $p < 0.05$

The two-way ANOVA results suggest that while the sealer materials' impact on the PBS was not significant, the final irrigation solutions and their interactions with the sealers had a noteworthy effect ($p < 0.05$) (Table 2). When root canals were filled with gutta-percha/EndoSequence BC sealer, the apple vinegar final irrigation group demonstrated the greatest PBS (mean = 3.44 MPa), significantly outperforming the GA (mean = 1.06 MPa) and saline solution groups (mean = 1.03 MPa). The second-highest PBS belonged to the EDTA final irrigation solution group (mean =1.24), though its difference from the other groups was not significant ($p < 0.05$) (Fig. 2). Therefore, the apple vinegar final irrigation group performed the highest PBS compared with all other groups.

The EDTA final irrigation solution group demonstrated the highest PBS (mean = 5.29 MPa), showing a statistically significant difference compared with the saline solution (mean = 1.29 MPa) and GA (mean = 0.52 MPa) groups when the root canals were filled with gutta-percha/Cerafill BC sealer. The apple vinegar final irrigation group, which had the second-highest PBS (mean = 1.85 MPa), also showed a significant difference compared with the GA group when the canals were filled with the same sealer ($p < 0.05$) (Fig. 3). Thus, there were no statistically significant differences between the PBS of the EDTA and apple vinegar final irrigation groups, and both groups performed higher PBS compared with other groups.

The failure analysis revealed that the cohesive mode of failure was prevalent in specimens obturated with gutta-percha/EndoSequence BC sealer, irrespective of the final irrigation solution used (Fig. 4A). Conversely, the specimens obturated with gutta-percha/Cerafill BC sealer primarily showed adhesive mode of failure (Figs 4B and C; Table 3).

Discussion

Adhesion of root canal sealer to dentin is vital for successful root canal treatment. Sufficient adhesion ensures the sealer's integrity and prevents its dislodgment during chewing or restorative processes. Hence, a stronger bond between dentin and sealer minimizes the chances of root canal system leakage and reinfection. The push-out test is a common method for evaluating the bond strength of root canal sealers even if other tests have been proposed for this purpose.[41,42] It operates on the principle of shear stress at the dentin–sealer interface, closely mimicking clinical conditions. The push-out test is able to evaluate the tensile bond strength at extremely low level.[43,44] While the test may not entirely replicate the sealer's clinical performance, it is instrumental in comparing various sealers or dentin surface treatments.

Sealer bond strength was reported to be impacted by the final irrigation that was used after root canal cleaning and shaping, aiming to remove the smear layer and reducing the microbial burden before obturation.[45] Ethylenediamine tetraacetic acid is typically applied at a concentration of 17% for less than 1 minute, and it is effective in eliminating the smear layer when in direct contact with the root canal dentin.[46] Despite EDTA's self-limited activity, dentin erosion has been shown to occur if it is kept in the canal for an extended period of time or if NaOCl is administered after EDTA.[46] Therefore, more biocompatible chelator agents need to be studied. Previous research has demonstrated that 17% GA and

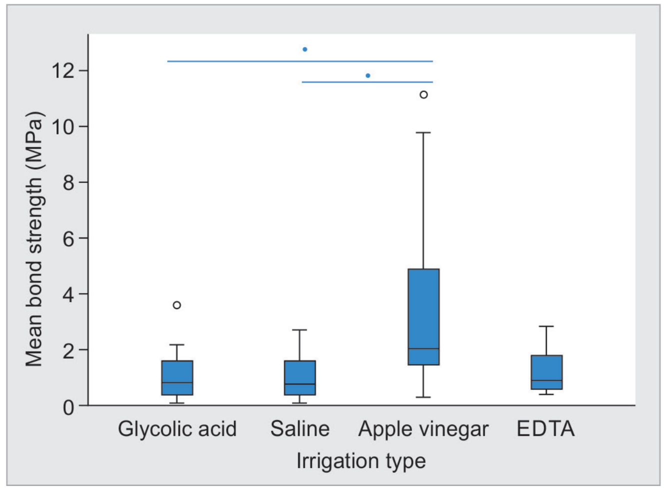


Fig. 2: The mean PBS values of the irrigation solutions for the EndoSequence sealer groups (*statistically significant at $p < 0.05$)

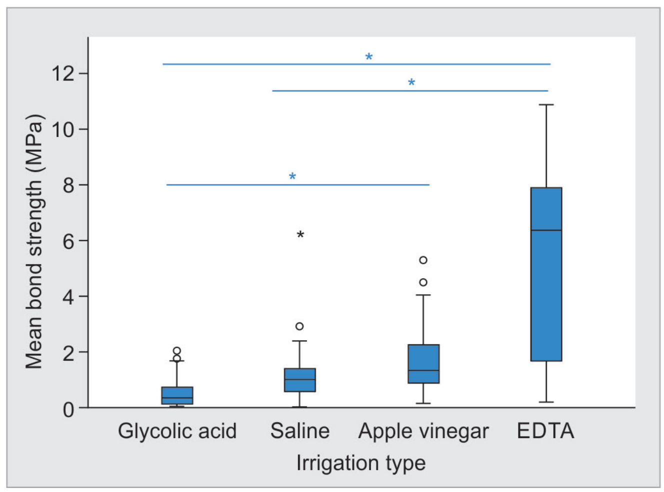
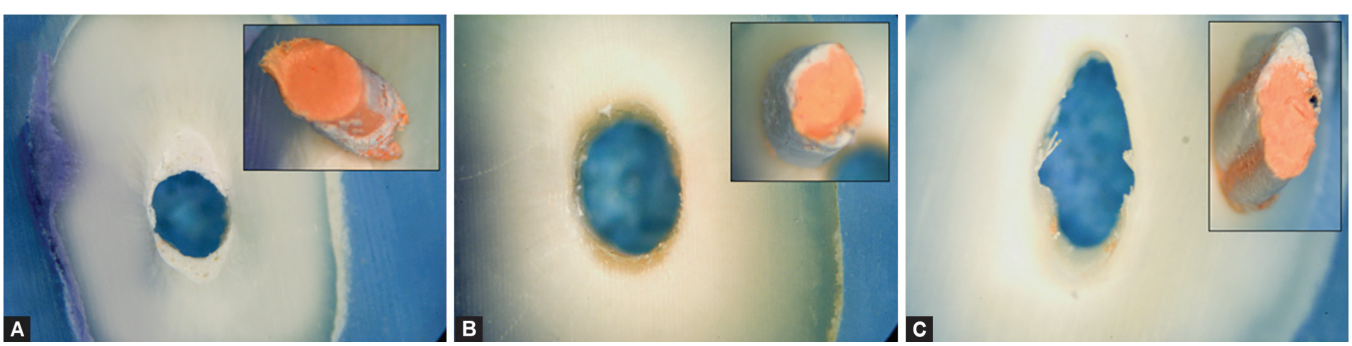


Fig. 3: The mean PBS values of the irrigation solutions for the Cerafill sealer groups (*statistically significant at $p < 0.05$)



Figs 4A to C: Stereomicrographs at 35× magnification of representative samples for each failure mode, displaying the extruded filling material after push-out test. (A) Cohesive failure: The failure within the sealer; (B) Adhesive failure: The failure occurs at the dentin/gutta-percha interface, and there is not sealer in the canal wall; (C) Mixed failure

Table 3: Comparison of the mode of failure between the types of irrigation solutions in each sealer

Sealer	*Irrigation*	*Adhesive*	*Cohesive*	*Combined*	*p-value**
EndoSequence	Glycolic acid	2 (10.0)	16 (80.0)	2 (10.0)	0.05
	Saline	6 (30.0)	9 (45.0)	5 (25.0)	
	Apple vinegar	1 (5.0)	15 (75.0)	4 (20.0)	
	EDTA	8 (40.0)	10 (50.0)	2 (10.0)	
Cerafill	Glycolic acid	12 (60.0)	5 (25.0)	3 (15.0)	0.09
	Saline	10 (50.0)	9 (45.0)	1 (5.0)	
	Apple vinegar	8 (40.0)	5 (25.0)	7 (35.0)	
	EDTA	12 (60.0)	7 (35.0)	1 (5.0)	

*****Chi-square test

5% apple vinegar have a chelating effect comparable with 17% EDTA.[13,39] Thus, in this investigation, these particular concentrations were considered.

This study examined two distinct bioceramic sealers with differing compositions. The EndoSequence BC sealer, as indicated by the manufacturers, consists of zirconium oxide, tricalcium silicate, dicalcium silicate, calcium hydroxide, and some undisclosed fillers.[26] The Cerafill BC sealer, in contrast, is composed of calcium silicates, calcium phosphates, zirconium oxide, bioactive glasses, calcium sulfate, and calcium oxide, along with unspecified fillers, accelerators, and thickening agents.[33] The sealers' compositions influence their physiochemical properties and bioactivity.[47,48]

Previous studies revealed that EndoSequence BC and Cerafill BC sealers exhibited divergent physiochemical and bioactivity characteristics.[25] They also differed in their particle size and setting times.[25] Both of these factors influence the sealers' adaptation and penetration into the dentin wall. Additionally, sealer–dentin adhesion is affected by elements like flowability and contact angle, though a direct comparison has not been made for these specific sealers.[49]

Conversely, the physical and chemical structure of the dentin surface is determined by the irrigation solution used in the cleaning and shaping of root canals.[50] Modifications to the dentin surface can impact its wettability and surface penetration by sealers.[51] The interplay between these factors related to sealers and irrigation may account for the significant effect of final irrigation and the interaction between sealer and final irrigation on the PBS observed in this study.

The current results indicated that using EndoSequence sealer yields the highest PBS when the canals are lastly irrigated with apple vinegar. This was followed closely by the group using EDTA for final irrigation. Notably, the difference between these two groups was statistically insignificant. Conversely, when using Cerafill sealer, the greatest PBS was achieved when EDTA was the final irrigant, followed by apple vinegar. The superior PBS of the EDTA group can be credited to EDTA's capability to eliminate the smear layer and expose the root dentin, enhancing contact between the bioceramic sealers and root dentin wall.[52–54] The bioceramic sealer permeates open dentinal tubules and forms tag-like microstructures. Over time, these tags solidify the sealer within them, providing mechanical retention at the interface.[34,55] Numerous studies have reported the formation of the mineral infiltration zone (MIZ) beneath the bioceramic sealer layer and ascribed it to hydroxyapatite deposition.[56] As for apple vinegar, this study also found high PBSs for bioceramic sealers when irrigation involved apple vinegar. This outcome, statistically equivalent to the EDTA group, held for both sealers. The chelating effect of apple vinegar results from its H^+ ion content. Solutions with higher H^+ ion concentrations deliver more effective chelation.[57] Previous studies evaluating apple vinegar, alone or combined with EDTA, for smear layer removal, have corroborated these findings.[16,57,58] Both standalone apple vinegar and its combination with EDTA are effective in smear layer removal when used as endodontic irrigating solutions. In accordance with the results of the present study, previous researchers have reported a comparable PBS of apple vinegar to that of EDTA when used as a final irrigating solution.[59] However, in controversial to our results, other study has reported higher values of PBS of EDTA final irrigating solution compared with that of apple vinegar.[60] This controversy may be attributed to different experimental designs and different sealers used.

Glycolic acid was the other final irrigation solution examined. It has been found to remove smears as effectively as EDTA.[13] It has an acidic pH, which demineralizes dentin, thus providing tags for sealer micromechanical retention.[13] As GA concentration increased, Bello et al. observed a decrease in the apatite/collagen ratio.[13,14] Despite this, the present study found the PBS of bioceramic sealers to be lower after GA and saline irrigation, irrespective of the sealer used. This could be explained by the chelating effect of GA interfering with calcium silicate hydration. Depletion of calcium at the sealer–dentin interface or a reduction in the calcium silicate fraction in the sealer may obstruct the formation of a MIZ; this could result in a less effective interaction between the sealer and root dentin.[56] Additionally, GA could potentially interfere with the structure of dentin collagen, thereby compromising sealer–dentin bonding.

Our findings on the final irrigation with GA differ from previous studies.[35,36] Küçükekenci and Veeramachaneni et al. assessed the impact of GA final irrigation on the PBS of bioceramic sealers, reporting an increased PBS after GA irrigation compared with EDTA.[35,36] However, since the materials tested were considerably different and the experimental designs varied significantly, a direct comparison with other similar PBS studies is not appropriate.

The push-out strength was low for each saline group tested, demonstrating the negative impact of the residual smear layer on PBS.[36,61]

The failure mode analysis of the specimens indicated that cohesive failure was the primary mode in EndoSequence sealer groups, regardless of the chelating agent used. Huffman et al.'s findings support this, showing that a calcium silicate-based sealer also demonstrated cohesive failure after a 7-day storage period.[62] Shokouhinejad et al. and Carvalho et al. further corroborated these findings, reporting a cohesive failure mode for bioceramic sealers after a 7-day incubation period.[63,64] However, when comparing this failure mode data with results from other studies where different experimental settings were used, one must exercise caution.

On the contrary, our current results revealed that the Cerafill sealer groups predominantly exhibited adhesive failure, irrespective of which chelating agent was used. This variation in failure modes between the EndoSequence and Cerafill sealers might be accounted for by their differences in composition, particle size, and physiochemical properties. These differences contribute to the variation in the sealers' ability to flow into dentin and their degree of interaction and adhesion to sealer–dentin.[65]

This study had several limitations. Firstly, the push-out test, as an *in vitro* examination, does not accurately represent the real-world forces that root canal fillings and sealers encounter in the mouth. Future studies should measure the bond strength of these fillings and sealers to dentin under multidirectional forces that emulate actual chewing pressure. A further limitation of this study was that tests were only carried out on dentin disks from the middle region of the root. As tubular density varies along the length of the root, it would be beneficial to test other root regions in subsequent research.

Conclusion

Within the context of this *in vitro* study, as a chelating agent in endodontic treatment, apple vinegar solution led to comparable PBS to that of EDTA regardless of the BC sealers used. Moreover, apple vinegar solution PBS was higher than GA and saline groups regardless of the BC sealers used. Thus, an apple vinegar solution may serve as an alternative chelating agent in final irrigation during endodontic treatment; however, this requires further examination.

Ethical Approval

The research project was approved by the King Abdulaziz University's Ethics Committee (#065-03-23).

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