RDE Restorative Dentistry & Endodontics

Research Article

eISSN 2234-7666
Restor Dent Endod. 2025 May;50(2):e16
https://doi.org/10.5395/rde.2025.50.e16

The effect of limonene extract on the adhesion of different endodontic cements to root dentin: an *in vitro* experimental study

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ABSTRACT

Objectives: The study aimed to evaluate the effect of limonene extract (LE) on push-out bond strength (BS) to root dentin in endodontically treated teeth.
Methods: Single-rooted teeth were selected and instrumented using the reciprocating technique, then divided into three groups based on the final irrigating solution: 2.5% sodium hypochlorite (NaOCl), 17% ethylenediaminetetraacetic acid (EDTA), and 5% LE. The roots were further divided ($n = 12$) and obturated using the single-cone technique with epoxy resin-based (ERB) or bioceramic sealer (Bio-C). After 3 days, the roots were sectioned into 2-mm slices, obtaining two slices from each root third. Push-out BS testing was conducted at 0.5 mm/min, followed by failure pattern and adhesive interface analysis using scanning electron microscopy. Push-out BS data were analyzed by three-way analysis of variance and Tukey *post-hoc* test ($p < 0.05$).
Results: ERB showed higher BS when irrigated with EDTA (5.0 ± 2.3 MPa) compared to NaOCl (1.8 ± 1.1 MPa) ($p = 0.0005$), particularly in the cervical third. LE yielded intermediate values without significant differences from the other irrigants (3.5 ± 1.9 MPa) ($p > 0.05$). For Bio-C, the highest BS was observed in the apical third, especially with LE (9.4 ± 5.0 MPa), differing from other thirds and final irrigating solutions ($p < 0.05$). Mixed failure patterns were most prevalent, regardless of the irrigant solutions.
Conclusions: The combination of LE with Bio-C demonstrated superior BS in the apical third, suggesting its potential as a final irrigating solution in endodontic treatments.

Keywords: Biocompatible materials; Dental cements; Dentin; Limonenes; Root canal irrigants; Tensile strength

Received: September 12, 2024 **Revised:** January 21, 2025 **Accepted:** March 28, 2025

Citation
Aguiar NLF, Soares EJ, Santos GNA, Pimenta ALA, Romano LK, Silva RG, Panzeri FC. The effect of limonene extract on the adhesion of different endodontic cements to root dentin: an *in vitro* experimental study. Restor Dent Endod 2025;50(2):e16.
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INTRODUCTION

Endodontic treatment is based on the biomechanical cleaning of the root canal system (RCS), involving the physical-chemical removal of inflamed and necrotic pulp tissue using instruments, along with irrigation, and obturation of the RCS [1]. The success of endodontic therapy relies on the elimination of microorganisms from the RCS and the prevention of reinfection. The objectives of instrumentation are to facilitate irrigation, cleaning, and shaping of the RCS, as well as three-dimensional obturation [2].

During and after instrumentation, irrigators facilitate the removal of microorganisms and the smear layer from the root canal by means of a flushing mechanism. Irrigators can also contribute to preventing the compaction of hard or softened tissue in the apical third, as well as to avoid debris extrusion into the periapical region [2]. Currently, instrumentation is widely regarded to provide access to apical anatomy for irrigant, which should perform most of the cleaning and shaping [3,4].

The most widely used irrigant is sodium hypochlorite (NaOCl), which is a highly destructive, nonselective oxidant that readily reacts with biomolecules, giving it the ability to dissolve biofilm components and necrotic debris [4]. Additionally, it contributes to reducing bacterial virulence factors such as endotoxins and lipoteichoic acids and serves as a lubricant for endodontic instruments [5].

NaOCl is used in dentistry as an irrigating solution in endodontic treatments, with its effect attributed to the free available chlorine (OCl^- and HOCl), which are strong oxidants whose properties depend on pH. The ideal concentration of NaOCl ranges from 0.5% to 8.25% [5]. Its limitations include cytotoxicity, inability to completely remove the inorganic smear layer, and adverse effects on the mechanical properties of roots, such as dentin brittleness at high concentrations [3,6].

Demineralizing or chelating agents, such as ethylenediaminetetraacetic acid (EDTA), constitute another group of endodontic irrigants. These agents bind to and form ring-shaped complexes with metal ions, allowing for the effective dissolution of inorganic material. EDTA does not affect organic tissue and has limited antimicrobial effects by chelating metal cations from the outer membrane of bacteria [2,7].

Monoterpenes are secondary plant metabolites commonly used as antiseptics [8]. Limonene, a type of monoterpene, is a monocyclic compound found in citrus oils, such as those from oranges, grapefruits, and lemons. It is widely used in perfumes, soaps, pharmaceuticals, and foods due to its citrus scent. Additionally, limonene is employed in medical practices [9–12].

Limonene, an organic compound found in citrus oils, has been studied in dentistry for its potential as a treatment for dental caries. A study has shown that limonene may have a stronger inhibitory effect on *Streptococcus sobrinus* than chlorhexidine and can potentially inhibit the progression of caries [10]. This compound can inhibit the growth of cariogenic bacteria, their adhesion, acid production, and other cariogenic virulence factors such as insoluble glucan [11,12]. Additionally, limonene exhibits antimicrobial, anti-inflammatory, anticarcinogenic, and anticariogenic activities. It is considered safe with low toxicity, and its lipophilicity allows for good cellular absorption, particularly in the intestines, providing good bioavailability in systemic circulation [8,10]. Studies have also demonstrated that d-limonene has relevant clinical activity against both gram-positive and gram-negative bacteria [13,14].

The aim of this *in vitro* study was to evaluate the effect of using limonene extract (LE) as a final irrigating solution during endodontic treatment in teeth obturated with different root canal sealers, namely epoxy resin-based (ERB) and bioceramic (Bio-C), regarding push-out bond strength (BS) and failure pattern after push-out using a stereomicroscope. The null hypotheses tested were that irrigation and obturation with different compositions do not interfere in the BS of endodontically treated teeth and in the failure pattern between the gutta-percha, the root canal sealer, and the intraradicular dentin.

METHODS

The sample size was calculated based on a pilot study, comparing means of push-out BS among the treatments and using OpenEpi app (www.openepi.com), with a 95% confidence interval and power of 80%. The minimum estimated number of specimens was 11 for each

https://doi.org/10.5395/rde.2025.50.e16

group; however, considering eventual sample loss, 12 specimens were included in each group.

This study used human tooth roots, such as maxillary and mandibular premolars, which were obtained from the teeth bank of the institution, where it was reviewed and approved by the Institutional Ethics Committee of Ribeirao Preto School of Dentistry (approval No. 67552923.8.0000.5419). The extracted teeth were initially stored at 4°C in 0.1% thymol solution until use to preserve their integrity during storage [15]. Following endodontic treatment, the teeth were transferred to artificial saliva at 37°C, mimicking the body's natural temperature to simulate clinical conditions prior to sectioning.

They were macroscopically analyzed and scanned using a cone-beam computed tomography scanner PreXion 3D (Prexion Co. Ltd, Tokyo, Japan), with an endodontic acquisition protocol of 90 kV, 4 mA, 37 seconds exposure time, isotropic voxel size of 0.10 mm, and a field of view of 5 × 5 mm. Two-dimensional morphometric data, including circularity and major and minor diameters, were obtained using the OnDemand 3D Project Viewer software (Cybermed Inc., Tustin, CA, USA) to determine the degree of root canal flattening [16–19]. Thus, 72 roots with oval-shaped root canals (major diameter/minor diameter ratio between 1 and 2), vestibulolingual/mesiodistal dimension ≤1.5 mm, and 16-mm root length were selected.

After sample selection, the root canals were irrigated with 2.5 mL of 2.5% NaOCl (Coltene/Whaledent AG, Altstatten, Switzerland) using a 21-mm disposable plastic syringe with a 27-gauge tip (Ultradent Products Inc., South Jordan, UT, USA) and explored with a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until the tip of the instrument emerged at the apical foramen. From this measurement, 1.0 mm was subtracted to establish the working length [19,20].

The biomechanical preparation was performed using the reciprocating motion instrumentation technique with WaveOne Gold files Medium 35.06 (Dentsply Maillefer), with torque and speed preset by the manufacturer. The final irrigation protocol was divided according to the final irrigating solution used for this purpose, into groups with 2.5 mL of 2.5% NaOCl, 2.5 mL of 17% EDTA (CanalPro EDTA; Coltene/Whaledent AG), and 2.5 mL of 5% LE (Sigma-Aldrich, St. Louis, MO, USA) (Table 1). The LE was diluted in distilled water to achieve the tested concentration (5%). For this, 0.5 mL of limonene was added to 95 mL of distilled water. Subsequently, 2.5 mL of the solution was used for the final irrigation of the root canal. A fresh limonene solution was prepared for each use. The irrigation process involved continuous flushing of the root canal with the solution for 5 minutes after instrumentation was completed. Canal drying was accomplished using a Capillary Tip aspiration cannula (Ultradent Products Inc.) and 35.06 absorbent paper points (Dentsply Maillefer).

The canals were obturated using the single-cone technique with WaveOne Gold Conform Fit Medium 35.06. The groups were divided into two subgroups: one obturated with ERB sealer (AH Plus; Dentsply De Trey, Konstanz, Germany) and the other with Bio-C Sealer (Angelus, Londrina, Brazil) (Table 2). The roots were stored in an incubator at 37°C for 3 days. All procedures, including biomechanical preparation and obturation, were performed by the same operator.

To obtain slices for the push-out BS test, the roots were embedded in self-curing acrylic resin (Jet; Clássico, São Paulo, Brazil) and adapted to a metallographic cutter (Isomet 1000; Buehler, Lake Bluff, IL, USA) to be sectioned perpendicularly to their long axis in the mesiodistal direction. A double-sided diamond disc with a thickness of 0.3 mm was used, operating at a speed of 350 rpm under continuous cooling.

From each root third, two dentin slices, each 2.0 mm (±0.2 mm) thick, were obtained, totaling six slices per

Table 1. Irrigating solutions with commercial name, manufacturer, and composition

Solution	Commercial name	Manufacturer	Composition
Limonene	Pro Lyks - Natural	Hydroplan EB, São Paulo, SP/Brazil	Citrus sinensis peel oil expressed
Sodium hypochlorite	NaOCl	Asfer Chemical Industry. São Caetano do Sul – SP/Brazil	2.5% Sodium hypochlorite base
Ethylenediaminetetraacetic acid	EDTA	Chemical and Pharmaceutical Biodynamics LTDA. Ibiporã – PR/Brazil	Disodium ethylenediaminetetraacetic acid, sodium hydroxide, and deionized water

Table 2. Endodontics root canal sealers

Material	Mainly compounds	Lot	Manufacturer
Epoxy resin-based: AH Plus	Paste A: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments	210400657	Dentsply De Trey, Konstanz, Germany
	Paste B: dibenzylamine, aminoadamantane, tricyclodecane-diamine, calcium tungstate, zirconium oxide, silica, silicone oil		
Bioceramic: Bio-C Sealer	Calcium silicate, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, and dispersing agent.	64766	Angelus, Londrina, Brazil

root for the push-out test and analysis of the adhesive interface using scanning electron microscopy (SEM).

Push-out bond strength test

The slices were positioned on stainless steel metal bases attached to the bottom portion of the universal testing machine model 2519-106 (Instron, Canton, MA, USA). Depending on the diameter of the root canal filling material in the cervical, middle, and apical thirds, metal bases with holes of 1.2-, 1.5-, and 2.5-mm diameter in their central portion were selected, along with metal rods with active tips of 0.8-, 1-, and 1.5-mm diameter. The specimens were positioned in the same direction as the hole in the metal base, with their cervical face facing downwards, and the rods were fixed to the upper portion of the testing machine and positioned over the intracanal material. The testing machine was operated at a constant speed of 0.5 mm/min until the maximum tension required for displacement of the material was reached [18–21].

The force required for displacement was measured in Newtons (N). To calculate the BS, the resulting force was converted into megapascals (MPa) by dividing it by the lateral area (LA) of the intracanal material. For the accurate calculation of the adhered LA, the geometric aspect of the intracanal material (sealer + gutta-percha) was considered based on the level of the slice obtained. For this purpose, before the test, the height of each slice was measured using a digital caliper, as well as the radius (major and minor) using a Leica M165C stereomicroscope (Leica Microsystems, Mannheim, Germany) with Las ver. 4.4 software (Leica Microsystems).

Thus, the area of adhesion of the sealer (in mm^2) was calculated using the formula for lateral area (LA):

$$\mathrm{LA} = \pi\,(R + r)\,\sqrt{(h^2 + [(R - r)]^2)}$$

In this formula, "R" is the measurement of the radius of gutta-percha and sealer in its coronal portion, "r" is the measurement of the radius of gutta-percha and sealer in its apical portion, and "h" is the height/thickness of the slice. From these data, the BS in MPa was calculated by dividing the force required for the displacement of the gutta-percha by its lateral area (BS = F/LA).

For the analysis of failure type, the slices were evaluated using the Leica M165C stereomicroscope at 25× magnification and the Las ver. 4.4 software. The observed failures were determined as percentages and classified as follows: (a) adhesive to dentin: when the obturation material detached from the dentin; (b) adhesive to sealer: when the gutta-percha detached from the sealer; (c) mixed: when the obturation material detached from both the dentin and the sealer; (d) dentin cohesive: when dentin fracture occurred; and (e) gutta-percha cone cohesive: when the gutta-percha cone fractured.

Scanning electron microscopy

For analysis by SEM, the second dentin slice from each root third (cervical, middle, and apical) was used. Preparation for SEM involved polishing the dentin specimens with progressively decreasing water sandpaper up to 1,200 grit. Subsequently, the specimens were rinsed in distilled water and superficially decalcified in 6-M hydrochloric acid (HCl) for 30 seconds and deproteinized in 2% NaOCl for 10 minutes. Afterward, the specimens were rinsed, dehydrated, and fixed onto aluminum cylindrical structures (10 × 10 mm) using double-sided adhesive tape as described in previous studies [19–23]. Following vacuum metallization, the specimens were analyzed using a scanning electron microscope (JSM 5410; JEOL Ltd., Tokyo, Japan) operating at 20 kV.

Data analysis

The BS data were initially analyzed for normal distribu-

https://doi.org/10.5395/rde.2025.50.e16

tion using the Shapiro-Wilk test ($p > 0.05$) and homogeneity using the Levene test, showing normal distribution and homogeneity.

The parametric three-way analysis of variance (ANOVA) was used to analyze the influence and interaction among final irrigating solutions (NaOCl, EDTA, and LE), root canal sealers (ERB and Bio-C), and root thirds (cervical, middle, and apical). The Tukey *post-hoc* test was used for multiple comparisons with a significance level of $p < 0.05$.

To analyze the prevalence of failure patterns found after the push-out BS test, the Fisher exact test was performed.

RESULTS

Bond strength

The mean and standard deviation values obtained from the push-out BS test for the variables of root canal sealers, final irrigating solutions, and root thirds are presented in Table 3.

The three-way ANOVA (Tukey, $p < 0.05$) showed that, for the ERB sealer, there was no significant difference in the BS of the sealer to dentin among the analyzed thirds ($p > 0.05$). Regarding the Bio-C sealer, there was a higher BS of the sealer when treated with LE in the apical third, a statistically different result ($p > 0.05$) compared to the other thirds, which did not differ from each other ($p > 0.05$). The other irrigating solutions did not result in BS differences between them ($p > 0.05$), regardless of the analyzed third.

Analyzing the BS of the root thirds in relation to the irrigating solutions performed for each of the root canal sealers, it was found that the ERB sealer exhibited higher BS to cervical dentin when treated with EDTA (5.0 ± 2.3 MPa). This result was statistically significantly different ($p = 0.0005$) compared to the irrigating solution with NaOCl (1.8 ± 1.1 MPa), which resulted in the lowest BS values among all solutions.

The irrigating solution with LE yielded intermediate values in relation to the other irrigating solutions, with no statistically significant difference compared to them ($p > 0.05$). Regarding the Bio-C sealer, there was a difference ($p < 0.0001$) in the BS to dentin in the apical third, such that after irrigating solution with LE, there was a higher BS (9.4 ± 5.0 MPa). This result was statistically different compared to the other irrigating solutions ($p > 0.0001$). NaOCl and EDTA did not show statistically significant differences among themselves ($p = 0.282$).

When comparing the BS of different sealers to dentin treated with the same type of irrigating solution and in the same third, it was found that, when dentin was treated with LE, the Bio-C sealer exhibited higher BS (9.4 ± 5.0 Mpa) in the apical third, with a statistically significant difference from the ERB sealer (2.2 ± 1.5 Mpa) ($p < 0.001$).

When the dentin was irrigated with EDTA, the ERB sealer exhibited higher BS (5.0 ± 2.3 MPa) in the cervical third, with a statistically significant difference ($p = 0.0008$) when compared to the Bio-C sealer (1.9 ± 1.5

Table 3. The bond strength (MPa) of endodontic root canal sealers to root dentin treated with different irrigating solutions in the cervical, middle, and apical thirds

Root canal sealer	Final irrigating solution		
	Limonene extract	EDTA	NaOCl
ERB			
Cervical third	3.5 ± 1.9^{abA}	$5.0 \pm 2.3^{aA\#}$	1.8 ± 1.1^{bA}
Medium third	2.5 ± 1.0^{aA}	3.4 ± 2.1^{aA}	1.4 ± 0.6^{aA}
Apical third	2.2 ± 1.5^{aA}	3.3 ± 2.1^{aA}	1.9 ± 0.9^{aA}
Bio-C			
Cervical third	2.4 ± 1.6^{aB}	$1.9 \pm 1.5^{aA*}$	1.4 ± 0.5^{aA}
Medium third	2.6 ± 1.9^{aB}	1.5 ± 0.9^{aA}	1.9 ± 1.4^{aA}
Apical third	$9.4 \pm 5.0^{aA*}$	1.4 ± 0.9^{bA}	3.0 ± 2.1^{bA}

Values are presented as mean ± standard deviation.
Bio-C, bioceramic; EDTA, ethylenediaminetetraacetic acid; ERB, epoxy resin-based; NaOCl, sodium hypochlorite.
Lowercase letters in the row and uppercase letters in the column, for the same sealer, indicate statistically significant differences ($p < 0.05$). Different symbols within the same final irrigating solutions and root third indicate statistically significant differences between sealers.

MPa). All other final irrigating solutions did not show statistically significant differences ($p > 0.05$), regardless of the root canal sealer used or the third analyzed.

Fracture pattern analysis

The results of the Fisher exact test analysis and the percentage data of the distribution of failure types for each group are described in Tables 4 and 5. The Fisher exact test demonstrated that, regardless of the root canal sealer used, there was a higher percentage of mixed failures to dentin compared to other types of failures. Analyzing the final irrigating solutions, it was found that, regardless of the root canal sealer used, the use of EDTA and LE resulted in almost 30% cohesive fractures to dentin and around 2% of adhesive fractures. Additionally, when treated with NaOCl, no adhesive fractures occurred, indicating that the irrigating solution is a significant factor for the prevalence of the fracture pattern with this type of sealer (ERB, $p = 0.020$; Bio-C, $p < 0.001$).

Analysis of the adhesive interface using scanning electron microscopy

The statistical analysis observed in the evaluation of the experimental groups can be confirmed by the qualitative analysis of the SEM images (Figure 1), which allowed the observation of areas of misfitting (yellow arrows) and adaptation at the adhesive interface between the gutta-percha cone, root canal sealers, and root dentin in the different irrigating solution types (NaOCl, EDTA, LE).

DISCUSSION

This study evaluated the BS and adhesive interface analysis of dentin irrigating solutions with LE, EDTA, and NaOCl as final irrigating agents in teeth filled with REB (AH Plus) and Bio-C (Bio-C Sealer). According to the results, the null hypothesis cannot be accepted, as teeth treated with LE showed higher BS values in the apical third compared to the other final irrigating solutions.

Prior to tooth selection, a thorough analysis of the teeth was conducted by evaluating images obtained from cone-beam computed tomography [16]. Root canal anatomy may exhibit varied cross-sectional shapes within the same dental group, depending on the ratio between buccolingual and mesiodistal dimensions. This analysis for sample selection is necessary because the single-cone technique may have limitations, especially in oval canals, regardless of the root canal sealer used. The sample was standardized by selecting circular

Table 4. Result of the Fisher exact test analysis of fracture pattern prevalence in relation to root canal sealers and final irrigating solution factors

Comparison group	χ^2 value	df	p-value (χ^2)	p-value (Fisher exact test)	Number of specimens
Failure pattern × ERB	11.9	6	0.064	0.020	232
Failure pattern × Bio-C	20.7	6	0.002	<0.001	234
Failure pattern × Total	22.3	6	0.001	<0.001	466

Bio-C, bioceramic; df, degree of freedom; ERB, epoxy resin-based.

Table 5. Failure rate following the push-out bond strength test for different root canal sealers and dentin irrigating solution

Root canal sealer	Failure pattern	LE (%)	EDTA (%)	NaOCl (%)
ERB	AD	1.4	1.3	0.0
	AC	0.0	1.3	0.0
	CD	12.8	29.5	14.5
	M	85.9	69.2	85.5
Bio-C	AD	0.0	1.3	0.0
	AC	2.6	3.9	0.0
	CD	9.0	26.9	36.8
	M	88.5	68.0	67.1

AD, adhesive to dentin; AC, adhesive to sealer; Bio-C, bioceramic; CD, cohesive in dentin; EDTA, ethylenediaminetetraacetic acid; ERB, epoxy resin-based; LE, limonene extract; M, mixed; NaOCl, sodium hypochlorite.

https://doi.org/10.5395/rde.2025.50.e16

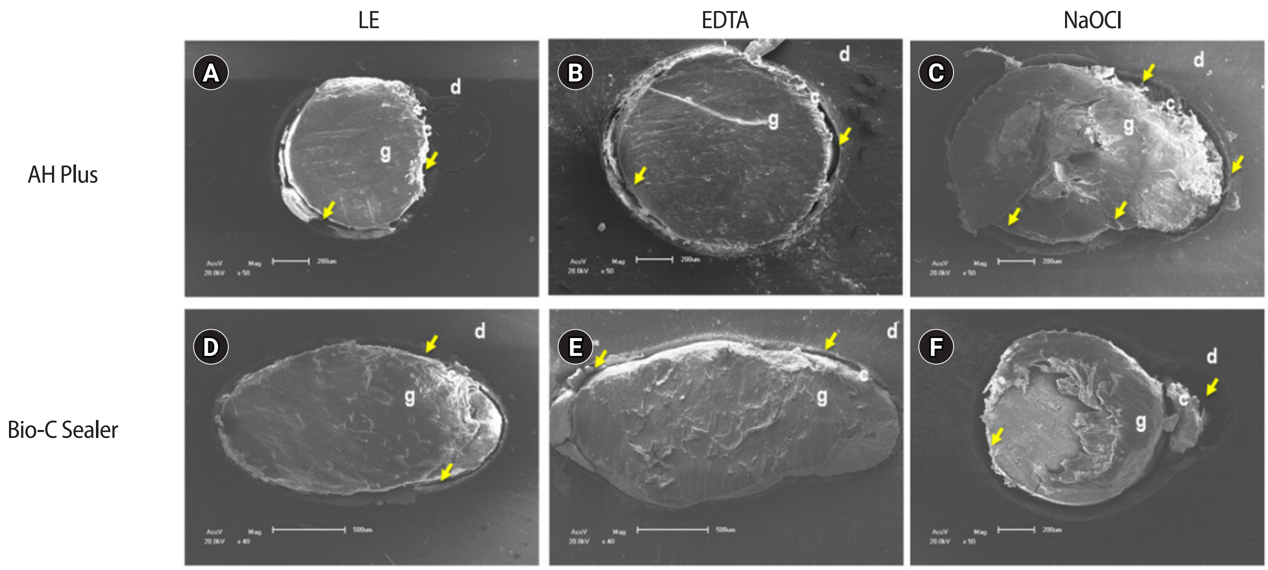

Figure 1. Areas of misfitting (yellow arrows) and adaptation at the adhesive interface between the gutta-percha cone (g), root canal sealer (c), and root dentin (d) in the different final irrigating solution types (limonene extract [LE], ethylenediaminetetraacetic acid [EDTA], and sodium hypochlorite [NaOCl]). For the epoxy resin-based sealer group (AH Plus; Dentsply De Trey, Konstanz, Germany) treated with LE (A) and EDTA (B), and Bio-C sealer group (Bio-C Sealer; Angelus, Londrina, Brazil) treated with LE (D) and EDTA (E), it is possible to observe adaptation at the adhesive interface, with integrity of the obturation margins and a lower percentage of gaps. In NaOCl, regardless of the experimental group, sealer predominance can be observed in the polar areas (C and F) with areas of misfitting.

root canals (ratio equal to 1) of single-rooted teeth to minimize bias risk.

Root canal irrigation in endodontic treatment is one of the steps in dentin preparation for the removal of the smear layer and intracanal disinfection. Different irrigation techniques are developed, studied, and observed in the literature, effectively removing debris and microorganisms from the root canals and thereby assisting in the adhesion between root canal sealers, gutta-percha, and root dentin [20–22,24].

In the present study, there was no statistically significant difference ($p > 0.05$) among the root canal sealers, except for the results of the LE groups in the apical third and EDTA groups in the cervical third. The use of LE for final irrigation followed by root canal obturation with ERB sealer yielded BS values like those obtained with Bio-C sealer, except in the apical third, where LE showed higher BS values with a mixed failure pattern in the obturation material. SEM photomicrographs allowed for the observation of a more uniform and thinner layer of endodontic root canal sealer at the adhesive interface.

In the remaining thirds, the use of LE did not lead to a decrease in BS compared to the other final irrigating solutions, resulting in values without statistically significant differences between them ($p > 0.05$). Furthermore, the analysis of fracture patterns showed that this irrigating solution resulted in a low prevalence of the number of adhesive fractures, with nonadhesive fractures being the most prevalent, demonstrating good bonding at the tooth/sealer interface. The use of EDTA for final irrigation, followed by obturation with ERB sealer and Bio-C sealer, showed BS without statistically significant differences with a mixed failure pattern. SEM photomicrographs allowed for the observation of a uniform and thin layer of ERB sealer and Bio-C sealer on the obturation material and the presence of gaps at the adhesive interface, with greater penetration into the dentinal tubules occurring regularly and homogeneously.

Dentin irrigating solution with NaOCl may result in lower BS values with a mixed failure pattern in the obturation material. This can be explained by the formation of an oxygen layer on the dentin surface, resulting from the cleavage of NaOCl into chlorine and oxygen

[25]. Studies support that NaOCl did not demonstrate a positive effect on adhesion to root dentin, which may increase tensions and generate larger gaps at the adhesive interface, resulting in less uniform root canal sealer penetration and more void spaces. Nonetheless, NaOCl is considered an endodontic irrigating solution with antibacterial effects and the ability to dissolve organic tissues [26–29]. According to a previous study [30], dentin samples treated with LE showed smear layer removal and open dentinal tubules. Considering this, the higher BS results of LE suggest an efficient removal of the smear layer from root dentin.

D-limonene, a monoterpene abundant in citrus essential oils, has attracted attention in dentistry for its low toxicity, sustainability, and diverse biological properties, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and anticariogenic effects [11]. Its antioxidant activity neutralizes reactive oxygen species (ROS), reducing oxidative stress and protecting cells, while its anti-inflammatory effects involve the inhibition of cytokines and prostaglandins via cyclooxygenase-2 and nuclear factor kappa-B pathways. Additionally, it disrupts microbial membranes and inhibits biomolecule synthesis in certain pathogens. Although widely studied in medicine for conditions like cancer and respiratory issues, its potential in restorative dentistry lies in its ability to inhibit matrix metalloproteinases through ROS scavenging, potentially improving restoration longevity [31].

Despite the promising results observed for the use of limonene as a final irrigating solution, the study is limited by having been conducted on teeth without microbiological contamination caused by caries progression. Therefore, further studies are needed to evaluate the effect of limonene on contaminated teeth.

CONCLUSIONS

This *in vitro* study concludes that combining LE with Bio-C sealer results in superior BS in the apical third of the root, while BS in the cervical and middle thirds is consistent across different root canal sealers when LE is used for final irrigation. The most common failure type between sealer and dentin was mixed, regardless of the irrigation method and root canal sealer type. Additionally, final irrigation with EDTA and LE enhances the adaptation of the adhesive interface, irrespective of the root canal sealer type used.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

FUNDING/SUPPORT

The authors have no financial relationships relevant to this article to disclose.

AUTHOR CONTRIBUTIONS

Conceptualization: Aguiar NLF, Panzeri FC. Methodology, Investigation: Aguiar NFL, Santos GNA, Romano LK. Validation, Formal analysis: Panzeri FC. Soares EJ. Supervision, Project Administration: Silva RG. Writing - original draft: Soares EJ, Santos GNA, Pimenta ALA. Writing - review & editing:

DATA SHARING STATEMENT

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

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https://doi.org/10.5395/rde.2025.50.e16