



Spectrophotometric evaluation of apical microleakage of three different endodontic sealers

Selma Jakupović¹, Amir Čaušević^{2*}, Adnan Šehić³, Anita Bajzman⁴, Vedran Jakupović⁵, Sabina Šegalo⁶, Fuad Julardžija³

¹Department of Restorative Dentistry and Endodontics, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, ²Department of Sanitary Engineering, Faculty of Health Studies, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, ³Department of Radiological Technology, Faculty of Health Studies, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, ⁴Department of Dental Morphology with Dental Anthropology and Forensics, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, ⁵General Department, Faculty of Health Studies, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, ⁶Laboratory Technology Department, Faculty of Health Studies, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

ABSTRACT

Introduction: The main purpose of root canal filling materials is to provide an apical sealing that prevents the movement of bacteria and the diffusion of their metabolic products from the root canal system to periapical tissue. The aim of this study was to evaluate the apical sealing ability of mineral trioxide aggregate's (MTA), epoxy resin, and zinc oxide eugenol-based sealer with single cone obturation techniques using the dye extraction leakage method.

Methods: Seventy-five extracted human single-rooted teeth were instrumented using the crown-down technique with the Mtwo rotary file system to file 25/6. The teeth were randomly divided into five experimental groups (n = 15); Group 1 obturated with AH plus, Group 2 obturated with MTA BIOSEAL, Group 3 obturated with Endoseal, Group 4 positive control (POS CTRL) root canals are processed but not obturated, and negative control- apex of teeth completely covered with nail varnish, canals are not treated or obturated. In the first three groups, the single cone obturation technique with gutta-percha 25/6 was used. Apical microleakage was assessed with the dye extraction method, where the absorbance of 2% methylene blue was measured using a spectrophotometer.

Results: The mean dye concentration values were MTA 22.79, AH plus 31.16, Endoseal 36.67, POS CTRL 280.15, negative control 9.01. Analysis of variance and pairwise comparisons indicated a significant difference in apical microleakage between the investigated groups (F = 3448, p < 0.001). The MTA BIOSEAL showed significantly less apical microleakage than the AH plus and Endoseal. The AH plus showed less apical microleakage than the Endoseal, although there was no significant difference between these two sealers.

Conclusion: With the limitation of the study, it was concluded that MTA sealers show statistically less apical leakage than AH Plus and Endoseal.

Keywords: Mineral trioxide aggregate's BIOSEAL; AH plus; Endoseal; dye extraction; spectrophotometry; single cone; endodontic sealer

INTRODUCTION

The primary objective of root canal filling materials is to establish an apical seal that inhibits bacterial penetration and the diffusion of their metabolic by-products from the root canal system into the periapical tissues (1-3).

The long-term efficacy of root canal therapy is predominantly attributed to comprehensive chemomechanical decontamination of the root canal system, alongside the durable sealing of both coronal and apical regions using a

core material and root canal sealer (RCS) that demonstrate dimensional stability and biocompatibility (4-6).

Gutta-percha is the most widely used core material, occupying the majority of the canal space, whereas the RCS fills the interface between the core material and the dentinal wall (7).

A large number of endodontic sealers are available on the market today, with different compositions and physical and chemical characteristics. According to Grossman's suggested ideal requirements for endodontic sealers, achieving three-dimensional obturation necessitates that the sealer be insoluble in tissue fluids, possess antibacterial properties, and demonstrate adequate flow into the dentinal tubules upon application (8). RCSs are classified according to their chemical composition, encompassing categories, such

*Corresponding author: Amir Čaušević, Department of Sanitary Engineering, Faculty of Health Studies, University of Sarajevo, Bardakčije 1, 71000 Sarajevo, Bosnia and Herzegovina, E-mail: amir.causevic@fzs.unsa.ba

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as zinc oxide eugenol-based, calcium hydroxide-based, glass ionomer-based, resin-based, and bioceramic formulations (9).

In certain cases, endodontic treatment may fail despite thorough cleaning, shaping, disinfection, and obturation of the canal system. Such failures are often attributed to the persistence of bacteria in apical regions inaccessible to treatment procedures. In addition, procedural errors during instrumentation – including ledge formation, perforations, instrument separation – along with canal calcifications and anatomical variations, can compromise effective debridement and shaping of the root canal system, ultimately resulting in treatment failure (10).

Every clinician strives to achieve the best outcome of endodontic therapy. Comprehensive knowledge of root canal morphology, together with a thorough understanding of the complexity of the root canal system, is of critical importance (11). Clinicians must also be aware of possible morphological variations, such as a single canal dividing into two branches that subsequently merge into one again (12,13).

The adhesion of root canal filling materials to dentinal walls plays a crucial role, both from a static and a dynamic perspective. Statically, any space that would allow fluid percolation between the filling material and the dentinal wall should be eliminated. Dynamically, material displacement due to consequent manipulation should be resisted (14).

Apical microleakage is leakage along the interface between the filling material and the canal wall (15). It is considered the most common cause of endodontic therapy failure. It can be caused by a whole range of variables, such as different filling techniques, the chemical and physical properties of the root canal filling material, and the presence or absence of a smear layer (16,17).

Immersion of teeth in different dyes (eosin, methylene blue, black India ink, Procion brilliant blue, etc.) was first described by Grossman in 1939. Today, it is perhaps the most widely used method for assessing apical leakage, due to the simplicity of its implementation. In this method, the phenomenon of capillarity is of crucial importance. Method is passive – the apex of the tooth is immersed in a dye that penetrates any space between the canal walls and the filling material (18).

Numerous studies have investigated the sealing ability of different endodontic sealers; however, no definitive consensus has been reached. Certain reports suggest that resin-based sealers exhibit superior sealing properties compared to other types of sealers (8).

Recently, bioceramic-based sealers have been introduced into practice. They are characterized by antibacterial properties and high biocompatibility, due to their adhesiveness; they enable good closure of dentinal tubules, which consequently reduces microleakage (19).

Given mineral trioxide aggregate's (MTA) favorable cohesive strength, biocompatibility, and osteoinductive properties, numerous MTA-based RCSs have been developed to capitalize on these advantages (20).

ITENA Clinical (Paris, France) has recently launched MTA-BIOSEAL, a novel sealer formulated with mineral trioxide

aggregate. According to the manufacturer, it demonstrates minimal expansion upon setting, low solubility in tissue fluids, and excellent flow characteristics (20,21).

Limited data are available regarding its effectiveness in establishing a reliable apical seal.

Therefore, the present study aims to evaluate the apical sealing ability of three different sealers – MTA BIOSEAL, AH Plus, and Endoseal – using the single-cone obturation technique in combination with the dye extraction method.

METHODS

Eighty-eight single-rooted permanent teeth with developed and closed root apex, without visible cracks or anatomical anomalies, were selected for the research. All teeth were cleaned of calculus and periodontal tissue using ultrasonic scaler, washed under running water, dried, and stored in 10% formalin solution.

Access cavity preparation was made using a #2 round diamond tip (NSK, Japan), and coronal preflaring was made using a Gates-Glidden bur (MANI, Japan). The canal orifices were prepared to assure a straight-line access to the canals. K-file #10 (DentsplyMaillefer, Bailiauges, SWITZERLAND) was introduced into the root canal until it was visible at the apex, and then 0.5 mm was subtracted from that point to determine the working length.

Biomechanical root canal preparation of all teeth was performed with the crown down technique using the Mtwo rotary system (VDW, Munich, Germany). The Mtwo files used were as follows: 10/04, 15/05, 20/06, and 25/06. Following instrumentation with each rotary file, the root canals were irrigated with 2 mL of 5.25% NaOCl (Ultradent, USA) and 2 mL of 18% Ethylenediaminetetraacetic acid (Ultradent, USA) using a disposable 30 G side-vented needle and subsequently rinsed with 5 mL of distilled water. The canals were then dried with 25/.06 paper points (VDW, Munich, Germany) until complete dryness was achieved.

The prepared specimens were divided into 5 experimental groups (Table 1).

Two control groups: Positive control (POS CTRL) – processed root canals, but not obturated and negative control (NEG-CTRL) – roots completely covered with 3 layers of varnish.

The root canals of the remaining 3 groups of specimens were obturated with 3 different endodontic sealers prepared in accordance with the manufacturer's instructions:

AH Plus Sealer (Dentsply DeTrey, Constance, Germany) epoxy resin-based sealer;

ITENA MTA BIOSEAL 8 (ITENA Clinical, Paris, France) MTA-based sealer;

ENDOSEAL (PREVESTDenPro, Jammu, India) zinc oxide eugenol-based sealer;

In all three groups, single cone obturation was performed with gutta-percha points 25/6 (VDW, Munich, Germany).

The crown cavity was temporarily restored with a Fuji IX glass ionomer (GC, Tokyo, Japan).

After obturation, the specimens were stored for 24 h at 37°C and 100% humidity in an incubator to allow setting of the sealer.

Quality of obturation is evaluated radiographically (Figure 1) and if deficiencies in terms of short or inhomogeneous obturation were noted, these specimens were excluded from the study. Finally, 75 teeth were included in the study, 15 in each group.

Then all samples were covered with nail varnish, with the exception of the apical 1 mm, fixed in special molds made of adhesive wax, and immersed into 2% methylene blue. Only the NEG CTRL group had the root apex completely covered. The teeth were immersed with two-thirds of the roots vertically. The samples were stored in a dark place at room temperature for 7 days to allow dye penetration. Subsequently, they were thoroughly rinsed under running tap water for approximately 30 min until the effluent was clear. After drying, the nail varnish was removed by carefully scraping with a scalpel blade. The apical 4 mm of each root was then sectioned using a diamond disk. Each specimen was placed in a glass test tube containing 1.0 mL of 65% nitric acid and left for 72 h.

Subsequently, the solutions were centrifuged at 14,000 rpm for 5 min to release the dye. The resulting supernatant was analyzed for optical density at 550 nm using a ultraviolet-visible spectrophotometer (DR 6000, HACH, Loveland, USA), with concentrated nitric acid serving as a blank (22).

The data obtained were subsequently subjected to statistical analysis.

Statistical analyses were conducted using Jamovi 2.6.25 (The Jamovi Project, Sydney, Australia). Continuous variables were summarized using means, standard deviations, medians, ranges, and 95% confidence intervals (CIs) for the means.

Group differences in means were assessed using one-way analysis of variance (ANOVA). When the ANOVA indicated statistical significance, *post hoc* pairwise comparisons were performed using *t*-tests, with Tukey's method applied to adjust for multiple comparisons.

The significance threshold (type I error rate) was set at $\alpha = 0.05$, with *p*-values below this level considered statistically significant.

RESULTS

The optical density of dye absorbance values of different endodontic sealers, measured in European brewing color (EBC) units, varied considerably between groups (Table 2). Among the tested sealers, MTA BIOSEAL exhibited the lowest mean of dye absorbance, with an EBC value of 22.79 (95% CI: 22.52-23.05, standard deviation [SD] = 0.457), indicating the lowest dye absorption potential. AH PLUS resulted in a higher mean EBC of 31.16 (95% CI: 30.82-31.51, SD = 0.596), while ENDOSEAL recorded the highest mean dye absorbance among the experimental groups at 36.67 EBC (95% CI: 36.49-36.84, SD = 0.303).

In comparison, the POS CTRL group demonstrated severe dye absorbance, with a mean EBC of 280.15 (95% CI: 270.75-289.54, SD = 16.276), surpassing all sealer groups. The NEG CTRL showed minimal dye absorbance, with a mean EBC of 9.01 (95% CI: 8.39-9.63, SD = 1.071).

Figure 2 illustrates the difference between groups, with ANOVA results shown in Table 2.

ANOVA test resulted in a statistically significant result ($F = 3448$, $p < 0.001$), with η^2 at 0.995, indicating a strong association between the type of endodontic sealer and EBC value (Table 3).

Pairwise comparisons of EBC values between endodontic sealers revealed significant differences across most groups (Table 4). MTA BIOSEAL demonstrated significantly lower dye absorbance compared to AH PLUS (mean difference: -8.38 , $t = -3.03$, $p = 0.028$) and ENDOSEAL (mean difference: -13.88 , $t = -5.03$, $p < 0.001$).

The difference between MTA BIOSEAL and the POS CTRL was substantial (mean difference: -257.36 , $t = -93.23$, $p < 0.001$), while the NEG CTRL exhibited significantly lower EBC values than MTA BIOSEAL (mean difference: 13.78 , $t = 4.99$, $p < 0.001$). AH PLUS resulted in higher EBC values than MTA BIOSEAL but did not differ significantly from ENDOSEAL (mean difference: -5.50 , $t = -1.99$, $p = 0.280$). However, AH PLUS showed significantly less dye absorbance than the POS CTRL (mean difference: -248.98 , $t = -90.20$, $p < 0.001$) and

TABLE 1. Experimental groups

Group	Canal preparation	Obturation -sealer	Obturation- Gutta-percha
GROUP 1 (n=15)	Crown-down technique Mtwo system	MTA BIOSEAL	Single cone obturation technique (gutta-percha 25/6)
GROUP 2 (n=15)	Crown-down technique Mtwo system	AH PLUS	Single cone obturation technique (gutta-percha 25/6)
GROUP 3 (n=15)	Crown-down technique Mtwo system	ENDOSEAL	Single cone obturation technique (gutta-percha 25/6)
GROUP 4 (n=15)	Crown-down technique Mtwo system	POS CTRL- root canals are processed but not obturated-no sealer	No gutta-percha
GROUP 5 (n=15)	Root canals were not processed	NEG CTRL- apex completely covered with nail varnish-no sealer	No gutta-percha

MTA: Mineral trioxide aggregate's, POS CTRL: Positive control, NEG CTRL: Negative control

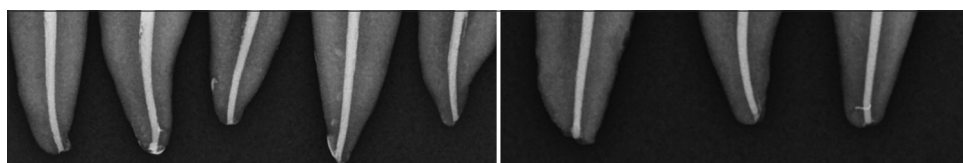


FIGURE 1. Quality of obturation evaluation.

TABLE 2. Optical density of dye absorbance values of different endodontic sealers measured in EBC units

Endodontic sealer	Mean	95% Confidence interval		Median	SD	Minimum	Maximum
		Lower	Upper				
MTA BIOSEAL	22.79	22.52	23.05	22.77	0.457	22.00	23.4
AH PLUS	31.16	30.82	31.51	31.19	0.596	29.97	32.1
ENDOSEAL	36.67	36.49	36.84	36.77	0.303	36.20	37.0
POS CTRL	280.15	270.75	289.54	280.98	16.276	254.39	303.5
NEG CTRL	9.01	8.39	9.63	9.04	1.071	6.85	10.7

MTA: Mineral trioxide aggregate's, POS CTRL: Positive control, NEG CTRL: Negative control

TABLE 3. One-way ANOVA

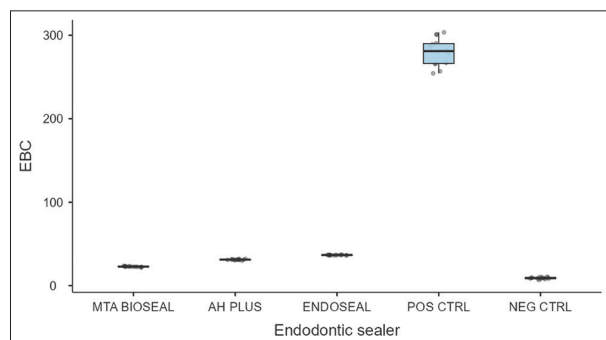
Variables	Sum of Squares	df	Mean Square	F	p	η^2
Endodontic sealer	735742	4	183935.4	3448	<0.001	0.995
Residuals	3467	65	53.3			

ANOVA: Analysis of variance

TABLE 4. *Post hoc* comparisons for endodontic sealer

Endodontic sealer	Comparison		t	ptukey
	Endodontic sealer	Mean difference		
MTA BIOSEAL	AH PLUS	-8.38	-3.03	0.028
	ENDOSEAL	-13.88	-5.03	<0.001
	POS CTRL	-257.36	-93.23	<0.001
	NEG CTRL	13.78	4.99	<0.001
AH PLUS	ENDOSEAL	-5.50	-1.99	0.280
	POS CTRL	-248.98	-90.20	<0.001
	NEG CTRL	22.15	8.03	<0.001
ENDOSEAL	POS CTRL	-243.48	-88.20	<0.001
	NEG CTRL	27.66	10.02	<0.001
POS CTRL	NEG CTRL	271.14	98.22	<0.001

Comparisons are based on estimated marginal means

**FIGURE 2.** European brewing color values between groups.

significantly greater dye absorbance than the NEG-CTRL (mean difference: 22.15, $t = 8.03$, $p < 0.001$). ENDOSEAL also exhibited significantly lower EBC values compared to the POS CTRL (mean difference: -243.48, $t = -88.20$, $p < 0.001$) but higher values than the NEG-CTRL (mean difference: 27.66, $t = 10.02$, $p < 0.001$). Finally, the POS CTRL showed the highest degree of dye absorbance, with a mean difference of 271.14 compared to the NEG-CTRL ($t = 98.22$, $p < 0.001$).

DISCUSSION

The present study evaluated the apical sealing ability of 3 different endodontic sealers: MTA BIOSEAL, AH Plus,

and Endoseal using a single cone obturation technique with dye extraction method.

The dye extraction method gives more reliable results than the dye penetration method because of its ability to measure all the dyes absorbed by the root. The dye extraction method consists of immersing the samples in dye, followed by acid treatment, which releases all the dye present at the interface. The optical density of the resulting solution is then measured using a spectrophotometer, allowing quantitative assessment of dye penetration along the margins of the restoration (23,24).

MTA BIOSEAL is a relatively new endodontic sealer on the market. According to the manufacturer, it is composed of salicylic resin, 40% MTA (including C3S, C2S, tricalcium aluminate, and calcium oxide), and radio-opacifiers. To reduce cytotoxicity, MTA BIOSEAL contains a lower aluminum content (≈ 0.05 wt%) and uses strontium and titanium as radio-opacifiers instead of bismuth, as found in MTA-Fillapex. Regarding its properties, only one study has evaluated the physicochemical characteristics of MTA BIOSEAL, reporting lower solubility compared to MTA-Fillapex (20).

AH Plus resin sealer (Dentsply DeTrey GmbH, Konstanz, Germany) is a widely used two-paste system that has been extensively studied. It is often considered the "gold standard" sealer due to its excellent adhesion to dentin, biocompatibility, and favorable physical properties (9), including sealing ability, strength, dimensional stability, and a setting time of approximately 8 h (25). The composition of AH Plus is as follows: Paste A contains bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, and iron oxide pigments; Paste B contains dibenzylamine, aminoadamantane, tricyclodecane-diamine, calcium tungstate, zirconium oxide, silica, and sili-cone oil (26).

Endoseal, produced by Prevest Denpro (Jammu, India), is a permanent RCS formulated with a zinc oxide eugenol base and enriched with components, such as dexamethasone, thymol iodide, hydrocortisone acetate, bismuth subcarbonate, epoxy resins, barium sulfate, and magnesium stearate. The manufacturer reports that it possesses antibacterial and anti-inflammatory effects, along with an extended working time and delayed setting characteristics (27).

Among the tested sealers, MTA BIOSEAL exhibited the lowest mean optical density of dye absorbance, with an EBC value of 22.79, indicating the lowest dye absorption potential. AH Plus resulted in a higher mean EBC of 31.16, while Endoseal recorded the highest mean dye absorbance among the experimental groups at 36.67 EBC.

MTA BIOSEAL demonstrated significantly lower dye absorbance compared to AH Plus (mean difference: -8.38 , $t = -3.03$, $p = 0.028$) and Endoseal (mean difference: -13.88 , $t = -5.03$, $p < 0.001$).

AH Plus resulted in higher EBC values than MTA BIOSEAL but did not differ significantly from Endoseal (mean difference: -5.50 , $t = -1.99$, $p = 0.280$).

In the study, we had two control groups to confirm the accuracy of the used techniques. The results of the present study showed the lowest dye absorbance with the NEG CTRL Group (9.01). The POS CTRL Group had the highest dye absorbance value of all tested groups (280.15).

Kataia and Kataia (14) assessed the sealing ability of AH Plus, iRoot SP sealer, and the Epiphany-Resilon root canal filling system using the dye extraction method. Their results revealed no significant differences in sealing ability among the tested sealers, which was attributed to their low solubility, minimal dimensional changes, strong adhesion to dentin, and overall effective sealing capacity. In addition, water sorption-induced swelling of the epoxy resin component in AH Plus may further enhance its adaptability and penetration into dentinal tubules.

Kaya et al. (22) compared dye extraction and dye penetration methods to quantitatively assess the microleakage of three RCSs. The sealers tested were Ketac-Endo (glass ionomer-based sealer), MetaSEAL (methacrylate resin-based sealer), and AH Plus JET (epoxy resin-based sealer). Their study showed that MetaSEAL performed better than Ketac-Endo, but exhibited higher leakage than AH Plus JET ($p < 0.05$). The authors attributed this to the fact that AH Plus JET, as an epoxy resin-based sealer, penetrates micro-irregularities more effectively due to its creep ability and long setting time, enhancing mechanical interlocking between the sealer and root dentin (28-30).

Salem et al. (8) conducted an *in vitro* study to evaluate the apical sealing ability of TotalFill BC and AH Plus using single-cone and continuous-wave obturation techniques, employing the dye extraction method. The study found that neither the type of sealer nor the obturation technique completely prevented apical microleakage. In addition, there was no significant difference in apical microleakage between TotalFill BC and AH Plus with either obturation technique.

Nepal et al. (10) evaluated apical microleakage of retrograde fillings with Glass Ionomer Cements (GIC), MTA, and Biodentine *in vitro* using spectrophotometric analysis. Both Biodentine and MTA exhibited lower microleakage compared to GIC, while no significant difference was observed between MTA and Biodentine.

Juez et al. (31) investigated microleakage following simulated apexification using White MTA, TotalFill bioceramic root repair material, and BioDentin in an *in vitro* study with spectrophotometric analysis. Their results showed no significant difference in leakage between MTA and calcium silicate-based materials.

In their study, Patil et al. (32) evaluated the apical sealing efficacy of three RCSs – MTA Fillapex, AH Plus, and zinc oxide eugenol – by employing the dye penetration technique. They reported dye infiltration in all groups, regardless of the sealer used. AH Plus exhibited the least

leakage, indicating superior sealing performance. No significant difference in apical leakage was observed between AH Plus and MTA Fillapex, suggesting comparable sealing properties, whereas the Zinc Oxide Eugenol (ZOE) sealer demonstrated the highest leakage among the tested materials. These findings underscore the importance of using an appropriate combination of RCSs and core materials during obturation to minimize apical leakage and highlight the critical role of sealer selection in achieving successful endodontic outcomes.

Kelmendi et al. (33) evaluated the coronal sealing quality of three RCSs using two obturation techniques through the bacterial penetration method. The sealers tested were zinc oxide eugenol-based Pulp Canal Sealer EWT (PC), epoxy resin-based AH Plus, and bioceramic-based Well-Root ST. Obturation was performed using cold lateral condensation and Thermafil techniques. The results showed no statistically significant difference between the two obturation methods. Although all root canals exhibited some degree of leakage, the bioceramic sealer demonstrated superior performance compared to both the epoxy resin-based and zinc oxide eugenol-based sealers.

From the studies cited above, it is evident that numerous factors influence the quality of endodontic obturation, including canal preparation techniques, irrigation protocols, and various obturation materials. The present study, however, specifically focused on evaluating the apical sealing ability of different endodontic sealers.

The results indicate that the type of sealer influences the extent of apical microleakage. In this study, the MTA-based sealer showed a significantly lower level of apical leakage compared to the other materials tested. Although no sealer can completely eliminate microleakage, some materials demonstrate superior sealing ability in the apical region. These findings highlight the importance of selecting an appropriate sealer to achieve optimal obturation and improve the long-term success of endodontic treatment.

This type of *in vitro* study has several limitations. While spectrophotometry allows for quantitative assessment of dye penetration, it does not provide information on the exact location or mechanism of leakage and may not fully reflect the behavior of fluids or bacteria *in vivo*. Variations in root canal anatomy, the chemical properties of the dye, and potential inconsistencies in obturation technique could have affected the results. In addition, the study evaluated leakage over a limited timeframe, without considering long-term material degradation.

CONCLUSION

Within the limitations of this study, it can be concluded that all three evaluated RCSs – MTA-based, AH Plus, and Endoseal – exhibited varying degrees of apical microleakage when used with the single-cone obturation technique. Among them, the MTA-based sealer demonstrated significantly superior sealing performance, showing the least apical microleakage. AH Plus performed better than Endoseal, although the difference between these two was not statistically significant. These findings highlight the influence of sealer composition on apical sealing efficacy. However, as the results are specific to the single-cone technique, further

investigations employing alternative obturation methods are warranted to comprehensively assess the sealing capabilities of these materials.

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DECLARATION OF INTEREST

Authors declare no conflict of interests.

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