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Bacterial Leakage Evaluation of Three Root Canal Sealers with Two Obturation Techniques: An in Vitro Study

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Abstract

Background: The purpose of this study was to compare the quality of the coronal seal of three root canal sealers and two obturation techniques using the bacterial penetration method.

Methods and Results: A total of 132 single-rooted human teeth with fully developed apices were used. The teeth were randomly assigned to three experimental groups according to the endodontic sealer used. Group 1: Samples (n=44) were obturated using a zinc oxide eugenol-based sealer, Pulp Canal Sealer EWT. Group 2: Samples (n=44) were obturated using an epoxy resin-based sealer, AH Plus. Group 3: Samples (n=44) were obturated using a bioceramic-based root canal sealer, Well-Root ST. Each group was subdivided into 2 equal subgroups in accordance with the obturation technique being used: the cold lateral condensation technique (CLCT) and Thermafil obturation technique (ThOT). Thus, 6 subgroups were formed: Sub-1A: Pulp Canal Sealer/CLCT; Sub-2A: AH Plus/CLCT; Sub-3A: Well-ROOT ST/CLCT; Sub-1B: Pulp Canal Sealer/ThOT; Sub-2B: AH Plus/ThOT; Sub-3B: Well-ROOT ST/ThOT. A dual-chamber device was used to evaluate bacterial leakage. Fresh medium and *E. faecalis* were added to the upper chamber every 4 days. The broth was monitored for color change daily for 33 days. Significant differences were found among Sub-2A vs. Sub-1B (P=0.023), Sub-1A vs. Sub-3A (P=0.014), Sub-1A vs. Sub-2B (P=0.024), Sub-1A vs. Sub-3B (P=0.002), Sub-3A vs. Sub-1B (P=0.003), Sub-2B vs. Sub-1B (P=0.005), and Sub-1B vs. Sub-3B (P<0.0001). There was no significant difference in the average occurrence of turbidity between CLCT and ThOT (P=0.718)

Conclusion: Regardless of the obturation technique, all root canal sealers exhibited leakage; however, the bioceramic-based root canal sealer appeared to perform better than the epoxy resin-based sealer and the zinc oxide eugenol-based sealer. (**International Journal of Biomedicine. 2022;12(4):584-590.**).

Keywords: microleakage • cold lateral condensation technique • thermafil obturation technique • root canal sealer

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Abbreviations

CLCT, cold lateral condensation technique; **CFU**, colony-forming units; **ThOT**, Thermafil obturation technique.

Introduction

Microorganisms are a major etiological factor in root canal infections, and eradicating them during root canal treatment by instrumentation, irrigation, and intracanal medication is fundamental. The root canals should be cleaned, shaped, and obturated with sterilized materials with antimicrobial properties.(1) It is not always possible to completely eliminate microorganisms from the root canals,(2) and microorganisms can also penetrate through coronal leakage after the obturation of root canals.[3] No available filling material or technique can achieve complete sealing of the entire root canal system.[4] Therefore, there is a need to develop root canal filling materials with an improved capacity to prevent bacterial ingress in the long term.[5]

Many studies involving dyes, radioisotopes, and bacteria have been performed to evaluate coronal leakage. The use of bacteria to assess substantial coronal leakage is considered to be of greater clinical relevance than dye penetration, according to Timpawat et al.[6] *Enterococcus faecalis* is commonly isolated from primary[7] and secondary endodontic infections.[8] However, its prevalence is higher in secondary endodontic infections. This microorganism is one of the most resistant in endodontic infections and is considered to be a possible cause of endodontic treatment failure.[9]

The success of endodontic treatment depends on adequate mechanical debridement of the root canal and quality obturation with a material that must be biocompatible. Gutta-percha is the recommended material because it has been shown to be biologically inert.[10] Gutta-percha does not bond to root dentin and must be used in conjunction with a sealer cement.[11] Cold lateral condensation of gutta-percha is one of the most widely accepted canal obturation methods and is taught by numerous dental schools. Nevertheless, its capability to adapt the gutta-percha to the internal surface of the root canal has been questioned.[12] Recently, a number of plasticized gutta-percha techniques have been introduced that have claimed to seal the root canal better.[13] The Thermafil obturation technique (ThOT) involves a plastic core covered by gutta-percha that is heated in an electric oven to ensure thermoplasticization. This carrier-guided gutta-percha technique is fast and effective in obturating the canal, exhibiting less leakage in vitro compared with the cold lateral condensation technique (CLCT).[14] The flow of endodontic sealers is related to their physical-chemical properties as well as to their root canal sealing ability during the procedure of root filling.[15]

AH Plus (epoxy resin-based sealer) has gained popularity due to its radiopacity, biocompatibility, ease of use, and availability. Pulp Canal Sealer (zinc oxide eugenol sealer) has antibacterial activity, but also exhibits some toxicity when placed directly on vital tissues.[16] New endodontic sealers based on bioceramic materials have been developed in an effort to create a biocompatible sealer with ideal physical, chemical, mechanical, and biological properties. Such an endodontic sealer is Well-Root ST.[17] Bioceramic sealers have stimulated strong interest because they contain calcium phosphate, silicates, and water-free thickening vehicles to enable the sealer to be applied as a premixed paste.[18] The inorganic ingredients of the sealer are mixed with thickening agents because water is needed for the setting response. The need for water can be attributed to the inherent properties of bioceramic materials or more precisely to the hydrophilic nanoparticles that allow more water molecules to come in contact with the sealer.[19] The differences between the materials change over time, and this change has clinical implications; therefore, this study investigated the need to measure microleakage over more extended periods.[20]

The purpose of this study was to compare the quality of the coronal seal of three root canal sealers and two obturation techniques using the bacterial penetration method.

Materials and Methods

Collection of teeth

A total of 132 single-rooted human teeth with fully developed apices were used. Exclusion criteria were teeth with cracks, root caries, internal/external resorption, and untreated root canals with open apices. The teeth were stored in a sterile saline solution until use. Bone, calculus, and soft tissue on the roots were removed with scalpel blades under running tap water, taking care not to damage the root surface.

Root canal preparation and filling

Patency and working length were determined by placing a #10 K-file (Maillefer, LD Caulk Co., Milford, DE, USA) in the root canal until it just penetrated the foramen, after which 1 mm was subtracted from this, and the length was recorded. Flaring was performed with Gates Glidden Drills #1 through #3.

Canals were instrumented using K-files via the step-back technique with 2% sodium hypochlorite (Parcan, Septodont, France) and 17% EDTA (Chelaton III, Lach, Czech Republic) used as irrigants. All root canals were enlarged to the size of a #40 K-file (master apical file) (Dentsply Maillefer, Tulsa, OK, USA) at the apical foramen. The samples were stored in normal saline until obturation. Absorbent paper points were used to dry the canals (Dentsply Maillefer). After root canal preparation, the teeth were randomly assigned to three experimental groups according to the endodontic sealer used. Group 1: Samples (n=44) were obturated using a zinc oxide eugenol-based sealer, Pulp Canal Sealer EWT (PCS) (Kerr Corporation, Romulus, MI, USA). Group 2: Samples (n=44) were obturated using an epoxy resin-based sealer, AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany). Group 3: Samples (n=44) were obturated using a bioceramic-based root canal sealer, Well-Root ST (Vericom, Gangwon-do, Korea).

Each group was subdivided into 2 equal subgroups in accordance with the obturation technique being used*:* CLCT and ThOT. Thus, 6 subgroups were formed: Sub-1A: Pulp Canal Sealer/CLCT; Sub-2A: AH Plus/CLCT; Sub-3A: Well-ROOT ST/CLCT; Sub-1B: Pulp Canal Sealer/ThOT; Sub-2B: AH Plus/ThOT; Sub-3B: Well-ROOT ST/ThOT.

Cold lateral condensation technique (CLCT)

The sealer was placed in the canal using a lentulo spiral (25 mm size 3 green, Dentsply-Maillefer), followed by inserting the gutta-percha master cone (Dentsply-Maillefer) to the predetermined working length. Nickel-titanium finger spreaders (Dentsply-Maillefer) were used to conduct the lateral compaction using accessory cones (Dentsply-Maillefer). Obturations were considered complete when the spacer could not enter the root canal area (10 mm).

Thermafil obturation technique (ThOT)

The samples of the Thermafil subgroups were obturated as specified by the manufacturer. We selected a Thermafil obturator the same size as the verifier. Sterile paper points were used to coat the walls of the canal to the working length with the allocated sealer of the subgroup. The Thermafil obturator was heated in the Therma Prep oven and inserted in the canal to the established working length. The shaft was severed level with the orifice using a round bur in a high-speed handpiece. The total time from checking the obturator until shaft removal was measured.

Bacterial leakage device

A scintillation vial was modified to create a dual-chamber device based on a similar two-chamber method. A round bur with a diameter of 9mm was used to bore a hole through the center of the screw cap, in which the tooth was suspended and secured with wax covered by two layers of nail varnish. The lower chamber of the device was filled with sterile tryptic soy broth (TSB). Using a sterile micropipette, 0.1ml of an overnight broth culture of E. faecalis ATCC-51299 (Liofilchem, Roseto degli Abruzzi, Italy) was inoculated into the root canal of each tooth via the coronal access cavity preparation. To prevent evaporation and contamination, the opening of the pipette tip was veiled with a sterile plastic cap. All laboratory procedures were carried out under aseptic conditions. Fresh medium and *E. faecalis* were added to the upper chamber every 4 days. The broth was monitored for color change daily for 33 days. An LS-722N-2000 Spectrophotometer (Qingdao Pharmacypro Co., Ltd. Shanghai, China) was used to measure the turbidity of the samples at an absorption rate of 590 µm. The measurements were all performed in the same room.

Statistical Analysis

Statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc, Armonk, NY: IBM Corp). The mean, standard deviation, standard error of the mean, median, interquartile range, and confidence interval (CI) were calculated. The means and medians for the turbidity presentation time according to obturation techniques and materials used for canal obturation were analyzed using Kaplan-Meier survival curves. Data were analyzed with One-way ANOVA & Tukey's (HSD) post-hoc test. Group comparisons with respect to categorical variables are performed using chi-square tests. A probability value of $P<0.05$ was considered statistically significant.

Results

On average, tooth turbidity was observed after 25.8 days. There was no significant difference in the average occurrence of turbidity between CLCT and ThOT ($P=0.718$) (Table 1 and Figure 1).

For both canal obturation techniques, the fastest turbidity presentation time was reported in Group 1 (Sub-1A=19.3 days and Sub-1B=18.2 days), and the slowest turbidity presentation time occurred in Group 3 (Sub-3A=30.2 days and Sub-3B=31.5 days). Significant differences were found among Sub-2A vs. Sub-1B ($P=0.023$), Sub-1A vs. Sub-3A ($P=0.014$), Sub-1A vs. Sub-2B ($P=0.024$), Sub-1A vs. Sub-3B ($P=0.002$), Sub-3A vs. Sub-1B ($P=0.003$), Sub-2B vs. Sub-1B ($P=0.005$), and Sub-1B vs. Sub-3B ($P<0.0001$) (Table 2 and Figure 2). CFU levels >300 were recorded in 31.8% of teeth for both canal obturation techniques (Table 3).

Among subgroups, the highest percentage of CFU>300 was registered for Sub-1B and Sub-1A (68.2% and 50%, respectively). The lowest percentage of CFU>300 was found in Sub-3B and Sub-3A (9.1% and 18.2%, respectively). CFU>300 was also found in 18.2% of Sub-2B samples (Table 4). No significant differences were found for the mean turbidity values using spectrophotometry for samples using the obturation techniques ($P=0.58$). The highest mean turbidity value was recorded in the CLCT group (CLCT=0.57 vs. ThOT=0.54) (Table 5 and Figure 3).

There were significant differences in the mean turbidity values according to the Subgroups ($P=0.008$). The highest mean turbidity values were recorded for Sub-1A and Sub-1B (0.64 and 0.58, respectively). The lowest mean turbidity values were recorded for Sub-2B and Sub-2A (0.42 and 0.44, respectively) (Table 5).

Table 1.

Mean and median for turbidity presentation time for Cold Lateral Condensation Technique vs. Thermafil obturation technique.

Means and Medians for Survival Time

Groups	Mean[a]				Median			
	Estimate	Std. Error	95% CI		Estimate	Std. Error	95% CI	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
CLCT	25.530	1.451	22.686	28.375	.	.	.	.
ThOT	26.061	1.422	23.274	28.847	.	.	.	.
Overall	25.795	1.016	23.804	27.787	.	.	.	.

a. Estimation is limited to the largest survival time if it is censored.
Log Rank (Mantel-Cox): Chi-Square=0.131, df=1, P=0.718

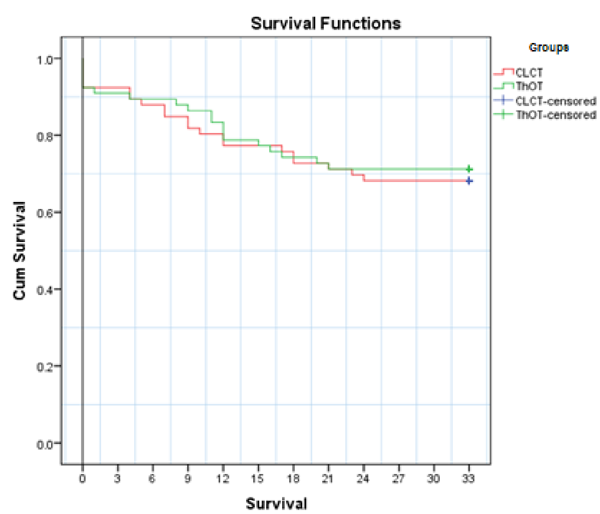


Fig. 1. *Kaplan-Meier survival curves: Turbidity presentation time for CLCT vs. ThOT.*

Table 2.
Mean and median for turbidity presentation time according to the obturation technique and paste subgroups.

Means and Medians for Survival Time								
Subgroups	Mean[a]				Median			
	Estimate	Std. Error	95% CI		Estimate	Std. Error	95% CI	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
2A	27.091	2.227	22.727	31.455	.	.	.	.
1A	19.318	3.030	13.379	25.258	18.000	.	.	.
3A	30.182	1.402	27.434	32.930	.	.	.	.
2B	28.545	2.142	24.346	32.745	.	.	.	.
1B	18.182	2.827	12.641	23.722	12.000	4.221	3.726	20.274
3B	31.455	1.090	29.317	33.592	.	.	.	.
Overall	25.795	1.016	23.804	27.787	.	.	.	.

a. Estimation is limited to the largest survival time if it is censored. Log Rank (Mantel-Cox): Chi-Square=24.754, df=5, P<0.0001

Pairwise Comparis: Log Rank Test (Mantel-Cox).

Subgroups	A / Group 2		A / Group 1		A / Group 3		B / Group 2		B / Group 1		B / Group 3	
	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
2A			3.001	0.083	0.643	0.423	0.453	0.501	5.153	0.023	2.425	0.119
1A	3.001	0.083			6.038	0.014	5.079	0.024	0.113	0.737	9.725	0.002
3A	0.643	0.423	6.038	0.014			0.011	0.915	9.106	0.003	0.740	0.390
2B	0.453	0.501	5.079	0.024	0.011	0.915			7.937	0.005	0.825	0.364
1B	5.153	0.023	0.113	0.737	9.106	0.003	7.937	0.005			13.01	0.000
3B	2.425	0.119	9.725	0.002	0.740	0.390	0.825	0.364	13.006	0.000		

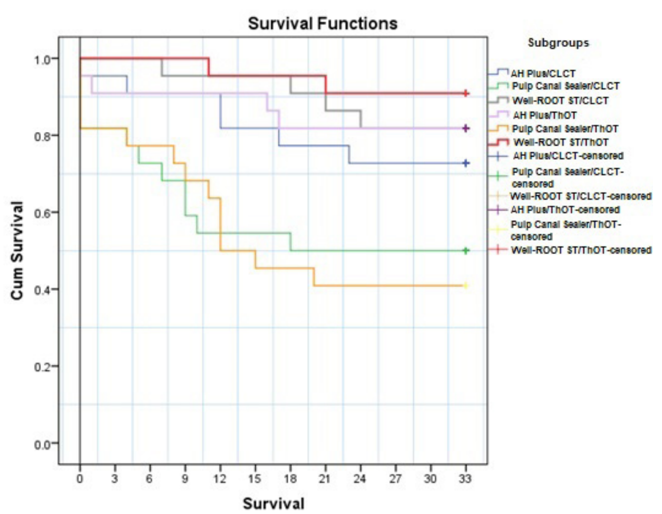


Fig. 2. *Kaplan-Meier survival curves: turbidity presentation time according to the obturation technique and paste subgroups.*

Table 3.
CFU in the teeth analyzed according to the canal obturation technique.

Groups	CFU	Frequency	Percent	Cumulative Percent
CLCT	Negative	45	68.2	68.2
	>300	21	31.8	100.0
	Total	66	100.0	
ThOT	Negative	45	68.2	68.2
	>300	21	31.8	100.0
	Total	66	100.0	

Table 4.
CFU according to the obturation technique and pastes used.

Subgroups*	CFU	Frequency	Percent	Cumulative Percent
AH Plus/CLCT	Negative	16	72.7	72.7
	>300	6	27.3	100.0
	Total	22	100.0	
Pulp Canal Sealer/ CLCT	Negative	11	50.0	50.0
	>300	11	50.0	100.0
	Total	22	100.0	
Well-Root ST/ CLCT	Negative	18	81.8	81.8
	>300	4	18.2	100.0
	Total	22	100.0	
AH Plus/ThOT	Negative	18	81.8	81.8
	>300	4	18.2	100.0
	Total	22	100.0	
Pulp Canal Sealer/ ThOT	Negative	7	31.8	31.8
	>300	15	68.2	100.0
	Total	22	100.0	
Well-Root ST/ ThOT	Negative	20	90.9	90.9
	>300	2	9.1	100.0
	Total	22	100.0	

** Pearson Chi-Square = 25.981[a], df=5, P<0.0001.*

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.00.

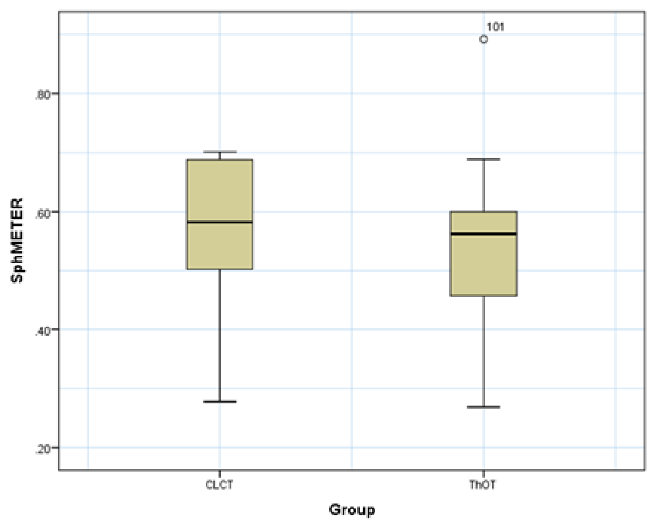
Table 5.
The mean value of turbidity using spectrophotometry according to the obturation techniques and subgroups.

Groups/ Subgroups	N	Minimum	Maximum	Mean	Std. Dev.	ANOVA	*P*-value
CLCT	21	0.28	0.70	0.57	0.124	F=0.311	0.58
ThOT	19	0.27	0.89	0.54	0.141		

Table 5 (continued).

The mean value of turbidity using spectrophotometry according to the obturation techniques and subgroups.

Groups/ Subgroups	N	Minimum	Maximum	Mean	Std. Dev.	ANOVA	*P*-value
AH Plus/ CLCT	6	0.28	0.55	0.44	0.113	F=3.763	0.008
Pulp Canal Sealer/CLCT	11	0.44	0.70	0.64	0.088		
Well-Root ST/ CLCT	4	0.48	0.59	0.56	0.054		
AH Plus/ ThOT	4	0.27	0.58	0.42	0.131		
Pulp Canal Sealer/ ThOT	13	0.38	0.89	0.58	0.138		
Well-Root ST/ ThOT	2	0.55	0.58	0.56	0.018		



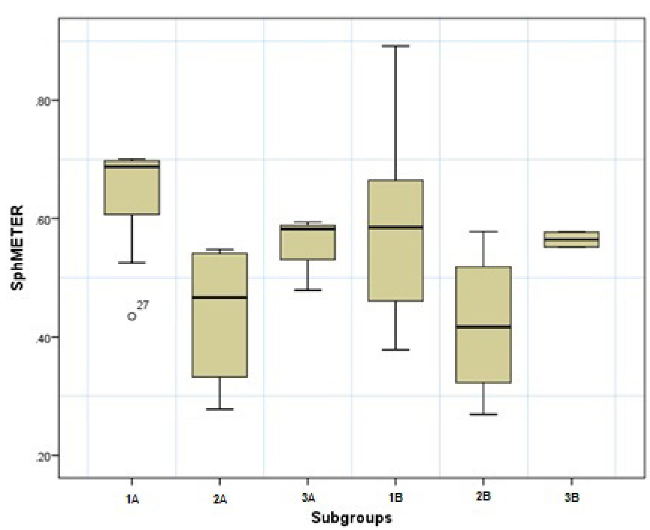


Fig. 3. *The mean value of turbidity using spectrophotometry according to the obturation techniques and subgroups.*

Multiple Comparisons						
Dependent Variable: SphMETER Tukey HSD						
(I) Subgroup	(J) Subgroup	Mean Difference (I-J)	Std. Error	Sig.	95%CI	
					Lower Bound	Upper Bound
PCS/CLCT	PCS/ThOT	0.19998*	0.05703	0.015	0.0279	0.3721
	WRST/CLCT	0.21832*	0.06560	0.024	0.0203	0.4163
* - *The mean difference is significant at the 0.05 level.*						

Discussion

Achieving an acceptable coronal seal is one of the key intentions in endodontic treatment. Many materials and obturation techniques are available. Each has its own advantages and disadvantages; therefore, the search for the ideal sealer and obturation technique continues. The present study used two obturation techniques to compare and evaluate coronal leakage in three root canal sealers.

We found no significant differences between the techniques of ThOT and CLCT. Our results, consistent with the findings of other researchers, indicated no significant differences in coronal sealing ability between ThOT and CLCT.[21,22] Gade et al.[23] found no significant differences in the quality of obturation between CLCT and ThOT. The turbidity of the samples appeared later in ThOT than in CLCT, which was in accordance with other studies in which the researchers concluded that ThOT provided better sealing than CLCT.[24,25] De Moor and Hommez[26] demonstrated that ThOT had significantly greater coronal leakage than three other condensation techniques. The important aspect of Thermafil is the margin of error permitted by the manufacturer in the production of plastic carriers. There is no evidence in the literature for the discrepancy percentage between different carriers.[27]

According to our results, Well-Root ST and AH Plus had similar leakage after 33 days, but Well-Root ST had the better sealing ability. Four of the Well-Root ST samples in the subgroup obturated with CLCT exhibited bacterial leakage, compared with only 2 samples in the subgroup obturated with ThOT. In the AH Plus/CLCT subgroup, 6 samples exhibited leakage, compared with 4 samples in the AH Plus/ThOT subgroup. In contrast, the Pulp Canal Sealer/CLCT subgroup included 11 samples with high leakage, and the Pulp Canal Sealer/ThOT subgroup included 13 samples with bacterial leakage. Our results indicated that Well-Root ST had less microleakage than other tested sealers, such as AH 26,[28] while zinc oxide eugenol had the highest microleakage, which agreed with previous findings.[29,30] One possible reason for the better sealing ability of Well-Root ST is its biocompatibility and antimicrobial effect.[31]

Turbidity in the lower chamber is an indicator of contamination by microorganisms. In the present study, the highest mean turbidity value was recorded in the CLCT group, but the differences were not significant. The highest turbidity value was recorded in the group with Pulp Canal Sealer, whereas the lowest turbidity value was in the group with AH

Plus. Because of its poor sealing and adhesion properties to dentin, Pulp Canal Sealer exhibited the highest amount of bacterial penetration compared with all groups, which is consistent with other studies.[32-34] Pommel and Camps [35] compared the lateral condensation technique and the ThOT with Pulp Canal Sealer and reported that Thermafil obturation had less leakage than lateral condensation. Our study showed no significant differences between the CLCT and ThOT subgroups in the Pulp Canal Sealer group.

This study found that regardless of the obturation technique, turbidity appeared most quickly in the groups obturated with Pulp Canal Sealer and AH Plus (18.75 and 27.82 days, respectively), compared with 30.82 days in the Well-Root ST group. This may be because the antibacterial effect of Pulp Canal Sealer and AH Plus is lower than that of Well-Root ST.[36,37] Apart from the antibacterial activity of the sealers, the physical properties, such as adhesion, adaptability, and solubility, are also important.

Obturation is the final step in root canal treatment. It can be achieved with different types of root canal sealers in combination with the core root canal filling material, such as gutta-percha cones, using different condensation techniques. In the present study, the samples were contaminated with *E. faecalis* and monitored for 33 days, which was long enough to detect early-stage leakage, although other studies monitored teeth daily from 30 to 90 days.[38,39] *E. faecalis* was used because it invades the dentin tubules and can survive in the root canal system after root canal treatment. Histological sections or high-resolution micro-CT imaging[40] should be used together with other testing methods to verify the mode of contamination for bacterial leakage.

There were some limitations in this study. The coronal restorations were missing, which increased the amount of leakage, even though the current clinical philosophy recommends that the tooth should be immediately restored to protect the root canal filling from coronal leakage. However, there are sometimes cases when the patient does not have time to finish the treatment session, or the dentist may need to postpone the placement of a coronal restoration until a subsequent appointment. In such instances, temporary filling cements are used, which may fall out of the cavity before the coronal restoration appointment, exposing the obturated root canal to saliva and oral bacteria.

Conclusion

Within the limitations of this study, our findings indicate no statistically significant difference between the CLCT and the ThOT. Regardless of the obturation technique, all root canal sealers exhibited leakage; however, the bioceramic-based root canal sealer appeared to perform better than the epoxy resin-based sealer and the zinc oxide eugenol-based sealer.

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Competing Interests

The authors declare that they have no competing interests.

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