ORIGINAL ARTICLES

Bacterial leakage of bioceramic versus resin sealers in different obturation techniques: an in-vitro study

ABSTRACT

Aim: *To compare bacterial leakage of Neo-sealer (a bioceramic sealer) and AH-Plus (an epoxy resin-based sealer) using different obturation techniques.*
Methodology: *Ninety mandibular molars without caries, cracks, fractures, resorption, open apices, or prior endodontic treatment were selected. Mesial roots were standardized in length; distal roots were discarded. Samples were randomly divided into six groups: (1) Neo-sealer + single cone; (2) Neo-sealer + lateral compaction; (3) Neo-sealer + warm vertical compaction; (4) AH-Plus + single cone; (5) AH-Plus + lateral compaction; and (6) AH-Plus + warm vertical compaction. Canals were prepared to size #30 with 4% taper and irrigated using 5% NaOCl and saline. Each group was obturated according to its protocol. Bacterial leakage was evaluated by a blinded operator, and the time to leakage was recorded. Statistical analysis was performed using two-way ANOVA and Tukey's test (α=0.05)*
Results: *Mean leakage times (in days) were: 9.64, 17.53, 13.21, 12.47, 7.73, and 8.53 for groups 1–6, respectively. No significant differences were found among sealers, techniques, or their combinations (P>0.05).*
Conclusions: *Neo-sealer and AH-Plus showed similar effectiveness in preventing coronal bacterial leakage across all obturation techniques. The single cone method was comparable to lateral and warm vertical compaction in sealing ability.*

Ali Akhavan[1]
Alireza Farhad[2]
Melika Sadat Araghbidi Kashani[1,*]
Mohammad Javad Tarrahi[3]
Fariba Heidari[4]
Carlo Rengo[5]
Mariateresa Milleri[5]
Parisa Soltani[5,*]
Francesco Traino[5]

[1] Department of Endodontics, Dental Materials Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran.
[2] Department of Endodontics, Dental Research Center, Dental Reearsch Institute, School of Dentistry, Isfahan University of Medical Science, Isfahan, Iran.
[3] Department of Epidemiology and Biostatistics, School of Health, Isfahan University of Medical Science, Isfahan, Iran.
[4] Dental Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Science, Isfahan, Iran.
[5] Department of Neurosciences, Reproductive and Odontostomatological Sciences, University of Naples 'Federico II', Naples, Italy.

[*] Corresponding author

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Corresponding Authors*
Parisa Soltani DDS, MSc Department of Neurosciences, Reproductive and Odontostomatological Sciences, University of Naples "Federico II,", Via Pansini 5, 80131 Naples, Italy | parisa.soltani@live.com |

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Introduction

Long-term endodontic success depends on many factors: instrumentation, chemical and mechanical preparation, isolation, obturation, and post-endodontic restoration. All of these factors have the same goal which is to prevent bacteria from leaking through the canal and affecting the periapical region. The role of obturation is the three-dimensional seals of the canal for the long term. Many materials and obturation techniques have been introduced for obturation (1-6).

Various factors can influence the occurrence of apical micro-leakage and the quality of root canal obturation, including the quality of the obturation materials, root canal obturation techniques, and the presence or absence of the smear layer, all of which can contribute to treatment failure (7).

Different techniques are used for filling root canals, such as cold lateral compaction, warm vertical compaction, hybrid Tagger, single cone technique, carrier-based gutta-percha, and thermoplastic injectable techniques (7)(8).

Many studies have identified the cold lateral compaction technique as the standard method for root canal filling (9). This technique is cost-effective, predictable, and safe, offering long-term success (10). However, common issues with cold lateral compaction include void formation, incomplete filling in curved root canals, and an increased risk of vertical root fracture due to high compaction forces (11,12).

As a simpler alternative, the single cone technique with an adaptive taper has been introduced. This method relies less on the operator's skill and poses a lower risk of damage to the dentin walls of the root canal (13). Other advantages include easy handling, lower costs, and reduced treatment time (14,15). However, its drawbacks include the potential for unfilled spaces, especially in large canals, as well as shrinkage during sealer setting (16). To optimize this technique, it has been suggested that sealers with good physical and chemical properties, such as proper flow along the canal walls and void spaces, should be used (13).

It is believed that the most important component of the obturation is the sealer used, as it is the only anti-microbial obturation component offering resistance against bacteria and leakage (8). Resin-based sealers, such as AH-Plus, have been used for a long time in endodontics and have their advantages: the ability to bond to composite resin restorations, good wettability, and penetration into dentinal tubules. It has some disadvantages: it has shrinkage and may cause voids (9, 10). The properties of AH-Plus have been known for a long time, and somehow it has become the gold standard in endodontic treatments (11, 12). Recently bioceramic sealers such as Neo-sealer have been introduced. Its advantages include biocompatibility and setting without shrinkage. It has been studied in single cone obturation technique, and some studies suggest that it has superior sealing ability compared to resin-based sealers with single cone obturation technique (13).

However, there are limited studies on bioceramic sealers used in different obturation techniques like warm vertical and lateral compaction obturations. Some studies have shown that these sealers may lose their properties in hot temperatures (14-16). Also, the lateral compaction obturation technique may have better sealing ability than the single cone technique used with bioceramic sealers (17).

Since there are limited and conflicting studies on this subject matter, the current experiment was designed to compare two types of sealers (AH-Plus as a resin-based sealer and Neo-sealer as a bioceramic sealer) in combination with different obturation techniques (single-cone, lateral compaction, and warm vertical obturation) in preventing coronal bacterial leakage.

Materials and Methods

Sample selection

This experimental study was approved by the Research Ethics Committee (IR.MUI.DHMT.REC.1403.109) at the Isfahan University of Medical Science and reported based on the PRILE 2021 checklist. A total of 90 mandibular molars were collected (15 samples in each group). The teeth included had closed apices and without any fracture, caries, resorption, and previous endodontic treatment. Teeth that did not match these criteria were excluded from the study. Teeth had Schneider curvature between 10-30 degrees based on placing a K-file #10 into the canal and taking a radiograph (18). The teeth were decornated at the level of the cementoenamel junction to match all roots for the same length, mesial roots were collected and distal roots were discarded. Mesiobaccal canals were treated for this study. Roots were stored in normal saline at room temperature.

Root canal preparation

At first, the glide path was determined using K-file #10 (Dentsply Maillefer, Ballaigues, Switzerland), and the working length was determined using K-file #10 passing through apical foramen and then canals were instrumented 1 mm shorter than the anatomical apex. Roots were instrumented using S1, S2, F1, and F2 ProTaper universal rotary files (Dentsply Sirona, Ballaigues, Switzerland) using the crown-down technique. Canals were irrigated with NaOCl %5 (Cerkamed, Stalowa Wola, Poland) with a syringe with a 25-gauge needle, and at the final stage irrigated with normal saline (DarouPakhsh, Tehran, Iran). Canals were dried with multiple paper points (Diadent, Chungcheongbuk-do, Korea).

Obturation

After preparation, the roots were divided into 6 groups: Group 1: roots were obturated using a single cone of gutta-percha and NeoSealer (Avalon Biomed, Texas, USA) as the sealer. First, the sealer was injected into the canal, then one gutta-percha point size #30 with %4 taper was coated with the sealer and then placed into the canal. Then obturation material was cut with a heat carrier (Fast Pack, Eighteeth) device.
Group 2: roots were obturated with NeoSealer as the sealer with the lateral compaction method. A master gutta-percha point size #30 with %2 taper was chosen, coated with the sealer, and placed into the canal. A spreader was placed into the canal to compact the obturation material and accessory gutta-percha points were used to fill the gap until the spreader could not enter the canal. Then obturation was cut using a heat carrier device.
Group 3: roots were obturated with NeoSealer as the sealer with the warm vertical compaction method. A gutta-percha point size #30 with %4 taper was coated with the sealer and then placed into the canal. Then it was cut with a heat carrier device 5 mm to the apex, after that a plugger (DiaDent, Cheongju, Korea) size #50 was used to downpack the obturation. Then, a thermoplastic gutta-percha was injected into the canal using a backfill device (Fast Fill, Eighteeth). A plugger size #60 was used to downpack the obturation.
Group 4: roots were obturated using a single cone of gutta-percha and AH-Plus (Dentsply Sirona) as the sealer. Obturation was done similarly to Group 1.
Group 5: roots were obturated with AH-Plus as the sealer with the lateral compaction method. Obturation was done similarly to Group 2.
Group 6: roots were obturated with AH-Plus as the sealer with the warm vertical compaction method. Obturation was done similarly to Group 3.

Bacterial leakage testing

The bacterial leakage test were accomplished according to the Yanpiset et al. protocol (19) by an operator blinded to the experiment. Briefly, all root surfaces were coated with two layers of varnish, except for the apical 2 mm,

which remained unsealed. A cut microcentrifuge tube (Eppendorf, Hamburg, Germany) was made for each obturated root. A segment of approximately 5 mm of the root was inserted through the cut end of a microcentrifuge tube, with about 9 mm positioned inside the tube. To ensure a tight seal between the root and the tube, cyanoacrylate adhesive (3M Super Glue Gel; 3M Company, Maplewood, MN, USA) was applied. Sterilization of the assembly was carried out using ethylene oxide gas. Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit, MI, USA) was sterilized in a glass bottle, into which the microcentrifuge tube was placed. Under aseptic conditions, approximately 2 mm of the apical root tip was submerged in the BHI broth.
A reference strain of Enterococcus faecalis (ATCC 29212) was cultured on BHI agar plates. Selected colonies were suspended in 3 mL of BHI broth and incubated overnight at 37 °C. The bacterial concentration was adjusted to match a 0.5 McFarland standard, equivalent to 1×10^8 CFU/mL. The upper chamber of the setup was inoculated with 400 µL of the E. faecalis suspension and incubated at 37 °C. To maintain bacterial viability, the culture medium was refreshed every 48 hours under sterile conditions.
Bacterial leakage was assessed daily over a 60-day period by observing turbidity in the BHI broth. Turbid samples were further analyzed using Gram staining and bacterial culturing to confirm the presence of E. faecalis. The time to leakage, measured in days, was recorded and validated

Statistical analysis
Data was entered into SPSS software version 22 (IBM; Chicago; IL, USA), and the mean and standard deviation of the days that leakage occurred for the samples were calculated. The normality of the data was checked by the Shapiro-Wilk test. The data did not have a normal distribution, so the Napierian logarithm of the data was calculated and analyzed.
The difference is measured by two-way ANOVA and Tukey-test, and a difference of more than 95% is considered significant.

Results

In this study, 90 mandibular molars were examined. When preparing the samples for the bacterial leakage test, 2 samples were excluded from the experiment due to not being sterile when applying the BHI culture. Six examination groups were tested, and each group contained 15 samples.

The variables were the sealer type (two types) and the obturation technique (three obturation techniques).
The mean and standard deviation of the groups were 9.64 (14.773), 17.53 (18.639), 13.21 (20.196), 12.47 (14.272), 7.73 (8.892), and 8.53 (10.398) days for group 1 to 6 respectively (Table 1).

By using the two-way analysis of variance, the means of leaked days were compared between groups for sealer and obturation variables. Based on these results, no significant difference was found between two types of sealer and three types of obturation, as well as the combination of sealers and obturations (Table 2).

Table 1
Mean and Standard Deviation of bacterial leakage (days)

Obturation Technique	Sealers	N	Mean	Std. Deviation
Single-Cone	AH-Plus	15	12.47	14.272
	Bio-C	14	9.64	14.773
	Total	29	11.10	14.326
Cold Lateral Compaction	AH-Plus	15	7.73	8.892
	Bio-C	15	17.53	18.639
	Total	30	12.63	15.190
Warm Vertical Compaction	AH-Plus	15	8.53	10.398
	Bio-C	14	13.21	20.196
	Total	29	10.79	15.783
Total	AH-Plus	45	9.58	11.347
	Bio-C	43	13.56	17.900
		88	11.52	14.960

Source	Type III Sum of Squares	df	Mean Square	F	P value
Corrected Model	5.580a	5	1.116	.927	0.468
Intercept	301.438	1	301.438	250.329	0.000
Obturation	1.227	2	0.613	0.509	0.603
sealer	.867	1	0.867	0.720	0.399
Obturation * sealer	3.389	2	1.694	1.407	0.251
Error	98.742	82	1.204		
Total	406.620	88			
Corrected Total	104.322	87			

Table 2
Tests of Between-Subjects Effects.

Discussion

The final aim of endodontic treatment is to prevent coronal bacteria from leaking through the canal and reaching the periapical region (20). Cleaning and shaping cannot sterilize the canal from bacteria and their by-products, so a sealed obturation is necessary to avoid bacterial leakage, leading to the long-term success of the treatment (21, 22). This study investigated the bacterial leakage of two resin (AH-Plus) and bioceramic (Neo-sealer) sealers in different obturation methods to identify the better root canal sealing protocol.
The bacterial leakage of AH-Plus and Neo-sealer in combination with different obturation techniques showed that there was no statistical difference between the groups. AH Plus is an epoxy resin-based sealer characterized by optimal flowability and viscosity, which facilitates deeper penetration into the root canal system. It interacts with the dentinal surface by creating a mild etching effect, exposing collagen fibers and thereby enhancing its adhesion to the dentinal walls. On the other hand, Neo-sealer is a bioceramic sealer that was introduced to endodontics recently and got more attention because of its biocompatibility, hydrophilicity, antibacterial properties, high pH, and no shrinkage after setting (2). In addition, it is usually recommended with the single-cone technique. This technique is easier to use and takes less time for application (23).

Kaul et al. study compared the sealing ability of resin, bioceramic, and silicon sealers. They concluded that the resin and bioceramic sealers had no differences in sealing ability when applied in the single cone obturation technique (5). Also, Lanker et al, concluded that bioceramic and resin sealers had the same sealing ability in lateral and vertical condensation techniques (1). The results of these studies are consistent with the current study. The similarity in the sealing ability results, in resin and bioceramic sealers, may be due to the thorough application of the sealer into the canal to prevent the formation of voids (1-5).
However, some studies have results contradictory to the current study. Antunovic et al. investigated the sealing ability of five sealers including four bioceramic sealers and a resin-based sealer, in single cone technique and lateral condensation technique. They concluded that TotalFill BCS sealer is superior to the other bioceramic sealers and resin-based sealers in sealing ability (24). They claimed that TotalFill BCS sealer releases more calcium hydroxide which leads to higher PH and antibacterial properties (24). In the other study, Trivedi et al. concluded that in comparing one resin-based and two bioceramic sealers, the Bio C sealer is superior to the others in sealing ability (6). They also claimed that Bio C sealer stimulates regeneration (6). Nevertheless, in these studies, some groups of bioceramic and resin-based sealers had no

differences in sealing ability.
The difference in chemical properties of different bioceramic sealers used in these studies may lead to different results obtained. Also, the evaluation method is one of the differences that may cause study results contradictory. Furthermore, the irrigation system used (using EDTA or maleic acid) in the studies can improve the sealing ability of the sealers (2). Standardization of canal morphology is also essential when comparing the sealing performance of different obturation protocols (25). Such variability may influence sealer distribution and leakage outcomes, underscoring the importance of using canals with comparable anatomy when evaluating sealing ability. To achieve more comprehensive results the sealers and obturation techniques should be examined mutually. This study tried to experiment with the sealers and obturation techniques in combination with each other but the results shouldn't extend to all the cases. For future studies, different bioceramic sealers in combination with obturation techniques should be investigated to achieve more reliable results.

Conclusions

The comparison between Neo-sealer as a bioceramic sealer and AH-Plus as a resin-based sealer in different obturation techniques revealed that no difference was found in coronal bacterial leakage. In clinical practice based on the situation, the clinicians can use either of the techniques and materials.

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