

Evaluation of Fracture Resistance of Roots Obturated with Three Different Sealers and Three Various Obturation Techniques

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ABSTRACT

Objective: The objective of this investigation was to assess and compare the fracture resistance of roots filled with AH Plus (AHP), Total Fill bioceramic (TFBC), and AH Plus bioceramic (AHBC) sealers using a single cone, warm vertical compaction (WVC), and Soft-Core techniques.

Methods: This study used the palatal root canals of eighty extracted human maxillary first molars. All roots were sectioned to maintain a uniform root length of 11 mm. The samples were mechanically prepared using EdgeFile X7 rotary files to size 40/0.04. Eight teeth were left unfilled as a control group, while the remaining teeth were classified into three primary categories according to the sealer used for obturation: AHP, TFBC, and AHBC. Each group was divided into three subgroups (n=8) based on the obturation technique: single cone, WVC, and Soft-Core. Every tooth was set into blocks of acrylic resin, and universal testing equipment (Instron Corp) with a metal-like spreader tip was used to measure the fracture force at a speed of 0.5 mm/min. The collected data were examined using ANOVA, which was succeeded by Tukey's test.

Results: The control group's fracture resistance values were significantly less than the obturated groups in the study. Overall, the fracture resistance of AHP and AHBC was significantly higher than that of TFBC sealers. The WVC and Soft-Core were significantly higher than single cone techniques. The obturation technique did not significantly influence the fracture resistance of AHP and TFBC. While the fracture resistance of AHBC was significantly impacted, WVC was significantly higher than the single cone group. When roots were obturated with the WVC technique, AHBC exhibited statistically significant higher values of fracture resistance than AHP and TFBC. There was no significant difference between the three sealers when a single cone and Soft-Core were used.

Conclusion: According to this in vitro investigation, Obturation with AHP and AHBC sealers enhanced the fracture resistance of the roots more significantly than TFBC sealer, while obturation with WVC and Soft-Core yielded greater fracture resistance compared to the single cone approach.

Keywords: AH Plus, AH plus bioceramic, fracture resistance, single cone technique, soft-core technique, total fill, warm vertical compaction

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HIGHLIGHTS

- Using AHP, TFBC, and AHBC sealers in conjunction with different obturation techniques, including single cone, warm vertical compaction, and Soft-Core, improved the fracture resistance of endodontically treated roots.
- Both AHP and AHBC sealers demonstrated markedly enhanced fracture resistance in comparison to TFBC.
- The thermoplasticised obturation techniques yielded enhanced fracture resistance relative to the single cone technique.
- Obturation using AHBC with WVC was a more effective combination providing the highest fracture resistance.

INTRODUCTION

Over the last decade, awareness of vertical root fracture following endodontic treatment has increased. The third most frequent cause for extracting endodontically treated teeth is vertical root fracture (1). The strength of teeth undergoing endodontic treatment is affected by tissue loss in both coronal and radicular areas resulting from previous pathology or trauma, as well as factors related to the treatment itself, such as over-instrumentation, irrigation, medicaments, and applying too much pressure during root obturation, all of which heighten the risk of tooth fracture (2).

It is believed that obturation of the root canal strengthens the tooth by making it more resistant to compressive stress. Consequently, root canal sealers that enhance the structural integrity of the root against fractures are of significant value (3). Recent research approaches have developed substances that improve adherence to the root canal system.

Adhesion and mechanical interlocking are thought to improve the integrity of the remaining structure of the tooth, hence diminishing the likelihood of fracture (4).

AH Plus sealer (Dentsply Sirona, Konstanz, Germany, AHP) is an epoxy resin-based sealant known as an easy-handling material with high dentin and gutta-percha surface wettability and effective sealing ability. Root canal sealer based on resin can penetrate dentinal tubules. It may form monoblocks between intraradicular dentine and root canal filling material, increasing fracture resistance by maintaining the integrity of the sealer-dentine interface (5, 6). Total Fill bioceramic Sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland, TFBC) is an injectable, pre-mixed 'calcium silicate-based bioceramic root canal sealer' that sets when it comes into contact with water. It possesses 'excellent antimicrobial ability, higher pH, and exceptional biocompatibility'. TFBC sealer easily penetrates dentinal tubules due to the existence of nanoparticles additionally it does not shrink while setting and demonstrates excellent physical properties (7). AH Plus Bioceramic (Dentsply Sirona, York, PA, USA, AHBC) represents a novel premixed calcium silicate-based sealant with 'hydraulic properties and adequate flowability. According to the manufacturer, the set time is fast and predictable. It has low solubility, increased washout resistance, biocompatibility, lower film thickness, better radiopacity, no tooth discolouration, and can be easily removed with a NiTi or hand file. AHBC differs from other calcium silicate-based materials because of its unique composition.

In contrast to other calcium silicate-based materials that include di- and tri-calcium silicates as reactive components, AHBC sealer comprises solely tricalcium silicate. AHBC contains dimethyl sulfoxide as a filler, a feature absent in other calcium silicate-based sealers. This leads to a reduced proportion of calcium silicates in comparison to premixed sealants like TFBC (8).

According to the literature, there was no data that evaluated the fracture resistance of teeth obturated using AHBC Sealer with different obturation techniques. Therefore, the goal of the current study was to assess and compare the fracture resistance of endodontically treated roots filled with various

sealants, including AHP, TFBC, and AHBC, using a single cone, warm vertical compaction (WVC), and Soft-Core techniques.

The null hypotheses of this investigation were as follows: first the root canal sealer does not influence the vertical root fracture resistance of endodontically treated teeth. Second, the obturation technique does not influence the root fracture resistance of endodontically treated teeth. Third, the sealer/obturation technique interaction does not influence the occurrence of vertical root fractures in endodontically treated teeth.

MATERIALS AND METHODS

The institutional Ethics Committee approved the protocol (Project NO. MUOPR29, Ref.NO. REC130 on 1/5/2023) for this current study. The study was conducted following the Declaration of Helsinki.

This study employed eighty extracted maxillary first molars distinguished by circular and straight palatal root canals and fully formed apices. To establish an accurate reference point for measurement, the palatal roots were vertically divided along the long axis at the furcation region. Sectioning the palatal roots resulted in a consistent length of 11 mm. The canal's initial size was defined using a size 20 K-file. The EDGEENDO X7 rotary system files were used for instrumentation, beginning with a size 20/0.04 rotary file, followed by sizes 25/0.04, 30/0.04, 35/0.04, and 40/0.04. The procedure was conducted at a speed of 300 rpm and a torque of 300 g-cm until the working length was reached, using two movements for each file through a gentle push-pull motion. Recapitulation was executed using a size #20 hand K-file between each rotary file to preserve the glide path and enhance the lubricant's penetration to the canal terminal. During canal preparation, 1 ml of 2.5% sodium hypochlorite (NaOCl) irrigation was employed between instruments using a 30-gauge side-vented needle, placed 2 mm shorter than the working length to aid in debris elimination. The canals were finally irrigated with 2 mL of a 2.5% NaOCl solution for 1 minute. Following that, a 2 mL solution of 17% ethylenediamine tetraacetic acid (EDTA) was administered for one minute. Finally, 5 millilitres of distilled water were used. After the irrigation procedures, the root canals were dried using paper points (40/0.04) (9). At this stage, eight randomly selected shaped but unfilled teeth were isolated to function as a control group. The other 72 teeth were randomly allocated into three main categories based on the sealer used (n=24). Group A: AHP, Group B: TFBC, Group C: AHBC. Each group subdivided into 3 subgroups according to obturation techniques. Were used (n=8). 1. Single cone 2. WVC 3. Soft-Core.

In the single cone procedure, after the apical fit of the master gutta-percha cone was verified via digital radiography, sealers were applied per the manufacturer's guidelines, A 40/0.04 master gutta-percha cone, demonstrating sufficient tug-back, was covered with sealant and progressively placed into the canal until the working length was reached. A plugger was used to carefully condense the cone after it was severed at the orifice level.

Regarding WVC, a plugger that is 4 mm shorter than the working length was used for a binding point. After applying the sealer per the manufacturer's guidelines, the master cone

TABLE 1. Two-way ANOVA test between subjects affect the fracture resistance

Source	Type III sum of squares	df	Mean square	F	Sig.
Sealer	59223.250	2	29611.625	9.777	<0.001
Obturation technique	66445.750	2	33222.875	10.970	<0.001
Interaction	60924.250	4	15231.063	5.029	0.001
Error	190804.625	63	3028.645		
Total	4680109.000	72			
Corrected Total	377397.875	71			

ANOVA: Analysis of variance, df: Degrees of freedom, Sig.: Significance

40/0.04 was put into the canal. A heated plugger was then inserted into the canal to trim the master cone, retaining only 4 mm of the apical gutta-percha. The canal was backfilled with warm gutta-percha injection using CV-Fill (Cicada) at a temperature of 200°C. The needle was positioned against the apical gutta-percha for 5 seconds before the extrusion of the gutta-percha. The bulk of the gutta-percha displaced the needle coronally to the canal orifice; A plugger was subsequently used to compress the gutta-percha at the orifice level.

For the Soft-Core technique, the Size Verifier was used to determine the proper size of the plastic core obturator from Soft-Core System Aps (Copenhagen, Denmark). The application of sealers according to the manufacturer's instructions, the obturators were heated using the Soft-Core DT Oven (CMS, Orange, Copenhagen, Denmark) set to a high temperature and were slowly inserted with gentle pressure until they reached the predetermined working length, maintaining their position for a few seconds. By twisting, the handle and insertion pin were separated. The extra plastic core material was eliminated with a small inverted cone bur, and any surplus gutta-percha was eliminated. After that, the gutta-percha was pressed down vertically with a plugger.

The specimens were incubated for seven days at 37°C and 100% relative humidity to verify that the sealers were fully set.

To simulate a periodontal membrane, a 0.2–0.3 mm thick wax material was applied to 9 mm of all roots apically. A digital calibre was used to determine the consistent thickness of the wax. The samples were placed vertically in self-curing resin cylinders (15 mm in height and 20 mm in diameter), embedding 9 mm of root length. Once the acrylic resin began to polymerise, the roots were removed from the acrylic, and then the wax was removed with the blade. A thin layer of polyvinylsiloxane impression material was applied to the root surfaces before returning them to the acrylic resin. A universal testing machine "Instron Corp." was used to conduct the fracture resistance testing. Samples were positioned at the base of the machine, and a specialised metal spreader featuring a tip diameter of 0.8 mm was affixed to the top. A vertical force was exerted throughout the long axis of the root, with the metallic tip positioned centrally over the canal orifice. A vertical force was gradually exerted at a rate of 0.5 mm/min until the root fractured. The fracture was identified by a sharp, sudden drop of more than 25% in the applied force. In most cases, a distinct sound was heard at the moment of fracture, and the force required to induce fracture was documented in Newtons (5, 10, 11).

Statistical Analysis

SPSS V.27 (IBM, New York, USA) was used to statistically analyse the fracture load data. The Shapiro-Wilk test was employed to assess data normality. A two-way ANOVA was used to evaluate the effects of sealer type and obturation technique on fracture resistance, followed by Tukey's Post Hoc test for multiple comparisons. One-way ANOVA was used to examine the differences between the obturated group and the control group, followed by Dunnett's two-sided test. The level of significance was set at 0.05 ($p \leq 0.05$).

RESULTS

The Shapiro-Wilk test was used to evaluate the normality of the data in this investigation and confirmed that the distribution was normal ($p > 0.05$).

The Two-Way ANOVA test indicated that both variables, the sealers, and the obturation techniques, as well as their interaction, had a highly significant effect on the resistance to fractures ($p < 0.001$, $p < 0.001$, $p = 0.001$, respectively) (Table 1).

The assessments of the sealer impact across the groups indicated that the fracture resistance in the AHP and AHBC groups was significantly greater than that in the TFBC groups ($p < 0.05$). Regarding the impact of the obturation technique, the fracture resistance for WVC and Soft-Core techniques was significantly higher than for single cone technique ($p < 0.05$), and There was no substantial difference between Soft-Core & WVC approaches ($p > 0.05$) (Table 2).

To better understand the impact of the obturation approach in conjunction with the sealant, all results were evaluated using the Tukey HSD test for pairwise comparisons at 95% confidence intervals (significance level 0.05) (Table 3).

The fracture resistance was not significantly affected by the obturation technique in the AHP and TFBC groups. This was verified by pairwise comparisons, indicating no significant differences among the groups (Table 3a, b).

In the AHBC groups, the obturation technique significantly influenced fracture resistance. Comparisons indicated that the fracture resistance of the WVC was markedly superior to that of the single cone, whereas no significant difference was observed between the Soft-Core and the other two techniques (WVC, single cone) (Table 3c).

When comparing the same obturation techniques using three different sealers, a significant difference was observed just in the WVC technique, where the AHBC sealer exhibited

TABLE 2. Mean±SD of the fracture resistance for the three obturation techniques tested with AH Plus, Total Fill, and AH Plus BC sealers with the total means and pooled SD with intergroup comparisons

Sealer	Obturation technique			Total mean*
	Single cone technique	Warm vertical compaction technique	Soft-core technique	
AH Plus	205.875±47.7327	254.25±61.23899	291±65.98485	250.292±71.10863 ^a
Total Fill	205.5±48.8233	211.75±51.0091	203±39.10426	204.792±48.48754 ^b
AH Plus BC	195.375±43.16393	351.5±68.37919	281.875±61.81872	276.167±90.20396 ^a
Total mean	202.25±44.84005 ^d	272.5±83.22625 ^c	258.625±67.69356 ^c	244.4583±72.90722

*: Total Mean values denoted by different superscript letters indicate statistically significant differences ($p < 0.05$). SD: Standard deviation

TABLE 3. Post hoc Tukey's test for multiple comparisons for the sealer-obturation technique interaction**A: Comparison between AH Plus groups**

Pairwise comparisons*	WVC-AH Plus soft-core -AH Plus	
Single cone -AH Plus	($p=0.709$)	($p=0.068$)
WVC-AH Plus		($p=0.917$)

B: Comparison between Total Fill groups

Pairwise comparisons*	WVC-TotalFill	Soft core -TotalFill
Single cone -TotalFill	($p=1.00$)	($p=1.00$)
WVC-TotalFill		($p=1.00$)

C: Comparison between AH plus BC groups

Pairwise comparisons*	WVC-AH Plus BC	Soft core -AH Plus BC
Single cone -AH Plus BC	($p=0.000$)	($p=0.060$)
WVC-AH Plus BC		($p=0.238$)

D: Comparison between the same obturation technique groups with the different sealers

Pairwise comparisons*	Single cone -AH Plus	Single cone -TotalFill
Single cone -AH Plus BC	($p=1.00$)	($p=1.00$)
Single cone -AH Plus		($p=1.00$)
Pairwise comparisons*	WVC- AH Plus	WVC-TotalFill
WVC-AH Plus BC	($p=0.021$)	($p=0.000$)
WVC-AH Plus		($p=0.830$)
Pairwise comparisons*	Soft core-AH Plus	Soft-core -TotalFill
Soft-Core-AH Plus BC	($p=1.00$)	($p=0.117$)
Soft-Core-AH Plus		($p=0.052$)

WVC: Warm vertical compaction

markedly greater fracture resistance than both the AHP and TFBC sealers (Table 3d).

The One-Way ANOVA test indicated a statistically significant difference between the control group and all obturated groups ($p < 0.001$) (Table 4). Obturated groups were statistically substantially greater than the control group ($p < 0.05$), according to Dunnett's two-sided test (Table 5).

DISCUSSION

The primary objective of obturation is to prevent the passage of bacteria and their by-products into the root canal space. Additionally, it aims to enhance the strength of roots by mechanically interlocking the obturating material with radicular dentine, thus increasing fracture resistance (12).

Statement of Principal Findings

The findings indicated that the sealer, obturation approach, and their interaction had a significant impact on the fracture

strength of the obturating materials in relation to the canal walls, resulting in the rejection of the null hypothesis.

Strengths and Weaknesses of the Study

In this study, to standardise roots of comparable size, length, and diameter were employed in this investigation. Standardised instrumentation and irrigation were implemented in all experimental groups. The study's principal weakness is the use of a single static load, which may not accurately represent clinical conditions; consequently, fractures could occur earlier under cyclic loading conditions.

Strengths and Weaknesses in Relation to Other Studies, Discussing Particularly Any Differences in Results

The unobturated group exhibited significantly less fracture resistance than the obturated groups, likely attributable to diminished dentine thickness resulting from root canal preparation and the absence of a filling substance to support the tooth structure, confirming findings from previous studies (5, 13).

TABLE 4. One-way ANOVA test for comparison of significance between the obturated groups and control group

	Sum of squares	df	Mean square	F	Sig.
Between group	304711.700	9	33856.856	12.174	<0.001
Within groups	194676.500	70	2781.093		
Total	499388.200	79			

ANOVA: Analysis of variance, df: Degrees of freedom, Sig.: Significance

The mean fracture resistance value with AHP sealer was substantially higher than that of TFBC sealer, irrespective of the obturation technique employed in this study. Multiple justifications are attributed to this outcome. AHP sealer, an epoxy resin-based material, demonstrates advantageous properties, specifically adhesion achieved through the formation of the covalent link between the exposed amino acids in collagen and the open epoxide ring. Additionally, because of its creeping tendency, AHP has a better ability to penetrate surface micro-uniformities, which results in increased fracture strength (14). These findings are consistent with previous research (15, 16).

The AHBC sealer exhibited significantly higher fracture resistance than the TFBC. While its performance was similar to AHP sealer, due to variations in physicochemical properties and composition. AHBC contained a greater quantity of zirconium compared to TFBC. The inclusion of zirconium may enhance flow properties and decrease the film thickness of the sealer (17). Also, the presence of zirconium exhibits notable properties such as higher fracture strength, elevated tensile toughness, and low Young's modulus (18). Additionally, AHBC contains dimethyl-sulfoxide, which is not present in TFBC. Dimethyl-sulfoxide serves as a solvent that enhances dentine wettability in sealers and contributes to the improvement of both immediate and long-term adhesive bond strength of a sealer (19, 20).

Irrespective of the sealer employed, the thermoplasticised gutta-percha obturation approach (WVC, Soft-Core) demonstrated significantly superior results compared to the single cone technique. These results may be attributed to an enhanced

flow of warm gutta-percha, which creates a uniformly mixed mass with minimal sealer (21), facilitating better material retention (22). Thermoplasticised techniques allow gutta-percha to become soft and pliable, enabling deeper penetration into difficult-to-reach areas of the root canal system, such as deep depressions and lateral canals. This process results in a higher volume of gutta-percha, reduced sealer usage, and fewer unfilled gaps compared to the single cone technique (23). Also, the core carrier used in the Soft-Core technique adequately supported the root dentine, thereby enhancing the fracture resistance of the roots (24, 25). This aligns with the findings of Mounes and Alhashimi in 2022 (26), who reported higher bond strength when using WVC and carrier-based techniques in comparison to the single cone method. This study challenges the conclusions of Al-Hiyasat et al. in 2023 (27) and Pandey et al. in 2024 (28), which indicated that filling with a single cone yielded greater fracture resistance than WVC. The difference may be due to the differing methodologies employed in the two studies. No substantial difference was seen between Soft-Core and WVC, which agrees with the findings of Topçuoğlu et al. in 2013 (29).

The evaluation of the impact of obturation techniques on the fracture resistance of the sealer revealed that obturation techniques did not significantly affect the fracture resistance of the AHP sealer, aligning with the findings of prior research (24, 25, 29), while contradicting the results of Al-Hiyasat et al. (27) which indicate that obturation with single cone yields greater fracture resistance than WVC. This difference may be attributable to variations in methodology. Additionally, there was no significant effect of obturation procedures on TFBC. This finding aligns with the result of previous studies (14, 25) but contradicts the results of Al-Hiyasat et al. (27), which indicated that obturation of the root canal using TFBC sealer with single cone yielded greater fracture resistance compared to WVC. The fracture strength of AHBC was significantly higher when WVC was employed in comparison to the single cone method. Eid et al. 2021 (30) found that the application of WVC enhanced the capacity of calcium silicate-based sealants to infiltrate dentinal tubules in comparison to the single cone approach. According to Alegre et al. in 2022 (31), the WVC showed more sealer penetration than the single cone technique. Furthermore, there was no significant

TABLE 5. Dunnett's (2-sided) test for multiple comparisons between obturated groups and control group

(I) Interaction	(J) Interaction	Mean difference (I-J)	Standard error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
AH Plus single cone	Control	89.50000*	26.36803	0.009	16.8064	162.1936
AH Plus + WVC	Control	137.87500*	26.36803	0.000	65.1814	210.5686
AH Plus+Soft-core	Control	174.62500*	26.36803	0.000	101.9314	247.3186
Total Fill+Single cone	Control	89.12500*	26.36803	0.009	16.4314	161.8186
Total Fill+WVC	Control	95.37500*	26.36803	0.004	22.6814	168.0686
Total Fill+Soft-core	Control	86.62500*	26.36803	0.012	13.9314	159.3186
AH plus BC+Single cone	Control	79.00000*	26.36803	0.027	6.3064	151.6936
AH Plus BC+WVC	Control	235.12500*	26.36803	0.000	162.4314	307.8186
AH Plus BC+Soft-core	Control	165.50000*	26.36803	0.000	92.8064	238.1936

WVC: Warm vertical compaction

difference between the Soft-Core technique and the other two methods, WVC and single cone. A direct comparison with previous results was impossible as no previous studies had examined the effects of these obturation techniques on AHBC sealer.

When comparing the fracture resistance of each obturation technique for the three distinct sealers, the fracture resistance of the three sealers in the single cone and Soft-Core approaches were comparable, exhibiting no significant difference. These findings were consistent with previous studies (14, 24, 29, 32–35). but differed from those of Mohammed and Al-Zaka in 2020(5) and Al-Hiyasat et al. in 2023 (27), who reported that filling with TFBC sealer enhanced the fracture resistance of the roots more significantly than AHP sealer. When WVC was employed for obturation, the fracture resistance of AHBC sealant significantly exceeded that of AHP sealant. Shieh et al. in 2023 (18) reported that the AHBC sealer exhibited significantly deeper penetration into the dentinal tubules compared to the AHP sealer, likely due to its superior fluidity, smaller particle size, and reduced film thickness (20 mm for AHBC versus 80.5 mm for AHP sealer), thereby enhancing the fracture resistance of the root (36). This outcome may also be related to the influence of heat on the composition and setting time of the epoxy resin sealer. Reports indicate that heat application during WVC expedites the setting reaction, hence diminishing the setting period, which could reduce the flowability and infiltration of the sealer into canal abnormalities and dentinal tubules (25).

Additionally, Atmeh and AlShwaimi in 2017 (37) revealed that the chemical structure of AHP was altered when exposed to heat. Additionally, it was found that AHBC with WVC had a higher fracture resistance than TFBC with WVC. This could be because the AHBC sealer had a finer particle size, a more densely packed structure, and a much thinner film than TFBC. The smaller particle sizes and lower viscosity improved its flowability inside dentinal tubules, which allowed for deeper penetration into canal irregularities (38). Lertchirakarn et al. (39) asserted that the infiltration of sealers correlated directly with their fracture resistance. It has been reported that the flowability of the TFBC sealer can be diminished by subjecting it to heat. The decreased flow could impair the mechanical interlocking links by preventing sealer particles from diffusing into the dentinal tubules (40).

- i. Meaning of the study: possible mechanisms and implications for clinicians or policymakers.

In order to improve the fracture resistance of weakened or thin roots and the long-term success of endodontic treatments, clinicians should prefer the use of AHP or AHBC sealers in conjunction with thermoplasticised obturation techniques (WVC or Soft-Core).

- i. Unanswered questions and future research.

Future research should focus on randomised clinical studies to validate *in vitro* findings, as well as cyclic fatigue testing to imitate long-term functional stress. Additionally, comprehensive studies are needed to evaluate under-explored properties of AHBC sealer (e.g., bioactivity, solubility, dimensional stability, retreatability) and compare its performance against emerging bioceramic and resin-based alternatives.

CONCLUSION

Within this study's constraints, several conclusions were drawn:

1. The application of AHP, TFBC, and AHBC sealers in conjunction with single cone, WVC, and Soft-Core enhanced the fracture strength of endodontically treated roots.
2. The fracture strength of AHP and AHBC was markedly superior to that of TFBC sealant.
3. The fracture resistance of WVC and Soft-Core was significantly greater than that of the single cone technique.
4. The obturation techniques did not significantly impact the fracture resistance of AHP and TFBC sealers.
5. The fracture strength of AHBC combined with WVC was significantly greater than that observed with the single cone technique.

Disclosures

Ethics Committee Approval: The study was approved by the Mustansiriyah University, College of Dentistry Ethics Committee (no: REC130, date: 01/05/2023).

Informed Consent: Informed consent was obtained from all participants.

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