

Physicochemical and Antimicrobial Properties of Bioceramic Sealer Enhanced with Silver Nanoparticles: An in vitro Evaluation

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Aim: The aim of this study was to determine the impact of different nanoparticle concentrations with endodontic bioceramic sealer. It was assessed the combination by analyzing the correlation between the degree of conversion (DC) and antibacterial efficacy. And assess the penetration depth into the lateral canals.

Materials and Methods: The AH Plus bioceramic sealers were mixed with silver nanoparticles (Ag) at different concentrations (2.5%, 5%, 10%). The antibacterial efficacy against *Enterococcus faecalis* was evaluated utilizing the direct contact test (DCT) at multiple time intervals (immediately, 30 minutes, 1 day, 2 days, and 7 days). The conversion degree was evaluated by FTIR. The penetration depth was evaluated using X-ray examination.

Results: The antibacterial activity increased with higher concentrations of nanoparticles, with silver nanoparticles demonstrating a pronounced effect on *E. faecalis*. The conversion degree increased with high quantities of silver nanoparticles. Positive, strong relation between the degree of conversion and antimicrobial activity. The penetration depth decreased as nanoparticle concentration increased. A statistically significant difference was seen between the antibacterial activity values and the degree of conversion at different time intervals ($P = 0.05$).

Conclusion: The highest nanoparticles concentration (10%) has the greatest effect on the antibacterial activity and degree of conversion, the highest nanoparticles concentration decreased the penetration depth into the lateral canals.

Keywords: antibacterial efficacy, conversion degree, *Enterococcus faecalis*, lateral canals, depth of penetration

Introduction

A primary cause of endodontic treatment failure is the persistence of bacteria within the intricate canal anatomy, which is challenging to eliminate entirely through cleaning and disinfection protocols.¹ During endodontic procedures, the bacterial population is significantly diminished through instrumentation, irrigation, and intracanal medicaments.^{2,3} Furthermore, the antibacterial properties of root canal filling materials, including endodontic sealers, are commonly employed to reduce the concentration of residual microorganisms in the root canal.⁴

Previous studies have demonstrated that *E. faecalis* is one of the microorganisms present in nonvital teeth, especially in teeth with recurrent endodontic infection.⁵ It is the most common microorganism isolated from periapical lesions⁶ and is therefore widely used to investigate the antimicrobial action of endodontic bioceramic sealers.⁷ Root filling is therefore a critical step of root canal treatment preferably using dental materials with antimicrobial properties.⁸ The use of root canal sealers with antimicrobial activity is considered beneficial in reducing the concentration of residual microorganisms,⁹ preventing recurrent root canal infection and assisting the healing of periapical tissues.¹⁰

The degree of conversion (DC) is defined as the extent to which monomers react to produce polymers or as the ratio of C=C double bonds transformed into C-C single bonds. In the polymerization of bifunctional methacrylate, complete conversion is

never achievable due to diffusional limitations in the later stages of the reaction, which hinder some monomer molecules from accessing reaction sites. Consequently, the degree of conversion in dental composites typically ranges from 50 to 80%.¹¹

The Fourier Transformed Infrared Spectroscopy (FTIR) examination of the bioceramic sealer indicated that the large peaks at 3350 and 3594 cm^{-1} correspond to the stretching vibrations of hydroxyl ions within the crystal network of the bioceramic sealer.¹² The detected peaks at 2935 and 2900 cm^{-1} , along with a diminished peak at 1476 cm^{-1} , signify the stretching vibrations of C-H within the alkane group. The peaks in 1733, 1619, 1607 cm^{-1} , and 1170 cm^{-1} correspond to the stretching bands of C=O and C-O, respectively. The distorted peaks between 828 and 892 cm^{-1} correspond to Si-O-Si stretching vibrations and Si-(CH₃)₃.¹³ The decrease in the absorption bands at 3350 and 3594 cm^{-1} on the FTIR spectra is directly related to the DC, shown in this equation: $\text{DC \%} = (A_0 - A_t/A_0) \times 100$. $\text{DC \%} = (A_0 - A_t/A_0) \times 100$. (A_0) denotes the absorption peak (area of the band) at 3350 cm^{-1} at time = 0 (base paste prior to mixing), whereas (A_t) represents the absorption area of the band at various time intervals subsequent to sealer mixing. The band region at 3350 cm^{-1} will be quantified utilizing Motic Images Plus 3.0 software for image analysis.¹⁴

The penetration depth of an endodontic sealer that can extend into the most distal regions of a root canal demonstrates superior efficacy in targeting residual bacteria. Confocal microscopy was employed to assess the penetration depth of three distinct sealers (Sealer 26, GuttaFlow, Seal apex). Seal apex had the most profound penetration at distances of 3 and 5 mm from the root apex. No statistically significant difference was seen between Sealer 26 and Gutta Flow at the 3 mm and 5 mm levels.¹⁵ Scanning Electron Microscope (SEM) research of various bioceramic endodontic sealers, including Cortisomol, iRoot SP, AH Plus, and SE, revealed that SE exhibited the greatest penetration depth, succeeded by AH Plus, iRoot SP, and Cortisomol. In terms of adaptability to root canal walls, AH-Plus exhibited the highest adaptability, succeeded by iRoot SP.¹⁶

Problem Statement and Justification of Study

The importance of focusing on AH plus bioceramic sealer primarily resides in two key aspects; the first is to assess the impact of integrating various nanoparticles concentrations into the performance of the bioceramic sealer, particularly regarding its antimicrobial efficacy. The second objective is to assess the impact of integrating various nanoparticles concentrations on the penetration depth of bioceramic sealers into lateral canals. The findings and results will update the current knowledge of some advantages of the use of nanoparticles in the field of endodontics. Thus, it will be an eye-opener for the researcher and every clinician in their daily clinical practice.

Study Hypotheses H₀

1. There are no differences in the amount of NP concentration to affect the antimicrobial activity of the bioceramic sealer.
2. There are no differences in the amount of NP concentration required to affect the bioceramic sealer's conversion degree.
3. There are no differences in the amount of NP concentration required to increase the depth of penetration of the bioceramic sealer into the lateral canals.

Materials and Methods

The pre-mixed AH plus Bioceramic sealer (Dentsply Sirona/Germany), silver nanoparticles size 30–50 nm (Chengdu Alpha Nano Technology/China). Simultaneously, the silver nanoparticles were utilized at three concentrations: 2.5%, 5%, and 10%; these concentrations were selected in this study according to previous study done by Inaam Baghdadi.¹³ The weight of each sample is presented in Table 1.

The sealers and nanoparticles were measured using a precision scale (Micronal S/A, model AB 204, São Paulo, SP, Brazil). The mixing and integration process of the sealer and nanoparticles was performed on an unpolished glass plate with roughness average (Ra) 2–5 micrometers. The roughness of the surface will enhance the dispersion of the nanomaterial granules, hence improving their integration.¹³ The mixing procedure was uninterrupted and uniform until achieving homogenous consistency. The materials were categorized into four primary groups according to the concentrations of silver nanoparticles, thereafter, separated into five subgroups based on varying time intervals. Table 2 and Table 3 present a summary of groupings and subgroups. The preparation of the mixture was conducted ten times at each interval that mean each group has 50 samples and total number for all groups is 200 samples.

Table 1 Show the Weight of Each Sample to Get the Nanoparticles percentages¹³

	Nanoparticles (Gram) Silver	AH Plus Sealer Pastes (Gram)
Group 1 Control group	0 g	0.1300 g.
Group 2 2.5% nanoparticles	0.0033 g.	0.1267 g.
Group 3 5% nanoparticles	0.0065 g.	0.1235 g.
Group 4 10% nanoparticles	0.0130 g.	0.1170

Table 2 Kappa Score Table

Kappa Score	Agreement
0–0.2 m	Poor
0.21–0.5 mm	Fair
0.51–0.7mm	Moderate
0.71–0.85mm	Good
0.86–1mm	Excellent

Table 3 Presents a Summary of Groupings and Subgroups for Degree of Conversion

		Group A1 Immediate	Group A2 30 Min.	Group A3 24 Hours	Group A4 48 Hours	Group A5 7 Days
Bioceramic Sealer AH Plus	Control group 100%	55.353%	73.165%	90.606%	94.672%	100%
	Group A 2.5% Ag	57.625%	77.536%	94.653%	98.511%	100%
	Group B 5% Ag	71.512%	80.506%	95.579%	99.154%	100%
	Group C 10% Ag	72.275%	84.252%	98.361%	100%	100%

Fourier Transform Infrared Spectroscopy (FTIR)

A limited quantity of sealer samples from each time interval was combined with potassium bromide (KBr) using a mortar and pestle, then placed in a specialized metal holder, subjected to a pressure of 8 tons, and compressed into a disc shape with a diameter of 1 cm before being incubated at 37°C.¹⁷ The DC will be assessed at five intervals: immediately after the combination, 30 minutes, 24 hours, 48 hours, and 7 days. The newly prepared samples were spread in a thin layer on a specialized glass slab, a component of the FTIR apparatus (FTIR 600 Biotech Engineering Co. Ltd England), for measurement purposes. The band region that measured in this study was at 3350 cm⁻¹ will be quantified utilizing Motic Images Plus 3.0 software for image analysis.¹⁴

Antimicrobial Efficacy (Bauer–Kirby Test)

E. faecalis ATCC BAA-2128 was chosen in accordance with the specifications set by the Development Organization, USA. The bacteria were acquired from the Media Microbiology Laboratory in Erbil, Iraq. A brain heart infusion broth agar was injected with two isolated colonies of *E. faecalis* and allowed to incubate for 30 minutes. Twenty milliliters of Muller–Hinton (MH) agar was aseptically dispensed into sterile Petri plates, followed by the application of a loopful of inoculated brain heart infusion (BHI) broth onto the Muller-Hinton agar, which was then incubated aerobically for 48 hours at 37°C. Each group of AH plus bioceramic sealer was administered, with repetitions occurring 10 times for each sample at varying intervals.¹⁸ The samples were applied on a 6 mm diameter filter disc, positioned on the surface of infected Muller-Hinton media and incubated at 37°C. The zone of bacterial growth inhibition will be measured using a millimeter ruler.¹⁹

Depth of Penetration into the Lateral Canals

Forty single-rooted upper anterior canine human teeth were obtained from clinics at Ajman University and private clinics in Iraq. All patients gave written informed consent for their extracted teeth to be used for research purposes, scientific or educational purposes, and for their patient details to be published anonymously. Ethical approval was obtained from Tishk International University, USM University and Ajman University UAE. Teeth exhibiting root resorption, open apex, root fractures, and calcification were removed from the study. The items were subjected to ultrasonic scaling, immersed in a 6% sodium hypochlorite solution for 5 minutes to eliminate adherent soft tissue, and subsequently sterilized in an autoclave for 40 minutes. A liquid chemical germicide (eg, sodium hypochlorite 3% diluted 1:10 with tap water) was employed to reduce bacterial proliferation during storage. All samples were preserved in a premium container till the commencement of laboratory tests.

Preparation of the Root Canal and Formation of an Artificial Lateral Channel

A size 10 K-File (Maillefer, Dentsply Sirona) was employed to navigate the canal until it arrived at the apical foramen. The corrected working length (CWL) was determined by measuring 1 mm short of the apical foramen. Thereafter, the glide path was created using a size 15 K File (Maillefer, Dentsply Sirona). The root canal instrumentation was performed progressively using ProTaper rotary Next files X1, X2, and X3 (Dentsply Sirona, Germany) to the working length. Extensive irrigation with 3% NaOCl was employed following each instrument. Patency was confirmed using a size 10 K-File.

Three artificial lateral canals (ALCs) were created on either side of the mesial and distal regions. The preparation was conducted from the external root region at the coronal, middle, and apical thirds. The Scout race reamers, Iso size 10, taper 0.02, and length 21 mm (FKG Dentaire/Switzerland), were utilized with the slow-speed handpiece set at 800 rpm and a torque of 2.5. The reamer was positioned perpendicularly to the main axis during the process at 3, 6, and 10 mm from the root apex.

The scout race file was angulated perpendicularly to the major canal as it drilled from the external surface towards the canal. The lateral canal, with a length less than 1.0 mm at the apical level or greater than 2 mm at the coronal level, was excluded. Modification in the custom-made device to receive the handpiece and to be sure that the preparation of the lateral canals is done at a right angle with tooth. The tooth was attached using a custom-made device to secure and stabilize the sample during the experimental test. In addition, a level device and L shape angle measurement were used to ensure that the insertion area was straight and that the scout race reamer insertion angle was about 90 degrees.

The level device was also used to check the handpiece with different levels by putting this level device on the top of the handpiece to ensure the level vertically is at 90 degrees and straight from the horizontal level; in addition, the holder of the handpiece has threads to ensure that the handpiece not moved from all direction during the drilling procedure. This checking is repeated after every drilling procedure. Two jaws of the custom-made device held the tooth to prevent movement of the tooth during the drill, and the amount of force of holding was determined through the same number of serrations used through the holding of teeth to ensure all teeth and all surfaces received the same compression force during holding. As shown in the [Figure 1](#) that contains subfigures ([Figure 1a](#) and [b](#)), and [Figures 2](#) and [3](#).

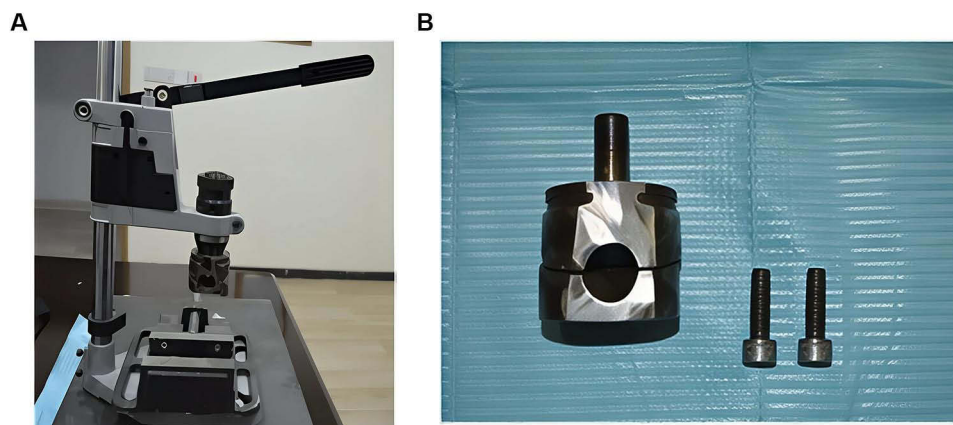


Figure 1 Contain subfigures (**A** and **B**) which explain the Custom-made device after modification of head to received handpiece for lateral canal preparation.



Figure 2 Level device that fixed on the top of handpiece during preparation of the lateral canals.

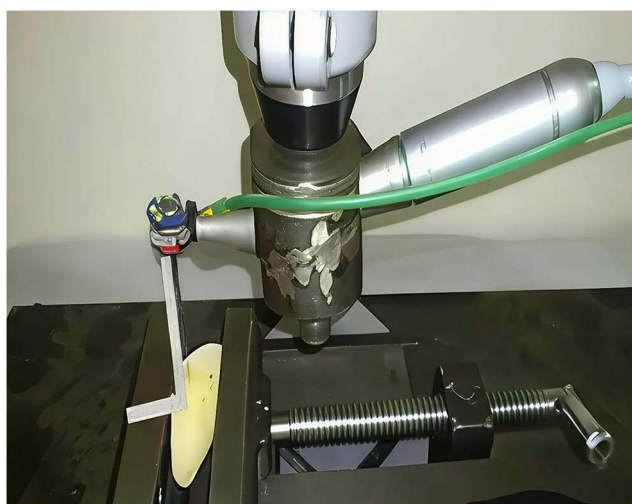


Figure 3 Experimental apparatus used for testing with leveler and L-shape angle measurement.

Application of Sealant into the Lateral Canal

The sample's main canals were dried using paper points (size x3) (Dia Dent/South Korea). The master gutta-percha with the size standardized to the X3 ProTaper Next (Dia Dent/South Korea) was selected to fit full working length. All samples were embedded in silicone putty to cover the root area to simulate the presence of a periodontal ligament (Coltene).²⁰ They were randomly assigned into 4 groups (Table 4). Ten samples for the control group and 10 samples for each experimental group (total groups samples are 40) The sealer was applied into the canal with a sterile paper point (size x2, Dia Dent/South Korea), rotated counterclockwise to form an even layer of a thin coating on the canal wall, repeated twice. The obturation process was completed with a single-cone technique. The apical part of the master cone was lightly coated with sealer and gently inserted into the canal.²⁰

Radiographical Assessment of the Depth of Penetration

The specimens will be imaged using a mounted X-ray device (Vatech EzRay Air wall/Italy, Ez Sensor classic 2.0 tech) to score the sealer's penetration depth (in millimeters) into the artificial lateral canals ALC. The depth was evaluated at different levels by 2 radiologist examiners depending on the scores of Table 2.

Table 4 Demonstrate the Significant Differences Between AH Plus Bioceramic Sealer Modified with Silver Nanoparticles with Different Concentrations (2.5%,5%,10%) Measured in Millimeters

	AH Plus	AH Plus + Silver 2.5%	AH Plus + Silver 5%	AH Plus + Silver 10%
Mean	1 mm**	0.85 mm	0.6 mm	0.02 mm
Chi-square + significance	57.50 *			

Notes: *Mean significant at p value = 0.05 according to chi- square analysis. **mm mean depth of penetration in millimeters.

Statistical Analysis

The data of this research were analyzed using one-way ANOVA for degree of conversion. Kruskal–Wallis test and Mann–Whitney *U*-test for antibacterial activity of AH plus bioceramic sealer. Pearson Correlation test and Linear Regression test (*R*) to evaluate the relation between degree of conversion and antibacterial activity of AH plus bioceramic sealer. The depth of penetration data was analyzed by the Kruskal–Wallis test.

Results

The measurements of this research were categorized into four primary groups according to the concentrations of silver nanoparticles, thereafter, separated into five subgroups based on varying time intervals. [Table 3](#) for degree of conversion and [Table 5](#) for antibacterial activity present a summary of groupings and subgroups.

The degree of conversion data of the AH Plus bioceramic sealer enhanced with silver nanoparticles were analyzed using the one-way ANOVA and Duncan multiple tests as presented in [Table 6](#). One ANOVA demonstrated a statistically significant difference for the sealer with modified groups. The silver Ag nanoparticles increased the conversion of AH plus bioceramic sealer, as the silver concentration increased, the degree of conversion increased.

This table illustrates the enhanced conversion degree of AH Plus bioceramic sealer modified with silver nanoparticles, observed at various time intervals and nanoparticle concentrations. There was a significant difference at $P = 0.05$. According to these data, we can see that as the silver nanoparticles concentration increases the degree of conversion will increase, and the highest and fastest degree of conversion occurs with 10% of silver nanoparticles.

Antibacterial Efficacy of AH Plus Bioceramic Sealer Enhanced with Silver Nanoparticles Against *Enterococcus faecalis* at Various Time Intervals

The antibacterial activity data of the AH Plus bioceramic sealer enhanced with silver nanoparticles were analyzed using the Kruskal–Wallis test as presented in [Table 7](#) and Mann–Whitney *U*-test as presented in [Table 8](#).

These tables illustrate the enhanced antibacterial activity of AH Plus bioceramic sealer modified with silver nanoparticles against *E. faecalis*, observed at various time intervals and nanoparticle concentrations (as shown in [Figure 4](#) that contain subFigure ([Figure 4A–E](#))). There was a significant difference at $P = 0.05$.

Pearson Correlation Test

All data for antibacterial activity and degree of conversion for AH plus bioceramic sealer modified with silver (Ag) nanoparticles were analyzed by Pearson correlation test to find the relationship between the antibacterial activity and degree of conversion as presented in [Table 9](#).

Table 5 Presents a Summary of Groupings and Subgroups for Antibacterial Activity Against *E. faecalis*

		Group A1 Immediate	Group A2 30 Min.	Group A3 24 Hours	Group A4 48 Hours	Group A5 7 Days
Bioceramic Sealer AH Plus	Control group 100%	5mm**	11.9mm	14mm	17mm	19.2mm
	Group A 2.5% Ag	7mm	13.9mm	15.9mm	20.9mm	21mm
	Group B 5% Ag	8.1mm	14.9mm	19.9mm	22mm	23.2mm
	Group C 10% Ag	9.1mm	16.2mm	20mm	24.2mm	25.2mm

Note: **mm refers to growth of inhibition zones in millimeters.

Table 6 One-Way ANOVA for the Degree of Conversion of AH Plus with Silver Nanoparticles

		Sum of Squares	Df.	Mean Square	F	Sig.
Control	Between Groups	13438.807	4	3359.702	2.962E4	0.000
	Within Groups	5.105	45	0.113		
	Total	13443.912	49			
2.5%	Between Groups	13036.183	4	3259.046	1.922E4	0.000
	Within Groups	7.629	45	0.170		
	Total	13043.812	49			
5%	Between Groups	6447.519	4	1611.880	1.147E4	0.000
	Within Groups	6.322	45	0.140		
	Total	6453.841	49			
10%	Between Groups	6123.295	4	1530.824	1.444E4	0.000
	Within Groups	4.772	45	0.106		
	Total	6128.067	49			

Table 7 Kruskal-Wallis Test demonstrates the antibacterial efficacy of AH Plus with different concentrations of silver Ag % nanoparticles against E. faecalis and the highest value with 10% of silver NP

	immediately	30 min	1 day	2 days	7 days
AH plus control group	5	11.9	14	17	19.2
chi-square + sig			46.846**		
AH plus 2.5 % Ag	7	13.9	15.9	20.9	21
chi-square + sig			45.168 **		
AH plus 5 % Ag	8.1	14.9	19.2	22	23.2
chi-square + sig			46.454**		
AH plus 10 % Ag	9.1	16.2	20	24.2	25.2
chi-square + sig			46.942 **		

Note: **mean statistically significant at P=0.05 according to chi-square analysis.

Table 8 Mann–Whitney U-Test Shows the Antibacterial Activity AH Plus with Silver Ag% Nanoparticles Against (E. faecalis) at Different Time Intervals with High Significant Differences Among the Variables

	Control		2.5% Ag		5% Ag		10% Ag	
	z value	p-value	z value	p-value	z value	p-value	z value	p-value
Immediately	3.871	0	3.921	0	3.851	0	3.924	0
30 mints								
Immediately	3.86	0	3.871	0	3.874	0	3.921	0
1 day								
Immediately	3.86	0	3.86	0	3.842	0	3.942	0
2 days								
Immediately	3.865	0	3.86	0	3.874	0	3.999	0
7 days								
30mint	3.633	0	3.794	0	3.874	0	3.892	0
1 day								
30 mints	3.842	0	3.891	0	3.842	0	3.896	0
2 days								

(Continued)

Table 8 (Continued).

	Control		2.5% Ag		5% Ag		10% Ag	
	z value	p-value	z value	p-value	z value	p-value	z value	p-value
30 mints	3.847	0	3.891	0	3.874	0	3.969	0
7 days								
1 day	3.832	0	3.842	0	3.863	0	3.892	0
2 days								
1 day	3.836	0	3.842	0	3.896	0	3.966	0
7 days								
2 days	3.627	0	0.281	0.779	2.887	0.004	3.197	0.001
7 days								

The amounts of Pearson correlation test are (-1, 0, +1) these numbers mean:

-1: negative relationship, 0: no relationship, +1: positive relationship.

Linear Regression Test (R)

All data of degree of conversion and antibacterial activity of the AH plus bioceramic sealers modified with silver nano particles were analyzed by linear regression test to find the strength of the correlation between the degree of conversion and antibacterial activity, as presented in Table 10. This table includes 3 subparts (Table 10a–c).

The amounts of (R) are (-1, 0, +1)

And according to the results of this research the readings were above zero, there are further explanations for R amounts as follows:

{0.1–0.4} consider a Weak relation between the variables.

{0.5–0.7} consider a Moderate relation between the variables.

{0.8–1} consider a Strong relation between the variables.

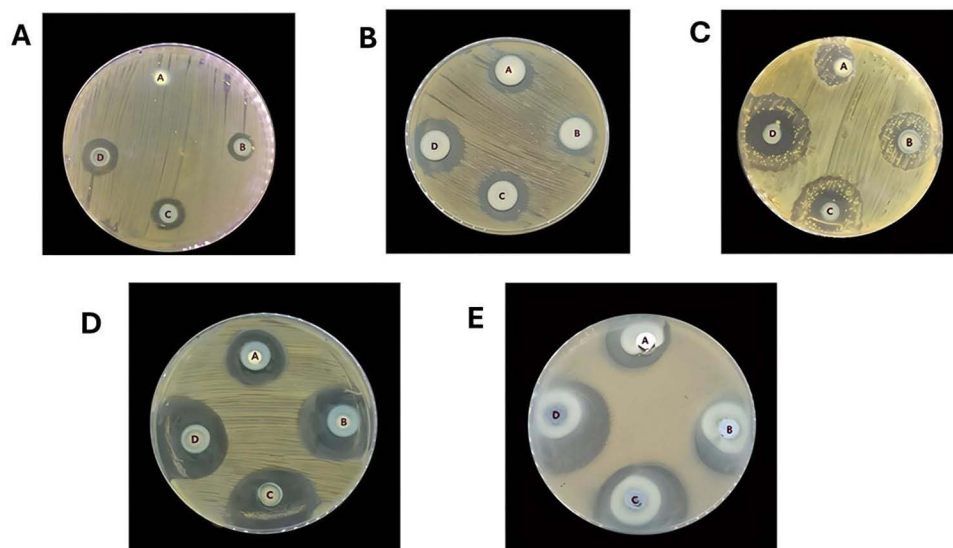


Figure 4 Contain subfigures (A–E) that demonstrate the antibacterial efficacy of AH Plus bioceramic sealer enhanced with silver nanoparticles against *E. faecalis* at various time intervals (immediately, 30 minutes, 1 day, 2 days, 7 days), the largest zone of growth inhibition with group D 10% silver NPs in all time intervals as shown in the subfigures. (A control group, B 2.5% silver NP group, C 5% silver NP group, D 10% silver NP group).

Table 9 Demonstrated There Was a Positive Relation Between the Degree of Conversion and the Antibacterial Activity of AH Plus Bioceramic Sealer Modified with Ag% Nanoparticles Against *E. faecalis*

		Antibacterial Activity AH Plus (<i>E. faecalis</i>) Ag%	Degree of Conversion AH Plus Ag
Antibacterial activity AH plus (<i>E. faecalis</i>) Ag%	Pearson Correlation	1	0.931**
	Sig. (2-tailed)		0.000
	N	200	200
Degree of conversion AH plus Ag%	Pearson Correlation	0.931**	1
	Sig. (2-tailed)	0.000	
	N	200	200

Note: **Correlation is significant at the 0.01 level (2-tailed).

Table 10 This Table Include 3 Subparts (Table 10a–c)

Table 10a Demonstrate the R value for linear Regression test that explains the strength of the relation between the antibacterial activity of AH plus bioceramic against E. faecalis (Ag%) and Degree of conversion AH plus bioceramic Ag% shows strong relation between the variables.

Model Summary					
Model		R	R Square	Adjusted R Square	Std. Error of the Estimate
1		0.931	0.867	0.866	5.25510

Table 10b demonstrate the high significance relation between the variables

Model		Sum of Squares	Mean Square	F	Sig.
1	Regression	35650.218	35,650.218	1.291E3	0.000
	Residual	5467.989	27.616		
	Total	41118.207			

Table 10c demonstrate the high significance relation between the variables

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	49.386	1.116		44.260	0.000
	VAR00001	2.306	0.064	0.931	35.929	0.000

Depth of Penetration

The data of depth penetration of AH plus bioceramic sealer modified silver nano particles were analyzed by Kruskal Wallis test as presented in Table 4.

In this table, we can see the depth of penetration of AH plus bioceramic sealer modified with silver nanoparticles decreased with different nano particles concentrations, as the concentration increased (2.5%, 5%, 10%) the depth of penetration decreased, and the number of the canals decreased at different levels (apical, middle and coronal third from the root) as shown in Figure 5 that contain subfigures (Figure 5A–D), and it was a significant difference at $P = 0.05$.

According to kappa score Table 2, the depth of penetration was measured by millimeter.

Discussion

This study demonstrated that incorporating silver nanoparticles into AH Plus bioceramic sealer significantly enhanced its degree of conversion (DC), with increasing silver concentrations correlating with higher conversion rates at all evaluated

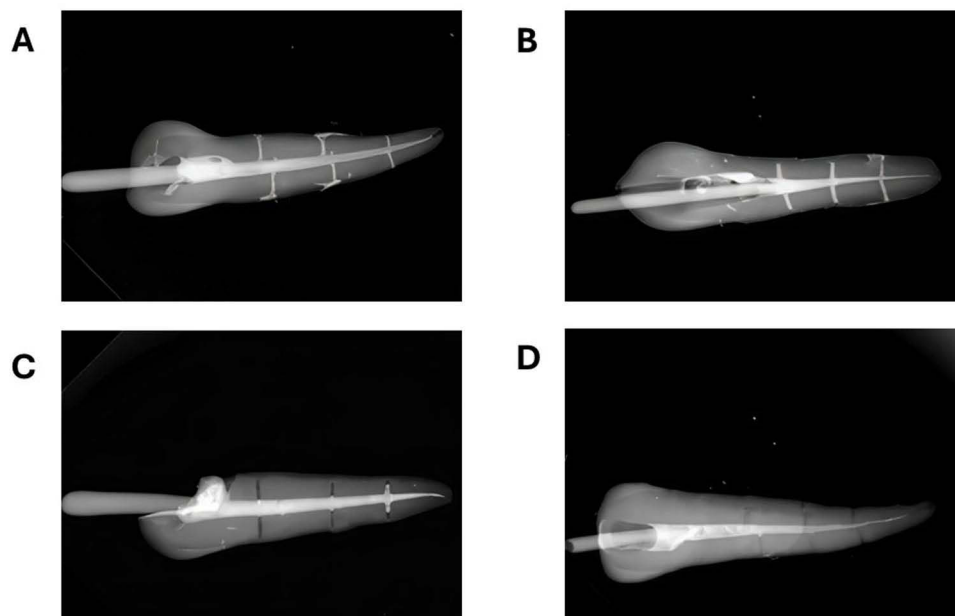


Figure 5 Contain subfigures (A–D), in the (A and B) that demonstrate the depth of penetration of AH plus bioceramic sealer control group 5a and 5b modified group with silver Ag 2.5% nanoparticles at different levels, while the (C) showed the lowest penetration and only at the apical level with 5% modified group. No penetration was with 10% modified group as shown in subFigure 5D.

time intervals. Notably, even though the unmodified sealer reached 100% conversion by day 7, the nanoparticle-enhanced versions exhibited superior performance throughout the curing process, suggesting that nanoparticles accelerate and possibly improve the polymerization kinetics.

The conversion rate of the sealer is contingent upon the experimental methodology employed. In root canal specimens, the process requires more time compared to mold or glass slabs, as molds provide a greater surface area of the sealer exposed to air and humidity.²¹ In the present study, sealers are positioned in molds, and the extent of surface setting is evaluated at various time intervals, considering the incubation of samples,²¹ mixing method of nanoparticles, as well as the type, concentration, and size of nanoparticles.²² All these factors will affect the degree of conversion.

The improved DC aligns with prior findings that nanoparticle additives can enhance setting behavior. For example, Baghdadi et al reported that nanomaterials such as multi-walled carbon nanotubes (MWCNTs) and titanium carbide reduced setting times in BioRoot sealers, indicating similar reinforcement behavior.²² However, unlike their study, our results focus specifically on silver nanoparticles and bioceramic sealers, highlighting a novel combination.

Enterococcus faecalis has been employed in many *in vitro* investigations to assess the antibacterial effectiveness of endodontic sealers and their efficacy in root canal treatment. Therefore, these microorganisms are utilized in the current study to test the antibacterial efficiency of the assessed AH Plus bioceramic sealer. The inconsistencies may primarily arise from variances in microorganism strains employed, time intervals, testing procedures (DCT or ADT), and bacterial resistance.²³ Incubation techniques (aerobic or anaerobic).²⁴ Categories of agar media (selective or non-selective), classifications of testing materials (bioceramic sealer), and the quantity of bacterial colonies. Moreover, there may be elements influencing the variances between our results and those of another research.²⁵ The kind, dimensions, and concentration of nanoparticles will influence the antibacterial efficacy of the sealer.²⁶ Iqbal et al found that incorporating particular types and amounts of nanoparticles into endodontic sealers demonstrated antibacterial effects *in vitro*. The imperative for meticulously organized clinical research to convert *in vitro* discoveries into clinical application is crucial. The incorporation of nanoparticles may enhance the antibacterial effectiveness of endodontic sealers and improve treatment outcomes.²⁶

The results of this study demonstrate that AH plus bioceramic sealer has excellent flowability and penetration into the lateral canal before the addition of nanoparticles. After the incorporation of silver nanoparticles, particularly with 2.5% and 5% concentrations, the penetration depth into the lateral canals was greater at the apical and intermediate levels compared to the coronal level, but with high concentration 10% the penetration depth decreased for all levels.

The variations in the results relate to the kind, size, and concentration of nanoparticles.²⁷ The type of sealer, namely the flow rate of AH plus bioceramic sealer, exceeds that of other bioceramic sealers, influencing penetration into lateral canals.²⁰ Additionally, canal instrumentation (manual or rotational), obturation methods, and the positioning of lateral canals (apical, middle, and coronal) significantly affect lateral canal penetration.²⁸ The diameter of the prepared lateral canal influences the depth of penetration. The technique for administering sealers to the canal, whether using a lentulo spiral file or manual application, affects the depth of penetration, as the lentulo file diminishes sealer viscosity, augments flowability, and promotes penetration capability into the ALCs.²⁹ Prior research supports this; for instance, Yuk et al and Öznur et al found that bioceramic sealers generally outperform resin-based sealers in canal penetration, particularly with activation techniques.^{20,30}

Limitations of this study were conducted under in vitro conditions, which do not fully replicate the complexity of the clinical environment. The use of molds for sample preparation may not reflect the actual behavior of sealers within the intricate anatomy of root canals, especially under variable moisture and temperature conditions. Additionally, only silver nanoparticles were evaluated, without direct comparison to other nanoparticle types that may offer different effects. The sample size and time intervals, while sufficient for preliminary evaluation, may limit the generalizability of the results. Furthermore, the antibacterial tests were limited to a single bacterial species (*Enterococcus faecalis*), and broader microbiological testing would be required for a more comprehensive understanding of antimicrobial efficacy. Future studies should involve clinical trials or ex vivo tooth models, including various nanoparticle formulations, and assess long-term performance and biocompatibility.

Conclusion

This research indicates that root canal bioceramic sealers combined with 2.5%, 5%, and 10% silver nanoparticles exhibited enhanced antibacterial efficacy compared to the sealer used alone. The incorporation of nanoparticles with endodontic sealers offers enhanced benefits by controlling and minimizing post-endodontic treatment infections, which is also advantageous in retreatment scenarios. There are many points we can conclude from this research:

1. The degree of conversion of AH plus bioceramic sealer was affected by the addition of silver nanoparticles with different concentrations at different time intervals.
2. As silver nanoparticle concentrations increased, the degree of conversion was increased in AH Plus bioceramic sealer.
3. The antibacterial activity of AH plus bioceramic sealer loaded with (2.5%, 5%, 10%) silver nanoparticles against *E. faecalis* were increased as the concentration of NPs increased at different time intervals.
4. Strong positive relation between antimicrobial action and conversion degree of AH plus bioceramic sealer.
5. The depth of penetration of AH plus bioceramic sealer loaded with silver nanoparticles into the lateral canals decreased as the concentration of silver NPs increased at different levels.

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Disclosure

The authors report there are no conflicts of interest in this work.

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