

Comparative Evaluation of Sealer Penetration in Dentinal Tubules After Side Firing Er:Yag Laser Tip and Diode Laser Activated Irrigation: A Confocal Laser Scanning Microscope Study

Vandana Kumari^{1*}, Savitha B Naik², Kiran Kumar N³, Parvati S Bangarimath⁴,
Tasmiya M Bhavikatti⁵

^{1*} Department of Conservative Dentistry and Endodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India. (Corresponding Author) Email: vndna1997@gmail.com

² MDS, Department of Conservative Dentistry and Endodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India.

³ PhD, Department of Conservative Dentistry and Endodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India.

⁴ Department of Conservative Dentistry and Endodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India.

⁵ Department of Conservative Dentistry and Endodontics, Government Dental College and Research Institute, Bangalore, Karnataka, India.

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ABSTRACT

Objective: This study sought to evaluate the penetration depth of AH Plus and Bioceramic sealers following the activation of the irrigant using three methods: Conventional syringe needle irrigation, a Diode Laser, and an Er:YAG Laser with a side-firing tip.

Background: The key to achieving endodontic success is thorough cleaning of the root canal space, followed by 3-dimensional obturation. Laser-activated irrigation has been shown to adequately remove the smear layer.

Method: Single straight-rooted 36 extracted noncarious teeth were included. Root canal instrumentation was performed. Samples were randomly divided into groups as follows: GROUP 1: AH Plus sealer group, GROUP 2: Bioceramic sealer group, followed by further division into three subgroups. The sealer was mixed with Rhodamine B dye and obturated using lateral condensation. After obturation, transverse sections were prepared and observed under a Confocal Laser Scanning Microscope to determine the penetration depth.

Statistical analysis: Data was analyzed using the statistical package SPSS 26.0 (SPSS Inc., Chicago, IL). Descriptive statistics was performed to assess the mean and standard deviation of the respective groups. Normality of the data was assessed using Shapiro-Wilk test.

Conclusion: Er:YAG laser-assisted irrigation significantly enhanced dentinal tubule penetration, particularly in the middle and apical thirds. AH Plus demonstrated greater depth of penetration compared to the bioceramic sealer under most conditions. Laser activation, especially with Er:YAG, may improve sealer penetration in deeper canal regions compared to conventional irrigation.

Keywords: Activated irrigation, Laser activation, Sealer penetration, Side firing tip.

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Introduction

The core objective of endodontic treatment is thorough cleaning and complete disinfection of the root canal system, followed by three-dimensional obturation to block fluid movement and prevent bacterial contamination or regrowth. Despite careful execution, conventional chemo-mechanical root canal preparation, along with syringe-needle irrigation using antimicrobial and chelating agents, has repeatedly been shown to fall short in consistently achieving thorough disinfection.

The smear layer is a non-homogeneous coating formed on root canal walls during instrumentation. Evidence indicates that elimination of this layer improves sealer bonding to dentin, underscoring the need for efficient smear layer removal methods to enhance endodontic treatment outcomes.¹ Sealer tags play a crucial clinical role by improving the adaptation and mechanical retention of the obturating material to the dentinal walls.²

Growing evidence emphasizes that effective agitation of irrigants within adequately prepared canals is essential for thorough cleaning and disinfection of the root canal system, including the dentinal tubules.³

The earliest applications of lasers in root canal treatment involved their use after canal shaping and drying to directly irradiate the root canal wall.⁴

Diode lasers are increasingly used in endodontic irrigation to enhance disinfection and cleaning of the root canal system⁵ and in recent years, the Er:YAG laser-activated irrigation (LAI) technique has emerged as a promising alternative to conventional needle irrigation (CNI).⁶

To enhance cleaning and disinfection during endodontic treatment and retreatment, Prof. Adam Shtabhols (The Hebrew University, Jerusalem, Israel) designed a specialized side-firing spiral Endo tip that directs the Er:YAG laser energy laterally toward canal walls, rather than straight ahead.⁷

AH Plus (Dentsply DeTrey, Konstanz, Germany) is among widely used epoxy-resin-based sealer that possesses positive handling characteristics and superior physical properties. The Mineral Trioxide Aggregate Bioceramic sealer (Prime MTA Bioceramic root canal sealer) is a calcium silicate-based sealer containing MTA with fine hydrophilic particles known for its biocompatibility and ability

to seal and promote tissue regeneration. Due to their differences in composition and adhesion mechanisms, comparing their dentinal tubule penetration is essential to determine whether bioactive sealers offer superior sealing ability over conventional resin-based sealers.

Therefore, the aim of this study was to compare the depth of AH plus and Bioceramic sealer penetration after activation of the irrigant with conventional syringe needle irrigation, Er:YAG laser with side-firing tip, and Diode laser.

METHODOLOGY

Sample size

A sample size of 36 specimens (n= 18) per group, 6 each per sub group was calculated using G*Power software for yielding 90% power to detect a significant difference, with an effect size of 1 and a significance level 0.05(5%).

Sample Preparation

Ethical approval was obtained from the Institutional Ethics Committee of the affiliated institution. The study was conducted in accordance with institutional guidelines for in-vitro research.

Single straight-rooted 36 extracted noncarious mandibular premolars having single canal with closed apex were included in the study. The extracted teeth were debrided of calcified deposits and residual soft tissues using an ultrasonic scaler and stored in distilled water until further use. The crowns were sectioned with a diamond disc under continuous water cooling to obtain a uniform root length of 14 mm.

Working length determination was carried out by introducing a size 10 K-file into the canal until its tip was just visible at the apical foramen, after which 1 mm was subtracted. Root canal preparation was completed up to an F4 master apical size using ProTaper Universal rotary files to match the diameter required for the side-firing tip. During instrumentation, each canal was irrigated with 2 mL of 2.5% sodium hypochlorite between successive files.

Interventions:

All the 36 samples were randomly allocated into two groups (n=18) and further into three subgroups with six samples in each using computer generated randomization.

GROUP 1: AH Plus sealer group

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Subgroup A) syringe needle irrigation with 2.5% NaOCl and 17%EDTA

Subgroup B) Er: YAG laser activated irrigation with side firing tip at a wavelength of 2940 nm

Subgroup C) Diode laser activation to perform the irrigation at 980 nm wavelength

GROUP 2: Bioceramic sealer group

Subgroup A) syringe needle irrigation with 2.5% NaOCl and 17%EDTA

Subgroup B) Er: YAG laser activated irrigation with side firing at a wavelength of 2940 nm

Subgroup C) Diode laser activation to perform the irrigation at 980 nm wavelength

Syringe needle irrigation with 2.5% NaOCl and 17% EDTA

Irrigation with 2 mL of 2.5% NaOCl was performed after each instrument change followed by 5 mL of 17% EDTA for one minute. A final rinse with 5 mL of 2.5% NaOCl for 1 minute and final flush with 5ml of saline was done to prevent any chemical interaction.

Er:YAG Laser activation

LiteTouch Er:YAG Laser (Light Instruments Ltd., Israel), was used for final irrigation using a laser activation at a wavelength of 2940 nm. A sterile side-firing spiral Endo tip from the same manufacturer was used for irrigation activation. The tip was inserted 1 mm short of the working length without binding. The very short-pulse mode was used to activate the laser at 1.5 W, 150 mJ per pulse at 10 Hz for a minute. The air and water of the laser system were turned off.

Diode laser activation

The root canals were first flushed with 2.5% sodium hypochlorite and subsequently activated using a diode laser (Lazon Solase Pro, 980 nm, peak power 2 W in continuous wave mode) with a 200- μ m fibre tip for 20 seconds. During laser activation, the optical fibre was introduced into the canal to a point 2 mm short of the working length and then withdrawn using slow, helical apicocoronal movements. This approach was adopted to counteract the forward-directed emission from the fibre tip and to ensure that all regions of the canal were exposed, thereby achieving uniform irradiation of the canal walls.

Post activated irrigation a final saline rinse was done for all the samples. The sealer was mixed with

Rhodamine B dye during manipulation at a ratio of 0.1% (wt)8 for creating fluorescence under a confocal laser scanning microscope (CLSM). A paper point was used to dry the canal and was obturated by lateral condensation.

Post obturation assessment

After obturation, transverse sections of 1 mm were made using slow-speed diamond discs at 3 mm (apical), 5 mm (middle), and 8 mm (coronal) from the root apex followed by observation under a Leica Confocal laser scanning microscope Stellaris 5 at 10x magnification and the penetration depth was measured using ImageJ Software (Confocal images of Group 1-subgroup A,B,C and Group 2- subgroup A,B,C). The average value was recorded as the penetration depth of the sealer in all the three sections.

STATISTICAL ANALYSIS

Data were analyzed using the statistical package SPSS 26.0 (SPSS Inc., Chicago, IL), and the level of significance was set at $P < 0.05$. Descriptive statistics were performed to assess the mean and standard deviation of the respective groups. The normality of the data was assessed using the Shapiro–Wilk test (Table 1). Inferential statistics to determine the difference between groups were performed using the Kruskal–Wallis test, followed by the Bonferroni post hoc test.

RESULTS:

Intragroup Comparison (AH Plus vs Bio Ceramic within Each Irrigation Protocol)

• EDTA + 2.5% NaOCl Group (Table 2)

AH Plus sealer demonstrated significantly greater penetration than Bio Ceramic sealer in the coronal region ($p = 0.004$).

However, no significant differences were observed in the middle and apical thirds.

• Er:YAG Laser Group (Table 3)

AH Plus sealer showed significantly greater penetration in the middle ($p = 0.037$) and apical regions ($p = 0.016$) compared to Bio Ceramic sealer. No significant difference was found in the coronal region.

• Diode Laser Group (Table 4)

A significant difference was noted only in the middle third ($p = 0.034$), favoring AH Plus.

No significant difference was observed in the coronal and apical thirds.

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Intergroup Comparison (Between Irrigation Protocols)

• Coronal Third (Table 5)

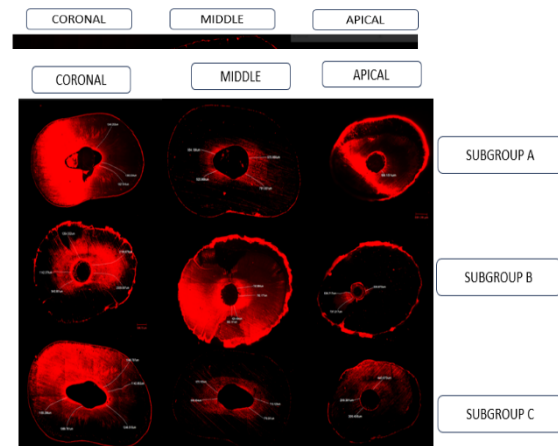
For AH Plus sealer group, a statistically significant difference was found among irrigation protocols ($p = 0.01$), with post-hoc analysis showing significant improvement between 2.5% NaOCl+ 17% EDTA and Er:YAG Laser activated groups.

No significant difference was observed among protocols for Bio Ceramic sealer.

• Middle and Apical Thirds (Table 6 and 7)

No statistically significant intergroup differences were found among irrigation protocols for either AH Plus or Bio Ceramic.

CONFOCAL IMAGES OF GROUP 2



CONFOCAL IMAGES OF GROUP 1

Table 1- Normality assessment

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
17%EDTA +2.5% NaoCL	.238	18	.008	.888	18	.035
Er:YAG LASER	.247	18	.005	.835	18	.005
DIODE LASER	.246	18	.005	.885	18	.031

Table 1 shows the results of normality tests for three groups. The Kolmogorov-Smirnov and Shapiro-Wilk tests revealed significant deviations from normality in all groups. For 17%EDTA+2.5% NaOCl, the Kolmogorov-Smirnov statistic was 0.238 ($p = 0.008$), and the Shapiro-Wilk statistic was 0.888 ($p = 0.035$). For the Er: YAG Laser, the Kolmogorov-Smirnov statistic was 0.247 ($p = 0.005$), and the Shapiro-Wilk statistic was 0.835 ($p = 0.005$). For the Diode Laser, the Kolmogorov-Smirnov statistic was 0.246 ($p = 0.005$), and the Shapiro-Wilk statistic was 0.885 ($p = 0.031$).

TABLE 2-ANALYSIS OF DEPTH OF PENETRATION -EDTA+ 2.5% NaOCl

		Mean	Std. Deviation	Difference	Z	P Value
CORONA L	AH PLUS	1890.300	297.04765	710.63333	5.139	.004*
	BIO CERAMIC	1179.666	505.99364			
MIDDLE	AH PLUS	1395.896	553.60878	438.84667	1.345	.237
	BIO CERAMIC	957.0500	563.35283			
APICAL	AH PLUS	448.9733	298.59211	-449.2266	-1.417	.216
	BIO CERAMIC	898.2000	667.35900			

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Table 2 shows the depth of penetration analysis for 17% EDTA + 2.5% NaOCl. In the coronal region, AH Plus (mean = 1890.30, SD = 297.05) significantly outperformed Bio Ceramic (mean = 1179.67, SD = 505.99) with a p-value of 0.004. In the middle and apical regions, no significant differences were found between AH Plus and Bio Ceramic (p = 0.237 and p = 0.216, respectively).

TABLE 3-ANALYSIS OF DEPTH OF PENETRATION – Er:YAG Laser

		Mean	Std. Deviation	Difference	Z	P Value
CORONAL	AH PLUS	2318.653	110.31471	432.62333	1.483	.198
	BIO CERAMIC	1886.030	770.45341			
MIDDLE	AH PLUS	1719.986	384.20456	838.63667	2.826	.037*
	BIO CERAMIC	881.3500	350.13185			
APICAL	AH PLUS	863.5200	75.99640	141.53667	3.582	.016*
	BIO CERAMIC	721.9833	144.11058			

Table 3 shows the depth of penetration analysis for Er:YAG Laser. In the coronal region, no significant difference was found between AH Plus and Bio Ceramic (p = 0.198). However, in the middle and apical regions, AH Plus significantly outperformed Bio Ceramic (p = 0.037 and p = 0.016, respectively).

TABLE 4-ANALYSIS OF DEPTH OF PENETRATION- DIODE LASER

		Mean	Std. Deviation	Difference	Z	P Value
CORONAL	AH PLUS	1969.660	271.64746	459.84000	1.586	.174
	BIO CERAMIC	1509.820	536.71428			
MIDDLE	AH PLUS	1991.626	406.99942	989.02000	2.891	.034*
	BIO CERAMIC	1002.606	507.45799			
APICAL	AH PLUS	848.8300	558.48014	-188.74000	-.706	.512
	BIO CERAMIC	1037.570	525.04845			

Table 4 presents the depth of penetration analysis for Diode Laser. In the coronal region, no significant difference was found between AH Plus (mean = 1969.66, SD = 271.65) and Bio Ceramic (mean = 1509.82, SD = 536.71) with a p-value of 0.174. In the middle region, AH Plus (mean = 1991.63, SD = 407.00) showed a significant difference of 989.02 compared to Bio Ceramic (mean = 1002.61, SD = 507.46), with a p-value of 0.034. In the apical region, no significant difference was observed (p = 0.512).

TABLE 5-ANALYSIS OF DEPTH OF PENETRATION-CORONAL

	AH PLUS		BIO CERAMIC	
	Mean	Std. Deviation	Mean	Std. Deviation
17%EDTA+ 2.5%NaOCl	1890.300	297.04765	1179.666	505.99364
Er:YAG LASER	2318.653	110.31471	1886.030	770.45341
DIODE LASER	1969.660	271.64746	1509.820	536.71428
P Value (Kruskal wallis test)	0.01*		0.17	
E vs Y	0.01*		0.14	

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Posthoc test	E vs D	0.83	0.63
	Y vs D	0.05	0.55

Table 5 shows the depth of penetration in the coronal region for AH Plus and Bio Ceramic. For AH Plus, significant differences were found between 17% EDTA + 2.5% NaOCl and Er:YAG Laser ($p = 0.01^*$), while no significant differences were found for Bio Ceramic ($p = 0.17$). Post-hoc tests for AH Plus revealed significant differences between EDTA + NaOCl and Er:YAG Laser ($p = 0.01^*$), but no significant differences between other treatment pairs.

TABLE 6-ANALYSIS OF DEPTH OF PENETRATION-MIDDLE

		AH PLUS		BIO CERAMIC	
		Mean	Std. Deviation	Mean	Std. Deviation
EDTA+ NAOCL		1395.896	553.60878	957.0500	563.35283
YAG LASER		1719.986	384.20456	881.3500	350.13185
DIODE LASER		1991.626	406.99942	1002.606	507.45799
P Value (Kruskal wallis test)		0.10		0.89	
Posthoc test	E vs Y	0.45		0.95	
	E vs D	0.09		0.98	
	Y vs D	0.56		0.89	

Table 6 shows the depth of penetration in the middle region for AH Plus and Bio Ceramic. The Kruskal-Wallis test revealed no significant differences for either material (AH Plus: $p = 0.10$, Bio Ceramic: $p = 0.89$). Post-hoc tests also showed no significant differences between treatment pairs.

TABLE 7-ANALYSIS OF DEPTH OF PENETRATION-APICAL

		AH PLUS		BIO CERAMIC	
		Mean	Std. Deviation	Mean	Std. Deviation
EDTA+ NAOCL		448.9733	298.59211	898.2000	667.35900
YAG LASER		863.5200	75.99640	721.9833	144.11058
DIODE LASER		848.8300	558.48014	1037.570	525.04845
P Value (Kruskal wallis test)		0.19		0.55	
Posthoc test	E vs Y	0.24		0.81	
	E vs D	0.26		0.87	
	Y vs D	0.99		0.52	

Table 7 shows the depth of penetration in the apical region for AH Plus and Bio Ceramic. The Kruskal-Wallis test revealed no significant differences for either material (AH Plus: $p = 0.19$, Bio Ceramic: $p = 0.55$). Post-hoc tests also showed no significant differences between treatment pairs.

DISCUSSION

Successful elimination of microorganisms from the root canal system is a primary objective of endodontic treatment. Nevertheless, because of the complex and variable canal morphology, mechanical instrumentation during chemomechanical preparation often fails to fully clean and shape all areas, particularly in teeth with irregularities or accessory canals. These

uninstrumented regions can harbour debris, bacteria, and their metabolic products, thereby limiting the depth of penetration of irrigating solutions into the dentinal tubules.⁹

Obturation of the root canal system helps compensate for the shortcomings of chemomechanical preparation, with the main objective of creating a hermetic seal that blocks any possible routes of leakage between the oral

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environment, the peri radicular tissues, and the canal space.¹⁰ Second, it also seeks to eliminate any voids within the root canal system and effectively entrap residual irritants that may persist despite thorough cleaning and shaping procedures.¹¹

The principle of bacterial entombment suggests that any microorganisms remaining within the root canal are inactivated because they are deprived of nutrients and physical space required for survival and multiplication.¹² Root canal sealers that penetrate into the dentinal tubules can aid in trapping any remaining microorganisms within these channels. In addition, the sealer's chemical constituents may exhibit inherent antimicrobial activity, which can be enhanced when the material is in close contact with bacterial cells.¹³

For evaluation of tubular penetration depth in this study, CLSM was the method of choice over Scanning Electron Microscopy because of its ability to create a 3D image, visualize sections at different levels, and make depth measurements more precise.¹⁴

AH Plus sealer exhibited superior penetration compared to the Bioceramic sealer in most experimental conditions, particularly in the coronal third with conventional irrigation and in the middle and apical thirds with Er:YAG activation.

Similar result of greater penetration depth for AH Plus sealer in the coronal section was observed in a study done by Gülmez et al which concluded that the greater depth may be due to its smaller particle size and homogeneous structure than MTA.¹⁵

A recent systematic review and meta-analysis of *in vitro* studies by Marcelo et al. concluded that bioceramic sealers and AH Plus exhibit comparable performance in terms of dentinal tubule penetration and antimicrobial efficacy. Evidence also indicates that both Bioceramic sealers and AH Plus demonstrate similar flow characteristics and comply with the requirements of ISO 6876:2012. This favorable flow behavior may account for their equivalent penetration abilities. Importantly, these properties are critical, as they promote more effective filling of the root canal system and thereby enhance clinical outcomes.¹⁶ Laser-assisted irrigation, particularly with Er:YAG laser, significantly enhanced penetration in the middle and apical thirds for AH Plus Group. This might be due

to laser-activated irrigation, featuring the innovative side-firing spiral and Endo tip, which significantly improves root canal disinfection through cavitation in areas that are usually not accessible by conventional syringe irrigation. The Endo tip is engineered to match the geometry and dimensions of prepared root canals, allowing efficient transmission of laser energy. The distinctive design incorporates a 3-mm flexible segment with lateral slits that allows sideward emission of the Er:YAG laser toward the canal walls reducing apical energy concentration and minimizing excessive temperature rise when used with continuous irrigation.¹⁷

Previous literature suggests that a temperature increase of more than 47°C may risk periodontal injury; however, Er:YAG laser activation under irrigation has demonstrated temperature increases within safe limits due to its high water absorption coefficient and superficial interaction with dentin.⁶ In the coronal third, however, conventional irrigation demonstrated comparable or superior performance for AH Plus. This may be because the coronal region inherently has wider canal dimensions and higher tubule density, allowing effective chemical debridement even without advanced activation. Therefore, the added benefit of laser activation may be less pronounced coronally. The diode laser group demonstrated significant improvement in the middle third but not consistently in the apical third. Unlike Er:YAG, diode lasers primarily produce photothermal effects rather than strong photoacoustic streaming. While they may enhance smear layer modification and partial tubule opening, their mechanism of action may not generate sufficient hydrodynamic agitation to consistently improve apical penetration.

As this study was an *in vitro* study, factors such as absence of periapical tissue resistance, lack of physiological fluid dynamics, and standardized canal preparation may limit direct clinical extrapolation.

Despite the demonstrated improvement in sealer penetration with laser-assisted irrigation, economic feasibility remains an important factor in clinical adoption. Er:YAG laser systems involve substantial initial investment, maintenance costs, and operator training. In comparison, diode lasers are relatively

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more affordable and widely available in dental practice. The improved penetration observed in the Er:YAG group must therefore be interpreted alongside cost-benefit considerations.

Conclusion

Within the limitations of this in vitro study, both the type of irrigation activation protocol and the sealer composition significantly influenced dentinal tubule penetration. AH Plus demonstrated superior depth of penetration compared to the bioceramic sealer under most experimental conditions.

Er:YAG laser-assisted irrigation significantly enhanced sealer penetration in the middle and apical thirds, particularly for AH Plus, indicating improved smear layer removal and irrigant activation in deeper canal regions. Conventional irrigation with 2.5% NaOCl and 17% showed favorable results in the coronal third but did not consistently improve penetration in the apical region. Diode laser activation demonstrated moderate improvement, primarily in the middle third.

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