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ORIGINAL ARTICLE

Assessing dentinal tubule penetration of an innovative bioactive glass-based root canal sealer through confocal laser scanning microscopy: an *in vitro* analysis

ABSTRACT

Aim: *To assess and compare the dentinal tubule penetration of zinc oxide eugenol (ZOE)-based, resin-based, bioceramic, and novel bioactive glass-based root canal sealers using a confocal laser 8 scanning microscope (CLSM).*
Methods: *A total of 48 single-rooted permanent teeth were categorized into four groups (n=12) and treated with gutta-percha along with ZOE sealer (Tubli-Seal EWT), resin-based sealer (AH Plus), bioceramic sealer (BioRoot RCS), and bioactive glass (Nishika Canal Sealer-BG). Cross sections of the roots at 3 mm and 6 mm from the apex were examined under a CLSM to evaluate dentinal tubule penetration.*
Results: *Results indicated that bioceramic sealers exhibited the highest depth of dentinal tubule penetration at both levels, followed by bioactive glass and resin-based sealers. ZOE-based sealer demonstrated the least tubule penetration. Bioactive glass displayed the highest percentage of sealer penetration at 3 mm and 6 mm, with no statistically significant difference observed between bioceramic and bioactive glass groups regarding depth and percentage of dentinal tubule penetration at both levels.*
Conclusions: *Of particular significance is the bioactive glass group, demonstrating the most substantial sealer penetration percentage compared to the other groups at both examined depths.*

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Introduction

Root canal treatment (RCT) is a fundamental procedure for preserving natural teeth, involving a series of steps such as shaping, cleaning, and filling the root canal system (1). Core materials and sealers, either used independently or in combination, play a crucial role in achieving comprehensive sealing of the root canal system (2). Grossman's non-staining zinc oxide eugenol (ZOE) sealer has been a long-standing choice (3). The AH Plus, a paste system, delivered through a double barrel syringe, contains Aerosil and radiopaque fillers in the epoxide paste (4, 5). Bioceramics, a subtype of biomaterials, are increasingly recognized for their promising role in endodontic applications, particularly in root canal filling. These materials offer significant advantages, including biocompatibility and the ability to promote periapical tissue regeneration (6). Moreover, there has been a growing interest in the use of "bioactive" materials in restorative and reconstructive dentistry. In restorative dentistry, the term "bioactive" typically refers to a material's capability to stimulate the formation of hydroxyapatite crystals on its surface. Beyond their structural properties, bioactive substances are valued for their ability to foster beneficial interactions with living cells and tissues from a biological standpoint (7-9). In recent years, a more reactive form of calcium-silicate-based bioactive glass, termed "bioactive root canal sealers," has emerged alongside traditional calcium-silicate-based sealers (10). Bioactive root canal sealers are specialized materials used in endodontic procedures to seal and fill the root canal system after it has been cleaned and shaped. Unlike traditional sealers, bioactive sealers possess the unique ability to interact with the surrounding tissues, promoting healing and regeneration. These sealers typically contain biologically active components that can stimulate tissue repair (9, 10). While bioactive glass has traditionally been used for regenerating dental hard tissues, its potential in treating various complex tissues has become evident (11). Specifically, calcium and silicate ions, essential in biological processes, have been found to accelerate both osteoinduction and angiogenesis, which are crucial for supporting periapical healing (12). Given the unique properties of bioactive root canal sealers and their potential to promote periapical healing, it is essential to evaluate their performance in critical aspects such as dentine tubular penetration. This parameter directly influences the effectiveness of the root canal filling by affecting its sealing ability and long-term stability (11, 12). Dentine tubular penetration plays a crucial role in providing a physical barrier against microbial invasion and enhancing the retention of the sealer within the root canal system. Therefore, assessing this aspect is vital, particularly for novel materials aiming to improve endodontic outcomes.

Confocal Laser Scanning Microscopy (CLSM) has emerged as a valuable tool for precisely assessing tubular penetration. Its high-resolution imaging capabilities enable researchers to visualize and quantify the extent of sealer penetration into dentinal tubules accurately. This technique has been widely adopted in numerous published studies across various experimental fields due to its reliability and effectiveness in evaluating material performance (13, 14).

This study aims to assess and compare the penetration of root canal sealers, including ZOE-based, resin-based, bioceramic, and bioactive glass-based sealers, into dentinal tubules. The evaluation will be conducted using CLSM.

Materials and Methods

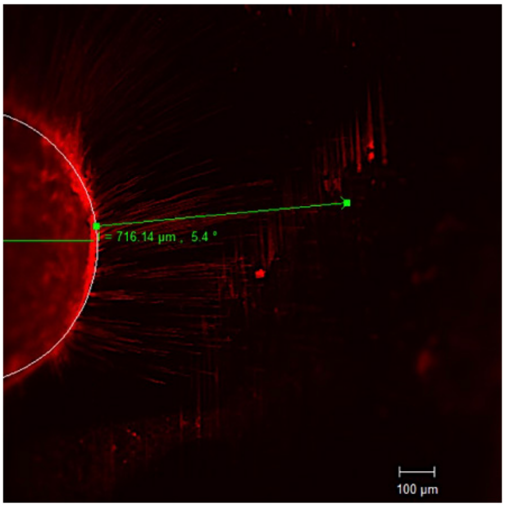
The study included 48 meticulously chosen intact single-rooted permanent teeth, which were divided into four groups, each comprising 12 teeth, through random allocation. The groups were designated as follows:

- Group 1: Ultrasonic activation of a ZOE-based sealer (Tubli-Seal EWT; Kerr, USA)
- Group 2: Ultrasonic activation of a resin-based sealer (AH Plus; Dentsply Maillefer, USA)
- Group 3: Ultrasonic activation of a bioceramic sealer (BioRoot RCS; Septodont, USA)
- Group 4: Ultrasonic activation of a bio-

active glass sealer (Nishika Canal Sealer-BG; Morita, Japan).
The penetration of dentinal tubules was evaluated and compared using CLSM with the Zeiss LSM 710 system.

Sample Preparation
Each tooth underwent digital radiography in both mesiodistal and buccolingual directions to confirm the presence of a single canal. Subsequently, meticulous cleaning was conducted using an ultrasonic scaler to eliminate calculus and tissue tags.
A disinfection process involving 3% sodium hypochlorite (NaOCl, Prime Dental Products, India) for 48 hours was applied, followed by decoronation to standardize the root canal length at 10 mm. The root canals were enlarged using the Protaper nickel-titanium rotary system up to size F3, while maintaining a distance of 0.5-1 mm from the apical foramen. Continuous irrigation with 3% NaOCl at 2 ml per file was employed during the shaping process. Final irrigation with NaOCl, lasting 1 minute using a U-file attached to an ultrasonic unit handpiece, was performed. To eliminate the smear layer, a 2 ml solution of 17% EDTA (Prime Dental Products, India) was applied for 3 minutes, followed by a final rinse of 2 ml saline. Each root was then dried with paper points before being randomly assigned to one of four groups based on the sealer used.
Sealer Placement
The sealers were prepared according to the manufacturer's instructions and labeled with Rhodamine B (HiMedia, Mumbai, India) at an estimated concentration of 0.1% to facilitate CLSM analysis. Using a tuberculin syringe, 0.05 ml of each sealer was dispensed into the canal. The root canals were then obturated using gutta-percha via the single cone obturation technique, and the orifice was sealed with Cavit (3M ESPE, Germany). Subsequently, all roots were stored at 100% humidity and 37 °C for 14 days to allow the sealer to set.

Sectioning and Image Analysis
The roots were precisely sectioned using a diamond disc at 200 rpm with continuous water cooling to prevent frictional heat. Horizontal sections were made at 3 mm and 6 mm levels from the apical foramen. To remove debris generated by sanding, the surface was polished using sandpapers numbered 400, 600, and 1,200 under running water. Following polishing, dentine segments with a thickness of 2 mm were air-dried and examined under CLSM at 10x magnification. The absorption and emission wavelengths of Rhodamine B dye were set at 540 nm and 590 nm, respectively.
Software analysis was used to measure the depth of sealer penetration, with the canal wall serving as the reference point. Sealer penetration into dentinal tubules was quantified using the built-in ruler, extending to its maximum depth (Figures 1, 2). Additionally, Figure 3 presents a compar-



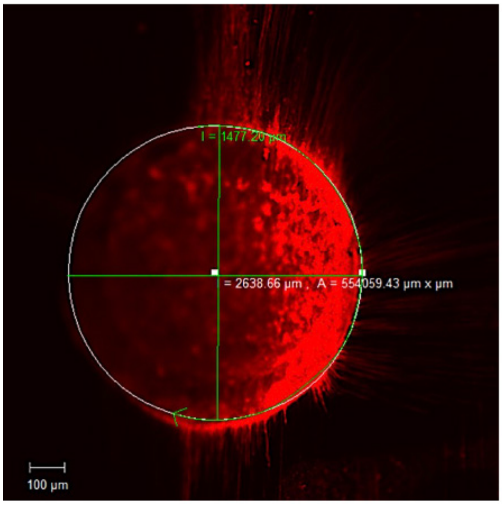


Figure 1
Analysis of sealer penetration depth.

Figure 2
Analysis of sealer penetration percentage.

ative analysis of dentinal tubule penetration at 3 mm and 6 mm levels.
The entire circumference of the root canal was delineated and measured using the built-in ruler. Then, the circumference with visible sealer penetration was marked with a distinct colored line and measured. The percentage of the circumference with sealer penetration was calculated for each sealer type at both 3 mm and 6 mm levels using the formula:
Percentage of Penetration = (y/x)*100
where *y* represents the circumference indicating sealer penetration and *x* denotes the total circumference.

Statistical Analysis
The data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows, Version 28.0 (IBM Corp, Armonk, NY). Confidence intervals were set at 95%, and statistical significance was determined at a p-value of ≤0.05. Continuous variables were expressed as Mean±Standard Deviation. Unpaired t-tests were employed to compare the depth of dentinal tubule penetration at 3 mm and 6 mm across all groups. One-way analysis of variance (ANOVA) was used to compare both the percentage and depth of dentinal tubule penetration, followed by Tukey's post hoc test for pairwise comparisons.

Results

Depth of dentinal tubule penetration at 3 mm level, resin-based and bioceramic sealers exhibited notably deeper penetration depths, measuring approximately 403 µm and 679 µm, respectively. Bioactive glass demonstrated a penetration depth of 504 µm. Notably, bioceramic sealers displayed the highest penetration depth overall. Conversely, at the 6 mm level, ZOE-based sealers exhibited the shallowest penetration into dentinal tubules, with an average depth of approximately 227 µm. Resin-based sealers showed improved performance with an average depth of approximately 731.41 µm, while bioceramic bsealers demonstrated the most substantial penetration, averaging approximately 1041.75 µm.
Bioactive glass exhibited an average penetration depth of 885.916 µm. Significant statistical differences ($p<0.05$) were observed between the 3 mm and 6 mm levels for all groups (Table 1). Pairwise comparisons between groups, as presented in Table 2, revealed statistically significant differences between all groups at 3 mm ($p<0.05$). Additionally, in Table 3, pairwise comparisons between different groups demonstrated a significant difference in the average depth of sealer penetration at 6 mm compared to ZOE ($p<0.05$). However, no statistically significant difference was

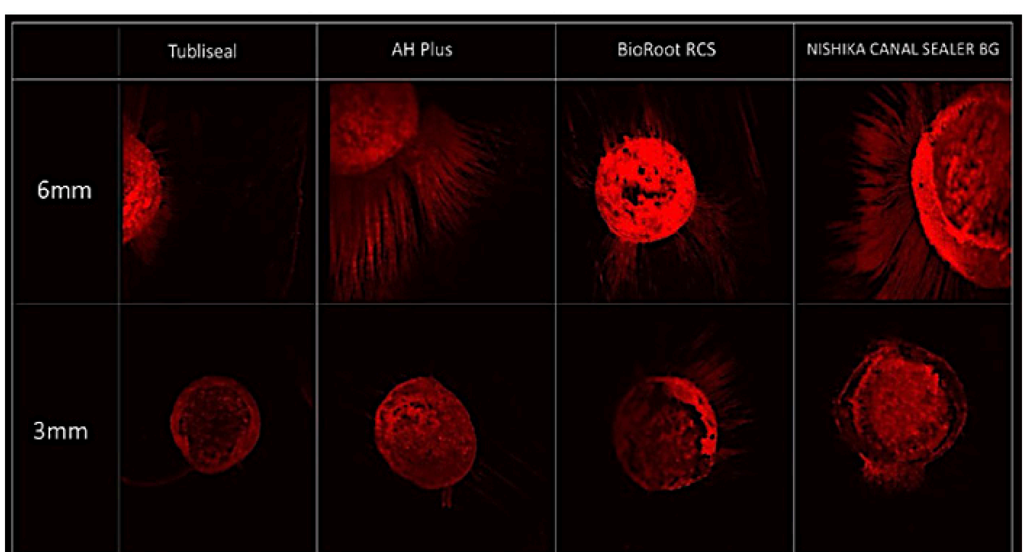


Figure 3
Comparative Analysis of dentinal tubule penetration by ZOE-based, resin-based, bioceramic, and bioactive glass-based root canal sealers using CLSM.

Table 1

Comparison of different sealer types for the depth of dentinal tubule penetration at 3 mm and 6 mm

Sealer Type	Groups		*p-value*
	3 mm	6 mm	
ZOE based	102.5833±8.72	227.5±21.90	0.001*
Resin based	403.6667±44.63	731.4167±109.36	0.05*
Bioceramic based	679±90.08	1041.75±275.92	0.004*
Bioactive glass	504.9167±116.80	885.91±167.44	0.001*

**indicates statistically significant difference (p<0.05)*

Table 2

Pairwise comparison of the average depth of dentinal tubule penetration at the 3 mm level

	Group (I)	Group (J)	Mean difference (I-J)	*p-value*
Depth of dentinal tubule penetration	Group I	Group II	301.08	0.001*
	Group I	Group III	576.42	0.001*
	Group I	Group IV	402.33	0.001*
	Group II	Group III	275.33	0.001*
	Group II	Group IV	101.25	0.001*
	Group III	Group IV	174.08	0.001*

**indicates statistically significant difference (p<0.05)*

Table 3

Pairwise comparison of the average depth of dentinal tubule penetration at the 6 mm level

	Group (I)	Group (J)	Mean difference (I-J)	*p-value*
Depth of dentinal tubule penetration	Group I	Group II	503.92	0.001*
	Group I	Group III	814.25	0.001*
	Group I	Group IV	658.42	0.001*
	Group II	Group III	310.33	0.005*
	Group II	Group IV	154.50	0.16
	Group III	Group IV	155.83	0.15

**indicates statistically significant difference (p<0.05)*

observed between resin-based, bioceramic, and bioactive glass groups ($p>0.05$).

Percentage of sealer penetration

At the 3 mm level, samples treated with the ZOE-based sealer displayed a mean percentage of sealer penetration along the root canal wall of approximately 44.8%, marking the lowest among all groups. In contrast, samples treated with resin-based and bioceramic sealers demonstrated mean percentages of sealer penetration of approximately 64.41% and 74.12%, respectively. Notably, the bioactive glass group exhibited the highest mean percentage of sealer penetration at 75.7%. At the 6 mm

Table 4

Comparison of sealer penetration percentages at 3 mm and 6 mm levels

Sealer Type	Groups		*p-value*
	3 mm	6 mm	
ZOE based	44.80±8.72	57.51±21.90	0.56
Resin based	64.41±44.63	68.1±109.36	0.63
Bioceramic based	74.12±90.08	77.97±275.92	0.56
Bioactive glass	75.70±116.80	79.09±167.44	0.56

**indicates statistically significant difference (p<0.05)*

Table 5

Inter-group comparisons for sealer penetration percentage at 3 mm depth

	Group (I)	Group (J)	Mean difference (I-J)	*p-value*
Percentage sealer penetration	Group I	Group II	19.61	0.001*
	Group I	Group III	29.32	0.002*
	Group I	Group IV	30.90	0.001*
	Group II	Group III	9.71	0.44
	Group II	Group IV	11.29	0.30
	Group III	Group IV	1.58	0.99

**indicates statistically significant difference (p<0.05)*

Table 6

Inter-group comparisons for sealer penetration percentage at 6 mm depth

	Group (I)	Group (J)	Mean difference (I-J)	*p-value*
Percentage sealer penetration	Group I	Group II	10.58	0.41
	Group I	Group III	20.46	0.002*
	Group I	Group IV	21.58	0.001*
	Group II	Group III	9.88	0.47
	Group II	Group IV	10.99	0.37
	Group III	Group IV	1.12	0.99

**indicates statistically significant difference (p<0.05)*

level, ZOE-based sealer-treated samples showed a mean percentage of sealer penetration along the root canal wall of approximately 57.51%, again representing the lowest among the four groups. In comparison, samples treated with resin-based sealers exhibited a slightly higher extent of penetration along the root canal wall at approximately 68.1%, while bioactive glass showed the highest mean percentage of sealer penetration at approximately 79.09%. No significant difference ($p>0.05$) was observed between the 3 mm and 6 mm levels for any of the groups, as indicated in Table 4.
The post hoc test results in Table 5 high-

light intergroup pair-wise comparisons specifically at the 3 mm level, revealing a significant difference in the percentage of penetration between ZOE and other groups ($p<0.05$). However, no statistically significant difference was observed between resin-based, bioceramic, and bioactive glass groups ($p>0.05$). Table 6 further demonstrates a statistically significant difference ($p<0.05$) between ZOE vs. bioceramic-based sealers and ZOE vs. bioactive glass-based sealers.

Discussion

Bacterial colonization tends to concentrate in the apical region of diseased root canals, posing challenges to effective treatment. Neglecting this area during cleaning may impede the healing of periapical lesions, while inadequate sealing of the apical root canal can create an environment conducive to bacterial proliferation, increasing the risk of endodontic failure (15). Endodontic sealers are indispensable in achieving successful endodontic therapy by completing root canal fillings during obturation procedures. Grossman outlined the characteristics of an ideal sealer, including tackiness for strong adhesion to the canal wall, the ability to create a hermetic seal, and radiopacity for easy radiographic visualization. Additionally, an ideal sealer should blend fine powder seamlessly with liquid, resist contraction upon setting, avoid tooth discoloration, exhibit bacteriostatic properties, remain insoluble in bodily fluids, demonstrate biocompatibility, and be soluble in common solvents (16).

These sealers, classified by composition as ZOE, calcium hydroxide, glass ionomer, silicone, resin, and bioceramic-based, have been extensively studied due to their significant biological and technical implications since their inception in the early twentieth century (2).

Root canal fillings typically involve a robust core material, such as gutta-percha, combined with a sealer to facilitate adaptation and ensure an effective seal of the root canal filling material (17, 18). The interface between the sealer and the root canal wall is pivotal for sealing the entire root canal system (19). The sealer plays a crucial role in filling irregularities on the root canal wall and within dentinal tubules, areas that gutta-percha alone may not reach. Enhanced sealer penetration into the tubules is associated with improved sealability, augmenting the contact surface between the filling material and dentin (20). Additionally, sealer penetration can contribute to an antimicrobial effect within the tubules, particularly when microbes are present nearby (21). Notably, sealer penetration observed in in vitro models closely mimics in vivo conditions (22-26).

The search for the best obturating materials and filling techniques relies heavily on a thorough analysis of the adhesion between the sealer and dentin, as well as its capacity to penetrate deep into dentinal tubules. This meticulous examination is crucial for ensuring successful endodontic therapy. Various microscopic techniques, including stereomicroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and CLSM, are utilized to explore the penetrating capabilities of sealers and the interaction between the sealer and dentin.

While SEM requires the desiccation of root sections, which can result in potential sealer loss and specimen deformation, CLSM offers distinct advantages as it enables evaluation without the need for destructive specimen preparation (27-32). The application of Rhodamine B dye in CLSM facilitates a rapid and detailed assessment of sealer penetration, permitting a closer examination at lower magnifications (33). It is noteworthy that the inclusion of 0.1% Rhodamine B dye into root canal sealers does not affect their flow characteristics (34).

While most root canal sealers do not chemically bond to dentinal walls, their tubular penetration enhances the mechanical retention of root-filling materials within the root canal space (35, 36). This study demonstrates that bioceramic and bioactive glass-based sealers exhibited deeper tubular penetration compared to resin and ZOE groups across all levels examined.

The superior penetration of bioceramic sealer over ZOE sealer may be attributed to its lower film thickness, potentially compensating for its reduced hydrophilic properties (37). Moreover, the deeper tubular penetration observed at the 6-mm section compared to the 3-mm section can be attributed to the greater thickness of dentinal tubules in the middle and coronal parts relative to the apical region of the root (38). The presence of a smear layer occludes tubular ostia and impedes sealer penetration into the tubular space; thus, the removal of the smear layer using 17% EDTA and 5.25% NaOCl enhances the tubular penetration of root canal sealers (39). Research findings indicate that passive ultrasonic irrigation surpasses manual irrigation techniques in eliminating smear layers effectively (40-42).

De-Deus et al. observed that while the vertical compaction technique led to deeper sealer penetration compared to lateral condensation or single-cone techniques, lateral condensation provided better distribution of the sealer, particularly in the middle and coronal thirds (43).

Conversely, Jeong et al. found that the warm vertical compaction technique did not affect the tubular penetration of calcium silicate-based sealers (32). In a separate study examining AH26 sealer, it was noted that sealer penetration significantly increased with the use of 17% EDTA, maleic acid, or citric acid as a final irrigation step following the removal of the smear layer (36).

Additionally, Chandra et al., using a confocal microscope, observed maximum tubular penetration in the RealSeal group, followed by the AH Plus, RoekoSeal, and EndoRez groups, with penetration being greatest in the coronal third, followed by the middle and apical parts (44).

Khader conducted an SEM study, which found comparable levels of tubular penetration between AH Plus and Apexit Plus sealers, while the Tubli-Seal group exhibited less penetration (45).

Conversely, Kuçi et al. observed in a confocal microscopic study that removing the smear layer enhanced the tubular penetration of MTA Fillapex, but not AH26 sealer. They noted deeper tubular penetration in the MTA Fillapex group compared to the AH26 group, suggesting differences in assessment methods and sealer placement techniques as potential factors influencing tubular penetration (46).

Conclusion

The bioactive glass group stood out for its remarkable performance, displaying the highest percentage of sealer penetration compared to the other groups at both depths. Importantly, our statistical analysis found no significant difference between the bioceramic and bioactive glass groups at the 3 mm and 6 mm levels in terms of both depth and percentage of dentinal tubule penetration. Conversely, the ZOE sealer exhibited the least tubule penetration at both levels examined.

Clinical Relevance

This study demonstrates that bioactive glass-based root canal sealers exhibit superior penetration into dentinal tubules compared to traditional sealers like ZOE and resin-based sealers. This enhanced penetration can lead to better sealing of the root canal system, potentially improving treatment outcomes in endodontic therapy. The findings highlight the importance of selecting sealers with optimal penetration capabilities to enhance the success of root canal treatments. Additionally, the study underscores the bioactive properties of novel sealers, which have the potential to promote tissue healing and regeneration, further enhancing their clinical utility.

Conflict of Interest

The authors have no conflicts of interest.

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