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Comparative Evaluation of Dentinal Tubule Penetration of Four Root Canal Sealers Using Scanning Electron Microscopy: An in Vitro Study

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Abstract:

- ***Background***

The long-term success of endodontic treatment relies on effective cleaning, shaping, and three-dimensional obturation of the root canal system. Dentinal tubule penetration of root canal sealers enhances mechanical interlocking, entombs residual microorganisms, and minimizes microleakage. Contemporary sealers differ significantly in physicochemical properties that may influence their tubular penetration ability.

- ***Aim***

To compare the dentinal tubule penetration depth of four different root canal sealers using scanning electron microscopy (SEM).

- ***Materials and Methods***

Fifty extracted human single-rooted teeth with mature apices were selected and decoronated to a standardized root length of 14 mm. Root canals were instrumented using the ProTaper Gold rotary system up to F2 and irrigated with 5.25% sodium hypochlorite and 17% EDTA. Samples were randomly divided into five groups (n=10) according to the sealer used: Group 1 – AH Plus, Group 2 – Sealapex, Group 3 – Bio-C Sealer, Group 4 – MTA Fillapex, and Group 5 – Eugeseal. Obturation was performed using the single-cone technique. Following incubation at 37°C and 100% humidity for one week, roots were sectioned at coronal, middle, and apical thirds. Dentinal tubule penetration was evaluated using SEM. Statistical analysis was performed using One-Way ANOVA and post hoc LSD tests with significance set at $p<0.05$.

- ***Results***

Statistically significant differences in dentinal tubule penetration were observed among all groups ($p=0.001$). MTA Fillapex demonstrated the highest penetration in the coronal (973.0±74.84 µm) and middle thirds (627.0±46.67 µm). Bio-C Sealer showed superior penetration in the apical third. AH Plus exhibited substantial coronal penetration but significantly lower apical penetration. Sealapex demonstrated moderate penetration, whereas Eugeseal exhibited the least penetration in all thirds.

- ***Conclusion***

Bioceramic and MTA-based sealers demonstrated superior dentinal tubule penetration compared with conventional resin- and zinc oxide eugenol–based sealers. MTA Fillapex showed the greatest penetration in coronal and middle thirds, while Bio-C Sealer exhibited superior apical penetration. Enhanced dentinal tubule penetration may contribute to improved sealing ability and long-term endodontic success.

Keywords: *Dentinal Tubule Penetration; Root Canal Sealer; Scanning Electron Microscopy; AH Plus; Bio-C Sealer; MTA Fillapex; Bioceramic Sealer; Endodontics.*

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I. INTRODUCTION

Successful endodontic treatment depends on complete debridement of the root canal system and the establishment of a fluid-tight seal that prevents reinfection. Although advances in instrumentation and irrigation have significantly improved canal disinfection, residual microorganisms may persist within dentinal tubules and anatomical irregularities.[1] Consequently, root canal sealers play a vital role in obturation by filling voids, enhancing adaptation between gutta-percha and dentinal walls, and entombing residual microorganisms.[2]

Root canal therapy aims to preserve teeth affected by pulpal and periapical pathosis through effective cleaning, shaping, disinfection, and obturation of the root canal system. The success of endodontic treatment is highly dependent on the elimination of microorganisms and prevention of reinfection within the complex anatomy of the root canal space. However, complete disinfection remains challenging because of the intricate morphology of root canals, including fins, isthmuses, lateral canals, and dentinal tubules, which may harbor residual bacteria even after thorough chemomechanical preparation.[3]

Dentinal tubules play a significant role in the persistence and progression of pulpal and periapical infections. Microorganisms such as *Enterococcus faecalis* have the ability to penetrate deeply into dentinal tubules, making them resistant to intracanal medicaments and irrigation procedures. Therefore, obturation materials capable of penetrating dentinal tubules may help entomb residual bacteria and reduce the risk of reinfection. Adequate sealer penetration also improves the adaptation between dentin and gutta-percha, thereby minimizing gaps and enhancing the sealing ability of the obturation system.[4]

Root canal sealers are used in conjunction with core obturating materials to fill anatomical irregularities and create a hermetic seal. An ideal sealer should possess excellent flowability, dimensional stability, biocompatibility, antimicrobial activity, radiopacity, and adhesion to dentinal walls. In recent years, significant advancements have been made in the development of endodontic sealers with improved biological and physicochemical properties.[5]

Dentinal tubule penetration by sealers is considered an important parameter influencing the quality of obturation. Increased tubular penetration improves mechanical retention, enhances sealer adaptation, and may reduce apical and coronal microleakage. Penetration depth is influenced by several factors including sealer viscosity, particle size, flow characteristics, smear layer removal, dentinal permeability, and obturation technique.[6]

Epoxy resin–based sealers such as AH Plus have been extensively studied and are considered a benchmark because of their low solubility, favorable sealing ability, and good adhesion to dentin. Nevertheless, the emergence of calcium silicate–based bioceramic sealers has introduced a new dimension in endodontic obturation. Bioceramic sealers are hydrophilic, bioactive, and capable of forming hydroxyapatite upon setting, thereby promoting chemical bonding with dentin. These materials also demonstrate excellent dimensional stability and alkaline pH, which may contribute to antimicrobial action and periapical healing.[7]

MTA-based sealers, including MTA Fillapex, have gained considerable attention due to their bioactivity and ability to stimulate mineralization. Their smaller particle size and favorable flow characteristics may facilitate deeper penetration into dentinal tubules. Similarly, calcium hydroxide–based sealers such as Sealapex have been widely used because of their antibacterial properties and potential to stimulate hard tissue formation. Conversely, zinc oxide eugenol–based sealers such as Eugeseal continue to be utilized because of their long clinical history and acceptable handling properties, despite limitations including higher solubility and lower flowability.[8]

Several factors influence the depth and quality of dentinal tubule penetration by sealers. These include the diameter and density of dentinal tubules, smear layer removal, irrigation protocol, obturation technique, sealer viscosity, particle size, and setting expansion. Removal of the smear layer using chelating agents such as EDTA exposes dentinal tubules and facilitates greater sealer infiltration. Furthermore, studies have shown that penetration is generally greater in the coronal third compared with the apical third because of anatomical variations in tubule density and diameter.[9]

Various imaging modalities have been employed to evaluate dentinal tubule penetration, including confocal laser scanning microscopy (CLSM), stereomicroscopy, and scanning electron microscopy (SEM). SEM provides high-resolution visualization of the sealer-dentin interface and enables accurate assessment of tubular penetration depth. Consequently, SEM remains one of the most reliable methods for analyzing the interfacial characteristics of endodontic sealers.[10]

Although numerous investigations have compared the penetration ability of different sealers, conflicting results continue to exist because of variations in study design, instrumentation systems, irrigation regimens, and obturation techniques. Therefore, further comparative studies using standardized methodologies are required to determine the performance of contemporary sealers under controlled conditions.[11]

The present study was designed to comparatively evaluate the dentinal tubule penetration depth of AH Plus,

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Sealapex, Bio-C Sealer, MTA Fillapex, and Eugeseal using scanning electron microscopy. The findings of this study may contribute to a better understanding of the sealing potential of contemporary endodontic sealers and assist clinicians in selecting suitable obturation materials for improved long-term treatment outcomes.[12]

II. MATERIALS AND METHODS

➢ *Study Design*

This in vitro experimental study was conducted in the Department of Conservative Dentistry and Endodontics in collaboration with the Sophisticated Analytical Instrumentation Facility (SAIF).

➢ *Sample Selection*

Fifty freshly extracted human single-rooted teeth with mature apices were selected.

➢ *Inclusion Criteria*

- Intact teeth extracted for periodontal or orthodontic reasons
- Single-rooted teeth with mature apices
- Non-carious teeth

➢ *Exclusion Criteria*

- Teeth with fractures, cracks, or craze lines
- Teeth with root caries or restorations
- Teeth with calcified canals
- Teeth with open apices or root resorption

➢ *Specimen Preparation*

- The crowns were decoronated using a diamond disc under water cooling to standardize root length to 14 mm.
- Working length was established 1 mm short of the apical foramen using a #15 K-file.

➢ *Chemomechanical Preparation*

Root canals were prepared using the ProTaper Gold rotary file system up to size F2. Irrigation during instrumentation was performed using:

- 5.25% sodium hypochlorite
- 17% EDTA
- Distilled water

Final irrigation consisted of:

- 5 mL 17% EDTA
- 5 mL 5.25% NaOCl
- 5 mL distilled water

Canals were dried with sterile paper points.

➢ *Group Allocation*

Samples were randomly divided into five groups (n=10):

- Group 1: AH Plus sealer
- Group 2: Sealapex sealer
- Group 3: Bio-C Sealer
- Group 4: MTA Fillapex sealer
- Group 5: Eugeseal sealer

➢ *Obturation Procedure*

Single-cone obturation using F2 gutta-percha cones was performed. Sealers were introduced using lentulospirals. Access cavities were sealed using Cavit G, and specimens were incubated at 37°C with 100% humidity for one week.

➢ *Sectioning and SEM Analysis*

Three horizontal sections of 1 mm thickness were obtained at:

- Coronal third (7–8 mm)
- Middle third (4–5 mm)
- Apical third (1–2 mm)

Sections were examined using scanning electron microscopy to evaluate maximum dentinal tubule penetration depth.

➢ *Statistical Analysis*

Data were analyzed using SPSS version 23.0. One-Way ANOVA followed by LSD post hoc analysis was used. Statistical significance was set at $p<0.05$.

III. RESULT

A statistically significant difference in dentinal tubule penetration was observed among the five root canal sealers at the coronal, middle, and apical thirds of the root ($p = 0.001$).

- Coronal third: MTA Fillapex demonstrated the highest mean penetration (973.0 ± 74.84 µm), followed by AH Plus (826.35 ± 87.43 µm), Sealapex (514.75 ± 27.99 µm), Bio-C Sealer (210.00 ± 41.63 µm), and Eugeseal (88.87 ± 12.03 µm). All pairwise comparisons were statistically significant ($p < 0.05$).
- Middle third: MTA Fillapex again showed the greatest penetration (627.0 ± 46.68 µm), followed by Bio-C Sealer (486.0 ± 38.64 µm), Sealapex (349.78 ± 24.75 µm), AH Plus (317.45 ± 39.51 µm), and Eugeseal (56.67 ± 7.87 µm). Most intergroup differences were statistically significant ($p < 0.05$).
- Apical third: Bio-C Sealer exhibited the highest penetration (696.0 ± 32.04 µm), followed by MTA Fillapex (295.0 ± 32.66 µm), Sealapex (105.18 ± 13.91 µm), AH Plus (36.74 ± 16.70 µm), and Eugeseal (16.20 ± 5.30 µm). Nearly all intergroup comparisons were statistically significant ($p < 0.05$).
- Intragroup comparison: AH Plus, Sealapex, MTA Fillapex, and Eugeseal showed progressively decreasing penetration from the coronal to the apical third, whereas Bio-C Sealer demonstrated the opposite trend, with maximum penetration in the apical third. These differences were statistically significant in all groups ($p = 0.001$).

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Overall, MTA Fillapex exhibited the greatest dentinal tubule penetration in the coronal and middle thirds, while Bio-C Sealer showed superior penetration in the apical third. Eugeseal consistently demonstrated the lowest penetration at all root levels.

➢ *Intragroup Comparison Between Coronal, Middle and Apical Third*

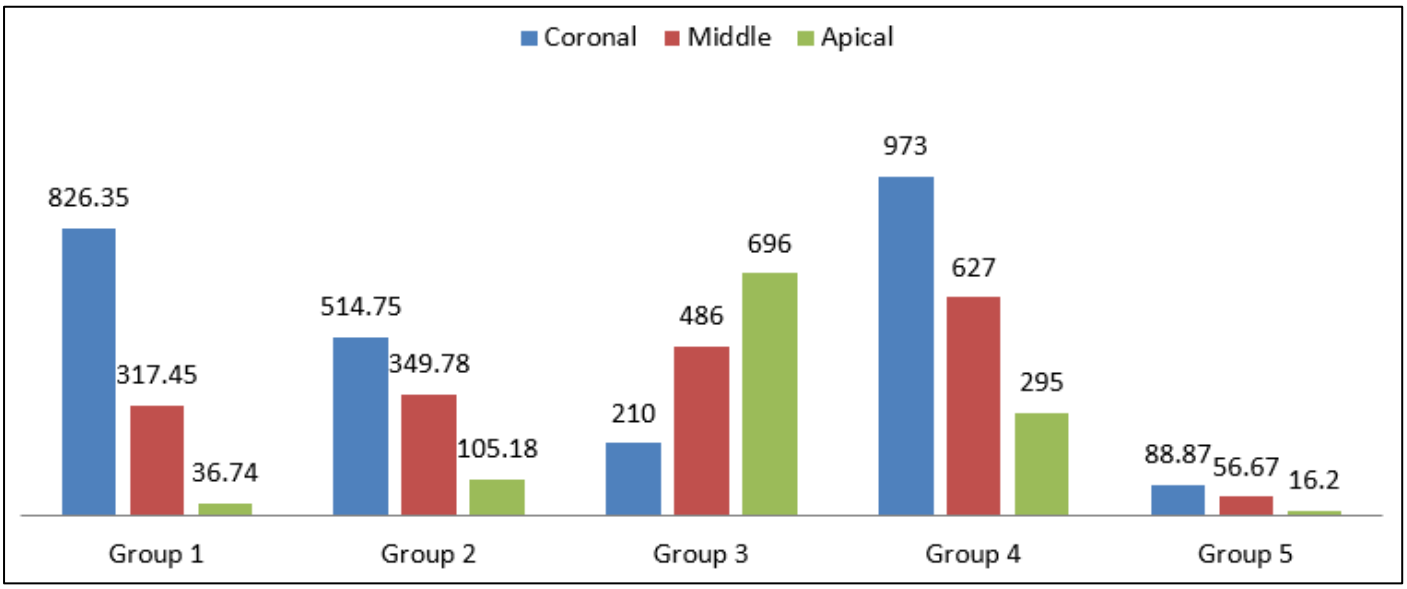

Fig 1 Intragroup Comparison Between Coronal, Middle and Apical Third

IV. DISCUSSION

The present in vitro investigation evaluated and compared the dentinal tubule penetration ability of AH Plus, Sealapex, Bio-C Sealer, MTA Fillapex, and Eugeseal using scanning electron microscopy. Effective penetration of root canal sealers into dentinal tubules is considered essential for improving the sealing quality of obturation systems because it enhances mechanical interlocking, entombs residual microorganisms, and reduces microleakage. The findings of the present study demonstrated statistically significant differences among the tested sealers in all radicular thirds, indicating that the physicochemical characteristics of each material considerably influence its penetration ability.[9]

Among all experimental groups, MTA Fillapex demonstrated the greatest dentinal tubule penetration in the coronal and middle thirds. This superior penetration may be attributed to its lower viscosity, higher flowability, smaller particle size, and hydrophilic nature. MTA-based sealers possess excellent adaptation properties and are capable of flowing into dentinal irregularities and accessory canals. Additionally, the resinous components present in MTA Fillapex may improve handling characteristics and facilitate deeper intratubular diffusion. Similar findings have been reported in previous studies where MTA Fillapex exhibited significantly greater tubular penetration compared with conventional sealers.[11]

Bio-C Sealer exhibited superior penetration in the apical third of the root canal. The enhanced penetration observed with Bio-C Sealer may be explained by its bioceramic composition, nanoparticle size, and hydraulic properties. Bioceramic sealers are highly hydrophilic and utilize the inherent moisture within dentinal tubules for setting reactions, thereby facilitating intimate adaptation to dentinal walls. Furthermore, these materials are capable of forming hydroxyapatite during setting, which may improve sealing ability and biomineralization. Previous investigations have similarly demonstrated deeper dentinal tubule penetration and superior adaptation of bioceramic sealers compared with resin-based and zinc oxide eugenol–based sealers.[12]

AH Plus demonstrated substantial penetration in the coronal region but comparatively reduced penetration in the apical third. AH Plus is an epoxy resin–based sealer known for its dimensional stability, long working time, and low polymerization shrinkage. These characteristics contribute to effective adaptation within wider dentinal tubules present in the coronal and middle thirds. However, the comparatively higher viscosity of AH Plus may limit its penetration into narrower apical tubules. Reduced tubule diameter and sclerosis in the apical region may further compromise sealer infiltration. Nevertheless, AH Plus continues to be regarded as a gold standard because of its excellent sealing ability and long-term clinical success.[10]

Sealapex demonstrated moderate dentinal tubule penetration in all thirds of the root canal. Calcium hydroxide–based sealers possess antibacterial activity and the ability to stimulate hard tissue formation because of calcium ion release. However, their comparatively lower flow characteristics and higher solubility may reduce their capacity for deeper tubular penetration. Although Sealapex demonstrated inferior penetration compared with MTA Fillapex and Bio-C Sealer, its biological properties may still contribute positively to periapical healing and tissue compatibility.[13]

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Eugeseal exhibited the least dentinal tubule penetration among all tested sealers. Zinc oxide eugenol–based sealers are associated with larger particle size, increased solubility, and lower flowability, which may limit their ability to penetrate deeply into dentinal tubules. In addition, eugenol-containing sealers may undergo shrinkage during setting, potentially compromising adaptation to canal walls. The lower penetration values observed in the present study are consistent with previous reports comparing conventional sealers with newer bioceramic materials.[14]

The present study also demonstrated a progressive reduction in dentinal tubule penetration from the coronal toward the apical third in all groups. This finding can be attributed to anatomical variations in dentin structure. Dentinal tubules are more numerous and wider in diameter in the coronal third, whereas the apical third contains fewer and narrower tubules with increased sclerosis. Additionally, restricted irrigant penetration and reduced sealer flow in the apical region may contribute to decreased tubular infiltration. Similar penetration patterns have been consistently reported in earlier endodontic investigations.[15]

Smear layer removal also plays a critical role in dentinal tubule penetration. In the present study, final irrigation with 17% EDTA followed by sodium hypochlorite was performed to eliminate the smear layer and expose dentinal tubules. Removal of the smear layer enhances dentin permeability and allows deeper sealer penetration. Previous studies have shown significantly greater penetration depth when chelating agents are used during final irrigation compared with irrigation protocols lacking smear layer removal.[16]

Scanning electron microscopy was utilized in the present study because it provides high-resolution imaging and precise evaluation of the sealer-dentin interface. SEM enables detailed observation of dentinal tubule penetration and interfacial adaptation at high magnification. Although confocal laser scanning microscopy has recently gained popularity for evaluating sealer penetration, SEM remains a reliable and widely accepted method for ultrastructural analysis in endodontic research.[17]

Despite the valuable findings obtained in the present study, certain limitations should be considered. The investigation was performed under in vitro conditions, which cannot completely reproduce the complex biological environment of the oral cavity. Factors such as blood contamination, periodontal ligament simulation, occlusal stresses, and long-term aging were not evaluated. Furthermore, only dentinal tubule penetration was assessed, whereas other important parameters such as bond strength, solubility, retreatability, and antimicrobial efficacy were not investigated. Future studies utilizing micro-CT and confocal laser scanning microscopy may provide additional information regarding three-dimensional adaptation and long-term sealing performance of contemporary sealers.[7]

The ability of endodontic sealers to penetrate dentinal tubules is closely associated with their physicochemical characteristics, particularly flowability and film thickness. Sealers with lower film thickness can spread more uniformly along dentinal walls and penetrate accessory anatomy more effectively. According to ISO specifications, root canal sealers should possess adequate flow to permit penetration into canal irregularities while maintaining dimensional stability after setting. In the present study, the superior penetration demonstrated by Bio-C Sealer and MTA Fillapex may be related to their favorable rheological behavior and enhanced wettability.[18]

Another important factor affecting dentinal tubule penetration is the obturation technique employed during canal filling. In the present investigation, the single-cone obturation technique was used to standardize sealer distribution among all groups. The hydraulic movement generated during single-cone obturation allows sealers with superior flow characteristics to spread more efficiently into dentinal tubules. Previous investigations have shown that obturation pressure and cone adaptation significantly influence sealer penetration depth and interfacial adaptation.[19]

The hydrophilic nature of bioceramic sealers contributes substantially to their enhanced penetration ability. Unlike hydrophobic resin-based sealers, bioceramic materials utilize residual moisture present within dentinal tubules to initiate and complete their setting reaction. This property improves their adaptability to moist dentin surfaces and promotes deeper tubular infiltration. In addition, calcium silicate–based sealers release calcium ions and create an alkaline environment, which may contribute to antimicrobial effects and hard tissue deposition.[20]

The particle size of endodontic sealers is another critical determinant influencing dentinal tubule penetration. Dentinal tubule diameters range from approximately 2–3 μm in the coronal region to less than 1 μm near the apex. Sealers with nanoparticle-sized components may therefore penetrate deeper into these microscopic spaces. Bio-C Sealer and MTA Fillapex contain finer particles compared with conventional zinc oxide eugenol–based sealers, which may explain their improved penetration characteristics observed in this study.[21]

The antimicrobial properties of root canal sealers are also clinically relevant because residual microorganisms within dentinal tubules may contribute to persistent periapical disease. Bioceramic and calcium hydroxide–based sealers release hydroxyl ions that increase local pH, thereby creating an unfavorable environment for bacterial survival. Enhanced tubular penetration combined with antimicrobial activity may improve the long-term prognosis of endodontically treated teeth. However, the relationship between penetration depth and antimicrobial effectiveness requires further investigation.[22]

The adaptation of sealers to root canal dentin is influenced not only by material properties but also by dentin moisture conditions. Excessive drying may collapse collagen fibers and reduce dentin permeability, whereas excessive moisture may interfere with sealer setting and adhesion. Bioceramic sealers are less sensitive to moisture variations because of their hydraulic setting mechanism. This property

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may partially explain the superior adaptation and apical penetration demonstrated by Bio-C Sealer in the present investigation.[23]

The findings of the present study are clinically important because inadequate sealer penetration may leave voids and unfilled areas that permit bacterial recolonization. Persistent microleakage remains one of the primary causes of endodontic failure. Sealers demonstrating superior penetration and adaptation may therefore improve the long-term integrity of root canal obturation. Nevertheless, clinicians should consider additional properties such as retreatability, radiopacity, setting time, and biocompatibility before selecting a sealer for routine clinical use.[24]

Recent advancements in endodontic biomaterials have focused on developing sealers capable of bioactivity and regenerative potential. Contemporary calcium silicate–based materials not only seal the canal space but may also stimulate mineralized tissue deposition at the dentin-sealer interface. Such biomineralization may improve long-term sealing ability and reinforce the structural integrity of the root canal system. Future biomaterials may further enhance dentinal interaction and antimicrobial performance through nanotechnology and bioactive additives.[25]

V. CONCLUSION

All tested sealers demonstrated varying degrees of dentinal tubule penetration.MTA Fillapex exhibited the greatest penetration in coronal and middle thirds.Bio-C Sealer demonstrated superior penetration in the apical third. AH Plus showed good coronal penetration but reduced apical penetration. Eugeseal exhibited the least penetration in all radicular thirds. Dentinal tubule penetration decreased progressively from coronal to apical regions. Bioceramic and MTA-based sealers demonstrated superior penetration characteristics compared with conventional sealers. These findings suggest that contemporary bioceramic and MTA-based sealers may provide improved dentinal adaptation and sealing potential in endodontic obturation.

➢ *Clinical Significance*

Enhanced dentinal tubule penetration may improve:

- Mechanical retention of obturation materials
- Entombment of residual microorganisms
- Resistance to microleakage
- Long-term success of root canal therapy

Selection of sealers with superior penetration characteristics may therefore contribute to improved clinical outcomes in endodontic treatment.

➢ *Future Scope*

Future studies should evaluate:

- Long-term sealing ability
- Antibacterial efficacy
- Push-out bond strength
- Retreatability of contemporary sealers
- Comparative evaluation using CLSM and micro-CT

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