

An In-Vitro Comparative Evaluation in The Depth of Penetration of Five Different Root Canal Sealers Using Five Different Sealer Placement Techniques in The Apical-Thirds of Root Canals Under Confocal Laser Scanning Microscope (CLSM) Analysis

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Abstract

Aim: Comparative evaluation in the depth of penetration of five different root canal sealers using five different sealer placement techniques in the apical-thirds of root

canals using Confocal Laser Scanning Microscope (CLSM) analysis.

Materials and Methods: One hundred and twenty-five extracted human mandibular single-rooted premolars were decoronated, root canal access obtained and canals

were instrumented using ProTaper Gold rotary files. The specimens were then divided into five groups, 25 teeth per group based on the root canal sealer used: Group 1: AH-Plus, Group 2: MTA-Fillapex, Group 3: Bio-C, Group 4: Endoseal, Group 5: Apexit-Plus. Each group was further divided into five sub-groups, 5 teeth per sub-group based on root canal sealer placement technique used: Sub-group A: ProTaper Gold Gutta-percha Point, Sub-group B: Lentulospiral, Sub-group C: Bidirectional spiral, Sub-group D: Pastinject system, Sub-group E: K-file. Each sealer was then labeled with Rhodamine-B dye. The root canals were then coated with respective sealer and sealer placement techniques as per the groups assigned and canals were obturated with gutta-percha following single cone technique.

All teeth were then incubated at 37°C for 7 days and were then horizontally sectioned at the apical-third, 4mm from the pre-determined working length, examined under CLSM to evaluate the depth of penetration of sealer.

Results: OneWay ANOVA and Tukey post-hoc tests were used for statistical analysis. P value <0.05 was considered as statistically significant. Bio-C and AH-Plus sealers when placed using Lentulospiral and Bidirectional spiral showed the greatest depth of penetration compared to other root canal sealers and sealer placement techniques.

Conclusion: Bio-C sealer showed the maximum depth of penetration compared to other sealers used. Lentulospiral instrument for the placement of sealers showed the maximum depth of penetration of all sealers used in our study. However, the use of Lentulospiral for the placement of Bio-C sealer showed the maximum depth of penetration compared to all other sealers and sealer placement techniques used.

Keywords: Bio-C sealer, Confocal laser scanning microscope, Depth of penetration, Root canal sealer, Sealer placement technique.

Introduction

Successful endodontic therapy relies on the complete eradication of microorganisms from the root canal system and the prevention of reinfection by achieving a fluid-tight three-dimensional seal. A proper root canal filling material prevents the penetration of microorganisms and their toxins into the root canal space, allows for periapical repair and prevents reinfection. The obturation process can be optimized by choosing an ideal root canal sealer combined with guttapercha following an effective obturation technique and a more efficient sealer placement method.¹

The primary purpose of a root canal sealer is aimed at providing a three-dimensional root canal seal with root canal dentin.² Various types of root canal sealers were used in conjunction with guttapercha for root canal obturation with varied success.³ The penetration of root canal sealer into the dentinal tubules of root canal dentin is considered to be a desirable outcome for a number of reasons; it increases the interface between guttapercha and dentin thus improving the sealing ability by mechanical locking, entombing any residual bacteria within the dentinal tubules and the chemical components of root canal sealer cements exert an antibacterial effect. Therefore, for a good obturation, it is important to have sealer/dentin interface as great as possible.⁴

Greater penetration of sealers, especially in the apical-third of the root canals contributes to improved sealing efficiency and long-term success of the root canal therapy. Endoseal (Prevest Denpro) is a Zinc oxide eugenol-based root canal sealer and it has been a standard in endodontics since its development. It contains zinc oxide powder and eugenol liquid, an essential oil derived

from cloves. It is well tolerated by the tissues and provides anti-inflammatory action.⁵

Apexit-Plus (Ivoclar Vivadent, Schaan, Liechtenstein) a calcium hydroxide-based endodontic sealer, The base paste of this sealer contains calcium hydroxide, calcium oxide, hydrated collophonium, highly dispersed silicon dioxide, and alkyl ester of phosphoric acid, while the activator paste contains disalicylate, bismuth hydroxide, bismuth carbonate associated to silicon dioxide and phosphoric acid alkyl ester. The higher pH of this sealer (12.5) is responsible for its antimicrobial effect.⁶ BIO-C sealer (Angelus, Londrina, PR, Brazil) is a ready-to-use bioceramic filling cement. It is a new root canal sealer composed of calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide and dispersing agent. It has optimal biocompatibility, bioactivity, high pH, radiopacity, flow values.⁷

AH-Plus (Amine Hydroxy) sealer (Dentsply, Maillefer, DeTray, Germany) is considered as a gold standard reference while considering desirable physicochemical properties of a sealer for root canal filling. This sealer gained popularity due to its biocompatibility, easy availability, radiopacity and ease of use with guttapercha as obturation material. It is an epoxy bis-phenol resin-based sealer containing calcium tungstate, adamantine and zirconium oxide, producing superior radiopacity and adhesion to root canal walls.⁸ MTA (Mineral Trioxide Aggregate) Fillapex (Angelus, Londrina, Parana, Brazil) is an MTA- based endodontic sealer with excellent biocompatibility, bioactivity and osteoconductivity. It is available in 2-paste system, can be very easily dispensed without any waste of the material and exhibits excellent handling properties with an efficient setting time. The two pastes upon mixing combines in a homogenous mix to form a rigid, but semi-permeable structure.⁹

The sealer placement techniques used significantly influences its distribution, penetration into dentinal tubules and adaptation to root canal walls. Conventional placement methods such as the use of master gutta-percha cone coating and K-file application often result in uneven sealer distribution and void formation, particularly in the apical third. Advanced techniques including lentulospiral, bidirectional spiral, and specialized paste carriers such as Past-inject have been introduced to improve sealer delivery, minimize air entrapment, and enhance penetration into complex canal anatomy. Application of an endodontic sealer may be optimized using an efficient sealer placement technique.¹⁰

The Lentulospiral, a flexible, spiral-shaped instrument attached to a slow-speed handpiece, offers a more efficient alternative to conventional placement methods. Its rotation generates centrifugal force that enhances lateral and apical penetration of the sealer, promoting deeper delivery throughout the canal and minimizing voids and air entrapment.¹¹ The invention of the Bidirectional spiral is a recent advancement in the sealer placement technique. This consists of coronal grooved spirals traveling in the apical direction and apical reverse spirals traveling in a coronal direction, bearing the cement coronally at the point of collision, causing the cement to be forced to travel laterally through the walls, lateral canals, and any other potential invaginations.¹² The MicroMega Pastinject system (Coltene Group) is a specially designed paste carrier that represents a more advanced sealer delivery method. When used with bioceramic sealers, Past inject has been shown to improve tubular penetration and adaptation.¹³

The apical-third of root canals presents an unique anatomical challenges due to reduced dentinal tubule density, presence of accessory canals. Since accessory

canals communicate with the periodontal membrane, they can create a periodontic-endodontic pathway for potential bacterial penetration to and from the periodontal tissues.¹⁴

Materials and Method

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics after obtaining the Institutional Ethical Committee clearance TIDSHRC/IEC/2022/D006. One hundred twenty-five freshly extracted human mandibular single rooted premolar teeth were collected, only the teeth extracted either for orthodontic reasons or periodontally compromised were used in the study. Teeth without any previous; Carious lesions, endodontically treated, fractured, cracks on the root surface, resorptive defects, calcifications, open root apices, single rooted and single canal as verified clinically and radiographically were only included in our study. Digital periapical radiographs of teeth were taken using Radiovisiography (RVG) (Kodak, Carestream Health India Pvt Ltd, Maharashtra, India) in bucco-lingual and mesio-distal directions to confirm the presence of a single straight canal and absence of any accessory canals, calcifications, resorptive defects and other anatomical anomalies and only the teeth with the degree of Root canal curvature between 10° to 20° according to Schneider SW¹⁵ criteria were only included in our study. All teeth were examined under Stereomicroscope (Olympus SZ61, Olympus Optical Co., Tokyo, Japan) at 10X magnification to ensure that they were intact and without any caries or non-caries lesions, devoid of restorations, clinically detectable fractures, cracks or enamel hypoplasia following strict inclusion criteria. Preliminary preparation involved diligent cleaning of all collected extracted teeth using ultrasonic instruments to remove superficial debris, calculus and residual tissue tags.

Occupational Safety and Health Administration (OSHA) and Center for Disease Control and Prevention guidelines were followed throughout specimen collection, sterilization and handling process. All specimens were stored in 0.5% Thymol at room temperature until use.

All teeth were then decoronated upto the level of 2mm coronal to cemento-enamel Junction using a diamond disc (DFS, Germany) attached to straight micromotor handpiece (NSK, Japan) at low-speed, to standardize length of all teeth. Endodontic access was done using round bur no. 2 (Mani, Japan) attached to high-speed contra-angled air-rotor handpiece (NSK, Japan) and canal orifices were located and pulp tissue was extirpated using barbed broaches (Mani, Japan). A no.10 K(Kerr)-file (Mani INC, Japan) was placed into the root canal to establish the patency and the working length was determined by subtracting 0.5mm from the length achieved with the tip of the trial file just visible at the apical foramen in each specimen. All specimens (n=125) were then numbered randomly from 1 to 125 and were then individually embedded in cylindrical self-cure acrylic resin blocks (DPI RR Cold Cure, Dental Product of India, Mumbai, India) upto a level 3mm apical to cemento-enamel junction for ease of handling of specimens during root canal instrumentation and root canal sealer placement. In all specimens, Root canal instrumentation was done using ProTaper Gold rotary file system upto F2 file as the master apical file. During instrumentation, 2ml of 17% EDTA solution (Ethylene Diamine Tetra Acetic acid) (Prime Dental Products Pvt Ltd, Thane, India), 2ml of 3% Sodium hypochlorite solution (Neelkanth Health Care Pvt Ltd, Jodhpur, India) and 2ml of 0.9% normal saline (Nivy Remedies Pvt. Ltd., West Bengal, India) were used as root canal irrigants per specimen. Each root canal was then finally flushed with 1 ml of 17% EDTA, 1 ml of 3% NaOCl solutions followed

by 2 ml of 0.9% normal saline. After the completion of root canal instrumentation, the canals were dried using sterile absorbent paper points (DiaDent, Group International, Korea).

All specimens (n=125) were randomly allocated into five groups, 25 specimens per group based on the type of root canal sealer used. Group 1: AH-Plus, Group 2: MTA-Fillapex, Group 3: Bio-C(Ceramic), Group 4: Endoseal, Group 5: Apexit-Plus (Figure no.1). Each group was further divided into five Sub-Groups (SG); A, B, C, D, E: 5 teeth each, based on the use of root canal sealer Placement techniques. In all groups, root canal sealers were manipulated following their respective manufacturer's instructions. In sub-group A and E, ProTaper Gold Guttapercha point (Master guttapercha point) and K-file (Master apical file) were used in passive mode for the placement of sealers on the walls of root canals. Whereas, in sub-group B, C and D, Lentulospiral (Paste carriers), Bi-directional spiral (EZ Fill) and Pastinject instruments were used respectively in active mode (attached to slow-speed micromotor handpiece) (Figure no. 2) following their respective manufacturer's instructions for the placement of sealers on the walls of root canals.



Figure 1: Five root canal sealers used in our study



Figure 2: Five root canal sealer Placement techniques used in our study

All canals were then obturated using ProTaper Gold; size F2 guttapercha point in single-cone technique. Guttapercha point was slowly inserted into the root canals of all specimens upto the predetermined working length and a heated finger plugger (Mani Inc, Japan) was used to remove the coronal excess of guttapercha and it was further vertically condensed with cold finger plugger and the coronal access of root canals was restored with Type-II Glass Ionomer Cement (GC Fuji, Japan). All specimens were then incubated in 100% relative humidity at 37°C for 7 days, to ensure complete setting of all the root canal sealers used. All specimens were then horizontally sectioned using a diamond disc attached to straight micromotor handpiece at low-speed to obtain cross-sections of apical-third of roots, at the level of 4mm from the predetermined working length. 2mm thick samples were collected from each specimen. From each selected sample, 300µm thick slices were obtained using a hard-tissue microtome (SP 1600, Leica Microsystems, Germany) and the depth of dye penetration was examined under Confocal Laser Scanning Microscope (CLSM) [Carl Zeiss Micro-imaging, GmbH, Jena, Germany] (Figure no.3) at 10X magnification in the Fluorescence mode using Image J software (Java based image processing program, LOCI, Wisconsin, U.S.A). The excitation and emission wavelength of Rhodamine-B

dye (red colour) was 568nm and all images were recorded with size 1000×1000µm at a resolution of 1024×1024 pixels. The depth of dye penetration was measured in micrometers(µm). The obtained data was tabulated, recorded and subjected to statistical analysis.

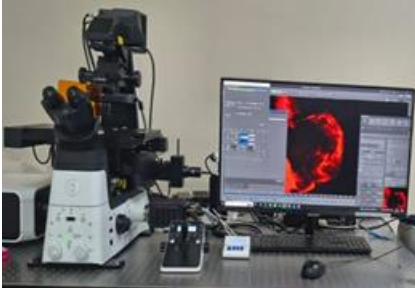


Figure 3: Confocal Laser Scanning Microscope (CLSM) [Carl Zeiss Micro-imaging, GmbH, Jena, Germany]

Results:

The obtained data in Microsoft excel was analyzed with Statistical Package for Social Sciences (SPSS) V.24 software in Windows 11 (SPSS Inc., Chicago, IL, USA).

The continuous variables are presented with Mean and Standard deviation. One Way ANOVA (Analysis of Variance) and Tukey’s Post-hoc tests were used for statistical analysis. The P value ≤ 0.05 was considered statistically significant. One-way ANOVA test was done for intra-group comparison to evaluate the depth of dye penetration into the dentinal walls at the apical-thirds of root canals among all the specimens of Groups; 1, 2, 3, 4 and 5 and its Sub-Group (SG); A, B, C, D, E. Bio-C sealer (Group 3) placed using Lentulospiral (Sub-group B) showed the maximum/greatest depth of sealer penetration into the dentinal walls of root canals followed by AH-Plus sealer (Group 1) placed using Bidirectional spiral (Sub-group C). Whereas, MTA-Fillapex (Group 2),

Table 1: One-Way ANOVA test: Group A: Intra-group comparison in the depth of sealer penetration (µm) in CLSM imaging.

Group A (n=25)	Sub-Groups (n=5)	Mean ± S.D. (µm)	P value
	A. Guttapercha	227.00±28.82	P=0.17
	B. Lentulospiral	356.00±38.77	

Endoseal (Group 4) and Apexit-Plus (Group 5) showed minimal/least depth of sealer penetration irrespective of the type of sealer placement techniques used in the study (Figure no. 4 and 5) (Table no. 1, 2, 3, 4, 5).

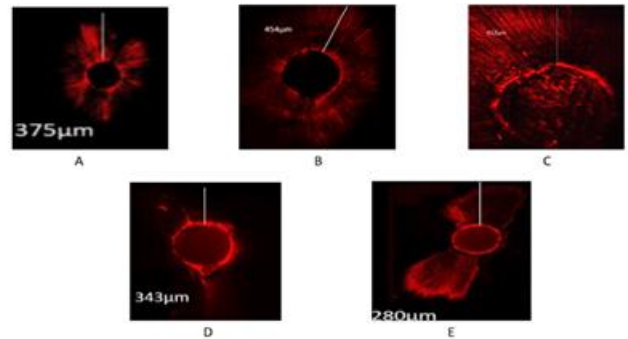


Figure 4: Bio-C sealer (Group 3); (Sub-groups: A- ProTaper Gold F2 guttapercha point, B- Lentulospiral, C- Bidirectional spiral, D- Pastingject, E- K-file) Depth of dye penetration into root canal dentin examined using Confocal Laser Scanning Microscope in Fluorescence mode.

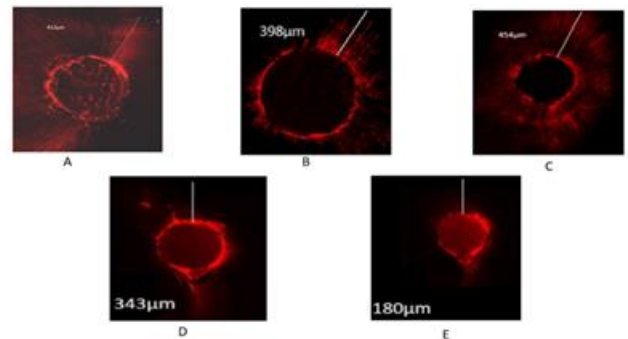


Figure 5: AH-Plus sealer (Group 1); (Sub-groups: A- ProTaper Gold F2 guttapercha point, B- Lentulospiral, C- Bidirectional spiral, D- Pastingject, E- K-file) Depth of dye penetration into root canal dentin examined using Confocal Laser Scanning Microscope in Fluorescence mode.

AH-Plus Sealer	C. Bidirectional-spiral	403.00±46.58	(Non-significant)
	D. Past-inject	305.00±49.76	
	E. K-file	280.00±51.64	

P: Probability, **SD:** Standard Deviation

Table 2: One-Way ANOVA test: Group B: Intra-group comparison in the depth of sealer penetration (µm) in CLSM imaging

Group B (n=25)	Sub-Groups (n=5)	Mean ± S.D. (µm)	P value
MTA-Fillapex Sealer	A. Guttapercha	203.00±34.56	P=0.093 (Non-significant)
	B. Lentulospiral	386.00±40.26	
	C. Bidirectional-spiral	310.00±50.94	
	D. Past-inject	274.00±45.77	
	E. K-file	247.00±39.08	

Table 3: One-Way ANOVA test: Group C: Bio-C Sealer: Intra-group comparison in the depth of sealer penetration (µm) in CLSM imaging

Group C (n=25)	Sub-Groups (n=5)	Mean ± S.D. (µm)	P value
Bio-C Sealer	A. Guttapercha	247.00±39.08	P=0.01 (significant)
	B. Lentulospiral	424.00±73.82	
	C. Bidirectional-spiral	389.00±55.94	
	D. Past-inject	321.00±66.56	
	E. K-file	292.00±40.04	

Table 4: One-Way ANOVA test: Group D: Endoseal Sealer: Intra-group comparison in the depth of sealer penetration (µm) in CLSM imaging

Group D (n=25)	Sub-Groups (n=5)	Mean ± S.D. (µm)	P value
Endoseal Sealer	A. Guttapercha	172.00±14.35	P=0.173 (Non-significant)
	B. Lentulospiral	285.00±29.20	
	C. Bidirectional-spiral	258.00±31.89	
	D. Past-inject	209.00±29.06	
	E. K-file	200.00±38.60	

Table 5: One-Way ANOVA test: Group E: Apexit-Plus Sealer: Intra-group comparison in the depth of sealer penetration (µm) in CLSM imaging.

Group E (n=25)	Sub-Groups (n=5)	Mean ± S.D. (µm)	P value
Apexit-Plus Sealer	A. Gutapercha	207.00±24.84	P= 0.140 (Non-significant)
	B. Lentulospiral	330.00±52.66	
	C. Bidirectional-spiral	290.00±45.43	
	D. Past-inject	234.00±38.08	
	E. K-file	221.00±33.90	

Table 6: Tukey Post-hoc test for Inter-group comparison.

Root canal sealer placement techniques used	Inter-group comparison of root canal sealers among the five groups	P value
Sub-group A: Guttapercha	Group 1 Vs Group 2	.693 (Not significant)
	Group 1 Vs Group 3	.999 (Not significant)
	Group 1 Vs Group 4	.024 (Significant)
	Group 1 Vs Group 5	.139 (Not significant)
	Group 2 Vs Group 3	.543 (Not significant)
	Group 2 Vs Group 4	.281 (Not significant)
	Group 2 Vs Group 5	.772 (Not significant)
	Group 3 Vs Group 4	.014 (Significant)
	Group 3 Vs Group 5	.087 (Not significant)
	Group 4 Vs Group 5	.899 (Not significant)
Sub-group B: Lentulospiral	Group 1 Vs Group 2	.990 (Not significant)
	Group 1 Vs Group 3	.964 (Not significant)
	Group 1 Vs Group 4	.282 (Not significant)
	Group 1 Vs Group 5	.645 (Not significant)
	Group 2 Vs Group 3	.796 (Not significant)
	Group 2 Vs Group 4	.526 (Not significant)
	Group 2 Vs Group 5	.887 (Not significant)
	Group 3 Vs Group 4	.020 (Significant)
	Group 3 Vs Group 5	.286 (Not significant)
	Group 4 Vs Group 5	.962 (Not significant)
Sub-group C: Bidirectional-spiral	Group 1 Vs Group 2	.939 (Not significant)
	Group 1 Vs Group 3	.01 (Significant)
	Group 1 Vs Group 4	.216 (Not significant)
	Group 1 Vs Group 5	.749 (Not significant)
	Group 2 Vs Group 3	.749 (Not significant)
	Group 2 Vs Group 4	.603 (Not significant)
	Group 2 Vs Group 5	.991 (Not significant)
	Group 3 Vs Group 4	.097 (Not significant)
	Group 3 Vs Group 5	.485 (Not significant)
	Group 4 Vs Group 5	.849 (Not significant)

Sub-group D: Past-inject	Group 1 Vs Group 2	.953 (Not significant)
	Group 1 Vs Group 3	.980 (Not significant)
	Group 1 Vs Group 4	.448 (Not significant)
	Group 1 Vs Group 5	.641 (Not significant)
	Group 2 Vs Group 3	.716 (Not significant)
	Group 2 Vs Group 4	.848 (Not significant)
	Group 2 Vs Group 5	.957 (Not significant)
	Group 3 Vs Group 4	.195(Not significant)
	Group 3 Vs Group 5	.326 (Not significant)
	Group 4 Vs Group 5	.997 (Not significant)
Sub-group E: K-file	Group 1 Vs Group 2	.975 (Not significant)
	Group 1 Vs Group 3	.968 (Not significant)
	Group 1 Vs Group 4	.305 (Not significant)
	Group 1 Vs Group 5	.890 (Not significant)
	Group 2 Vs Group 3	.739 (Not significant)
	Group 2 Vs Group 4	.632 (Not significant)
	Group 2 Vs Group 5	.997 (Not significant)
	Group 3 Vs Group 4	.103 (Not significant)
	Group 3 Vs Group 5	.548 (Not significant)
	Group 4 Vs Group 5	.813 (Not significant)

Tukey Post-hoc test was done for inter-group comparison in the depth of sealer penetration into the dentinal walls at the apical-thirds of root canals among all specimens of Group; 1, 2, 3, 4, 5, P-value < 0.05 considered as statistically non-significant, except between Group 1 and Group 4 when respective sealers were placed using Guttapercha, Group 3 and Group 4 when respective sealers were placed using Lentulospiral, Group 1 and Group 3 when respective sealers were placed using Bidirectional-spiral (Table no.6). Bio-C sealer placed using Lentulospiral and Bidirectional-spiral instruments showed the maximum depth of penetration into the apical root canal dentin followed by AH-Plus sealer placed using Bidirectional-spiral and Lentulospiral.

The maximum depth of penetration of all five different root canal sealers was seen when they were placed using Lentulospiral instrument compared to other four sealer placement techniques. However, irrespective of the five different sealer placement techniques used, MTA-Fillapex (Group 2), Endoseal (Group 4) and Apexit-Plus (Group 5) sealers showed least depth of penetration into the apical root canal dentin.

Discussion

One of the important objectives of endodontic therapy is to totally fill the root canal system for obtaining a fluid-tight seal and attaining this three-dimensional seal is a major pre-requisite for the success of endodontic treatment. Modern endodontic treatment employs chemo-mechanical approach to disinfect the root canal space and

obtain a fluid-tight seal both coronally and apically. The sealing ability of root canal sealer is one of the key factor that determines the sealing ability of root canal filling. Guttapercha is used as the core root canal filling material and the root canal sealer promotes better adhesion between root canal walls and guttapercha points. Root canal sealer will also help to fill any empty areas where gutta-percha points fails to completely fill the root canal space, thus providing a three-dimensional obturation of the root canal space and also entomb the remaining microorganisms in the canal space and favor periapical healing.¹⁶

The penetration of root canal sealers into radicular dentin is considered to be highly desirable, as it will improve the sealing ability of sealers with the dentin, improved mechanical locking of sealers into the dentinal tubules, entomb any residual microorganisms within the tubules and also exhibit antimicrobial effect. Therefore, for a good obturation, it is important for deeper penetration of sealers into root canal dentin.⁴ The depth of sealer penetration into dentinal tubules depends on many factors such as smear layer removal, the number and diameter of tubules, root canal dimensions, physical and chemical properties of the sealers. The flow is one of the main physical factors to influence the tubular penetration. Very few studies were conducted on the effect of sealer activation/placement on the depth of penetration root canal sealers.⁴ Therefore in the present study, the depth of penetration five different root canal sealers were compared when placed using five different placement techniques, three active placement techniques (Lentulospiral, Bi-directional spiral and Pastinject) and two passive placement techniques (ProTaper guttapercha point, K-file) analyzed using CLSM examination.

The apical 5mm of root canal is a critical area for placement of root canal sealer, as accessory canals are most often found in this area. Since accessory canals communicate with the periodontal membrane, they can create a periodontic-endodontic pathway of communication for potential bacterial penetration to and from the periodontium into the root canal.¹⁴ Thus, the apical-third (4mm) of root canals was chosen in our study for evaluation in the depth of sealer penetration.

Bio-C(Ceramic) (Brasseler, USA) is a calcium silicate-based root canal sealer. It is insoluble, radiopaque and requires presence of water to set and harden. It is a premixed, ready-to-use sealer and its used in both single-cone and lateral condensation obturation techniques. The findings of our study showed that Bio-C sealer demonstrated maximum depth of penetration compared to other sealers, especially placed with Lentulospiral instrument. The setting reaction of Bio-C (Ceramic) sealer is moisture-activated, producing a final set consisting of calcium silicate-hydrate gel and calcium hydroxide. The calcium hydroxide interacts with phosphate ions of dentin to form hydroxyapatite and water, the water generated continues to react with the calcium silicate, leading to the precipitation of additional gel-like calcium silicate hydrate.¹⁷

The findings of our study are in accordance with Shinde A et al¹⁸ reported significantly greater penetration of Bio-C compared to AH Plus root canal sealer across all radicular thirds. Ishita G et al¹⁹ also reported Bio-C deeper depth of penetration compared to Apexit-Plus and MTA-Fillapex sealers. Chougule PG et al²⁰ also reported Bio-C sealer demonstrated deeper depth of penetration than AH-Plus and MTA-Fillapex sealers.

AH-Plus an epoxy resin based sealer. Although epoxy sealers have high fluidity and low polymerization shrinkage, but they are hydrophobic, so moisture

negatively affect its ability to penetrate deeper and adapt to root canal dentin. The findings of our study showed that AH-Plus sealer also showed deeper penetration into dentin and these findings are in agreement with Trivedi S et al²¹ that compared AH-Plus with Bio-C and MTA-Fillapex sealers and concluded that both AH-Plus and Bio-C sealers showed deeper penetration into dentin than MTA-Fillapex. Similarly, Mokashi P et al²² also reported AH-Plus sealer achieved maximum penetration depth in both coronal and apical root canal dentin compared to MTA-Fillapex. However, the sealer placement technique has shown to significantly affect the depth of penetration of sealers into root canal dentin.

The findings of our study showed that all five root canal sealers penetrated deeper into root canal dentin when placed using Lentulospiral and Bidirectional spiral instruments. The findings of our study agree with the findings of Kim SY et al²³ observed greater depth of sealer penetration when placed with these instruments compared to K-file. However, the findings of our study also showed both Bio-C and AH-Plus sealers placed using Lentulospiral placement followed by Bidirectional-spiral demonstrated deeper penetration into dentin compared to other sealers and sealer placement techniques used. These findings are in accordance with Dash AK et al²⁴ reported Lentulospiral followed by Bidirectional-spiral achieved greater penetration depth. Using lentulospiral, there is rapid insertion of sealer into the walls of root canal causing increased pseudoplasticity, increased flow and devoid of voids.²⁵ Lentulospiral with its action pushes the sealer centrifugally within the root canal space, thus pushing the sealer deeper into root canal dentin.⁴

Several techniques are available to evaluate the penetration of root canal sealers into dentin including stereomicroscopy, scanning electron microscopy,

transmission electron microscopy and Confocal laser scanning microscope. CLSM offers several advantages like the use of non-decalcified samples that do not require a specific section technique (sputter coating). It also provides detailed information about the presence and distribution of sealers at relative low magnification through the use of fluorescent dye (Rhodamine-B) marked sealers and allows the exclusion of artifacts from the sample.²⁶

The findings of our study suggest that newer Bioceramic sealer such as Bio-C is a promising alternative to other sealers for a more predictable and successful endodontic treatment. Importantly, the technique used for sealer placement is as critical as the root canal sealer used, the use of Lentulospiral and Bidirectional spiral for sealer placement in our study showed deeper penetration of all sealers used, especially with Bio-C and AH-Plus sealers.

Conclusion

Under the parameters of our in-vitro study, both the type of root canal sealer and the sealer placement technique used significantly influence the depth of penetration of sealer into the root canal dentin. Bio-C and AH-Plus sealers showed maximum depth of penetration into the root canal dentin compared to other sealers used. Among the sealer placement techniques, the use of Lentulospiral showed enhanced depth of penetration of all sealers used, especially with Bio-C sealer showed maximum depth of penetration into root canal dentin. Further in-vivo and long-term clinical studies are recommended to validate the findings of study under dynamic oral conditions.

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