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Comparative evaluation of sealer penetration depth into radicular dentinal tubules using confocal scanning microscope: an *in vitro* study

Ambika Sigadam[1,*], Kalyan Satish R[2], Girija S Sajjan[2], Madhu Varma K[2], Sita Ram Kumar M[3], Praveen D[3]

[1]Postgraduate Student, [2]Professor, [3]Reader, Department of Conservative Dentistry and Endodontics, Vishnu Dental College, Bhimavaram—534202, Andhra Pradesh, India.

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ABSTRACT

Background: Endodontic treatment involves the removal of the vital and necrotic contents of the root canal through chemo-mechanical means followed by obturation of the prepared root canal to prevent the ingress of fluids and avoid bacterial infection or regrowth. Root canal sealers and core filling materials are used together to fill the irregularities in the root. Penetration into the dentinal tubules also results in the inhibition of bacterial regrowth and increases the success of root canal therapy.

Aim: This study aimed to evaluate the penetration depth of various sealers into the dentinal tubules using a confocal microscope.

Materials and methods: A total of 65 specimens were decoronated to standardize the root length of 13mm. Working length was determined, and Biomechanical preparation for all the samples was done with a rotary ProTaper file till F4. Samples were randomly divided into five groups containing 13 teeth in each group based on the sealer used, namely Group 1: Endomethasone (n=13), Group 2: AH-Plus (n=13), Group 3: Roekoseal (n=13), Group 4: MTA Fillapex (n=13), Group 5: Endosequence BC (n=13). All the sealers were labelled with Rhodamine -B dye, and samples were obturated using cold lateral compaction technique. The specimens were sectioned orthogonally at coronal, middle, and apical thirds. All the samples were examined with a Zeiss Pascal Laser Scanning Microscope to examine the sealer penetration depth into the dentinal tubules. The data were subjected to statistical analysis using one- way Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) tests.

Results: Endosequence BC showed the highest penetration into dentinal tubules, followed by MTA Fillapex and Roekoseal, AH-Plus, and Endomethasone exhibited the least penetration.

Conclusion: Endosequence BC sealer exhibited maximum penetration. All the groups showed maximum penetration at coronal third, followed by the middle and apical third.

1. Introduction

Pulpal and periapical diseases are primarily related to microorganisms and their by-products in the root canal system, which occurs due to the invasion of bacteria through caries or fracture. The main objective of endodontic treatment is to

Correspondence: *Corresponding author Email Address: ambika.sigadam@gmail.com

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eliminate microorganisms from the root canal space and also prevent it from reinfection [1]. Chemo-mechanical preparation is considered the most effective step in the management of the infected root canal space, followed by 3-dimensional obturation with a biocompatible material [2]. Hence, there is a need to obturate the root canal space thoroughly to prevent leakage and to entomb residual debris and recalcitrant bacteria. Obturation eliminates all avenues of leakage from the oral cavity and the periradicular tissues into the root canal system by creating a fluid-tight seal. Root canal sealers are used along with a core-filling material to attain an impervious seal between the core material and root canal wall [2,3]. Commercially there are many sealers available in clinical practice. Variations in the mechanical and chemical properties of sealer cement also influence the depth of penetration [4]. Therefore, it is essential to compare the penetrability of various sealers that are used in routine clinical practice. Endomethasone N is a zinc oxide eugenol sealer with anti-inflammatory activity due to the presence of hydrocortisone acetate. AH Plus, which is resin-based cement has excellent mechanical properties and low polymerization shrinkage. RoekoSeal is a silicon-based sealer with no shrinkage and excellent sealing property. MTA Fillapex is MTA based sealer with tissue recovery property and a lack of inflammatory response. Endoseqence BC sealer which is a bioceramic sealer which sets in the presence of moist dentine. Hence this in vitro study aimed to evaluate the penetration efficiency of five different sealers into dentinal tubule using Rhodamine B dye under confocal laser scanning microscopy.

2. Materials and methods

A total of 65 extracted human mandibular premolars with a single root and single canal were included in the study. For standardization, all the samples were decoronated to a length of 13mm by using a double-faced diamond disc (KG Sorensen, Barueri, SP, Brazil). Pulpal tissue extirpation and working length were determined. Biomechanical preparation for all the samples was done in crown-down motion using ProTaper rotary nickel-titanium files (Dentsply Maillefer). Canals were irrigated between the use of files with 5ml of 3% Sodium hypochlorite (Prime dental PVT LTD., India). To remove the smear layer, all canals were irrigated with 3mL of 17% ethylenediamine tetraacetic acid (DESmear, Anabond Stedman Pharma research, India). Final rinse performed by using 5 mL of distilled water to remove any remaining irrigating solution. All the irrigation procedure was followed using a side vented needle placed 1mm short of the apical foramen. The canals were dried with sterile absorbent paper points (Prime dental PVT LTD., India.) after irrigation. All intracanal procedures were done by a single operator to eliminate inter-operator variability. Teeth were then randomly divided into five experimental groups using computer-generated sequence allocation, consisting of 13 teeth in each (n=13) sealer group. Sealers used in this study were Endosequence (Brasseler USA, Savannah, GA), MTA-Fillapex (Angelus, Londrina, Brazil), Roekoseal (Coltene/Whaledent, Langenau, Germany), AH-Plus (Dentsply-Maillefer, Tulsa, OK, USA), Endomethasone (Septodont, Saint-Maur, France). Rhodamine B dye was labelled to all the sealer groups. All the sealers groups were manipulated according to manufacturer instructions and were coated on to the teeth using lentulospirals. Later all the samples were obturated using cold lateral compaction technique. The teeth sealed with intermediate restorative material (PREVEST DenPro) at the coronal end.

2.1 Sample preparation for confocal microscope

All the samples were sectioned orthogonally using double-sided diamond disk under continuous water cooling and obtained with a thickness of 1mm.

2.2 Evaluation of sample by using a confocal laser microscope

All the samples which sectioned at coronal, middle, and apical thirds examined with a Zeiss Pascal Laser Scanning Microscope (Carl Zeiss, Gottingen, Germany). Measurements were recorded using the digital measuring ruler, in CLSM image recorder software. The data were averaged to obtain a single value for each section. All analyses were recorded and evaluated by a single operator to rule out any discrepancies.

The data were subjected to statistical analysis using the statistical package for the social sciences IBM SPSS Statistics version 22.0 software and Oneway Analysis of Variance test for intragroup examination and Tukey's posthoc test for intergroup examination.

3. Results

The mean and standard deviation of penetration depth (mm) of the five sealers in the three different zones;

coronal, middle, and apical, are given in table 1. Among the sealers tested, Endosequence exhibited the maximum penetration at coronal, middle, and apical levels (Figures 1-3), whereas Endomethasone showed the least penetrability into the dentinal tubules. One-way ANOVA showed a significant difference (p=0.000) in the depth of penetration in the coronal and apical thirds in all the sealers. Maximum depth of penetration was observed at the coronal third, which was significantly higher than the depth of penetration observed at the middle and apical thirds for all five sealers tested.

In posthoc analysis, both Endosequence and MTA-Fillapex showed significant differences (p<005) with the other sealant materials in the coronal region (Tabe 2). However, Roekoseal exhibited no significant differences with AH-Plus and Endomethasone. In the middle zone, significant differences (p<0.05) observed between all the sealant materials (Table 3). In the apical zone, both Endosequence and MTA-Fillapex displayed significant differences (p=0.000) with all the sealants. Significant differences were also observed between AH-Plus and Endomethasone, whereas no significant differences were observed between Roekoseal and Endomethasone (Table 4).

4. Discussion

Factors influencing sealer depth penetration in dentinal tubules are the presence/absence of smear layer, dentinal permeability (the number and the diameter of tubules), root canal dimension, presence of water, and physical and chemical properties of the sealer [5]. In the present study, removal of the smear layer was done using 3ml of 17% ethylenediaminetetraacetic acid (DESmear, Anabond Stedman pharma research, India), which enhances the sealer penetration into the dentinal tubules.

Confocal laser scanning microscope (CLSM) images are distinctly higher than those obtained with the conventional optical microscope because the produced images contain volumetric and texture details that are impossible to achieve with the conventional microscope. The advantage of using CLSM is its higher resolution, greater contrast, three dimensions of reconstruction, image analysis. Hence in the present study, CLSM was used to measure the sealer penetration.

Results in the present study showed that in all the radicular portions Endosequence BC sealer showed the highest amount of penetration into the dentinal tubules followed by MTA Fillapex, AH-plus, Roekoseal and Endomethasone. The higher penetration of the Endosequence BC root canal sealer can be attributed to its extremely small particle size (less than 2 µm). Also, its low initial viscosity level and hydrophilic nature allow it to flow into all aspects of the canal anatomy. These specifications may improve the flow of the sealer into dentinal tubules, anatomic irregularities, and gutta-percha [6,7]. Moreover, Endosequence BC exhibits minimal or no shrinkage during the setting phase [8].

Table 1: Comparison of Surface roughness using One-way ANOVA

Sealers	Coronal third		Middle third		Apical third	
	Mean±SD#	Significance	Mean±SD	Significance	Mean±SD	Significance
Endosequence	1399.46±88.99	0.000*	1105.01±67.44	0.000*	591.89±66.52	0.000*
MTA Fillapex	1119.68±99.40		820.64±3.60		353.32±53.01	
AH-Plus	978.89±44.29		725.89±3.15		264.60±58.00	
Roekoseal	951.81±170.06		609.24±53.81		249.67±55.02	
Endomethasone	853.85±118.03		453.98±118.12		196.46±28.67	

* Significant differences were observed among the groups.

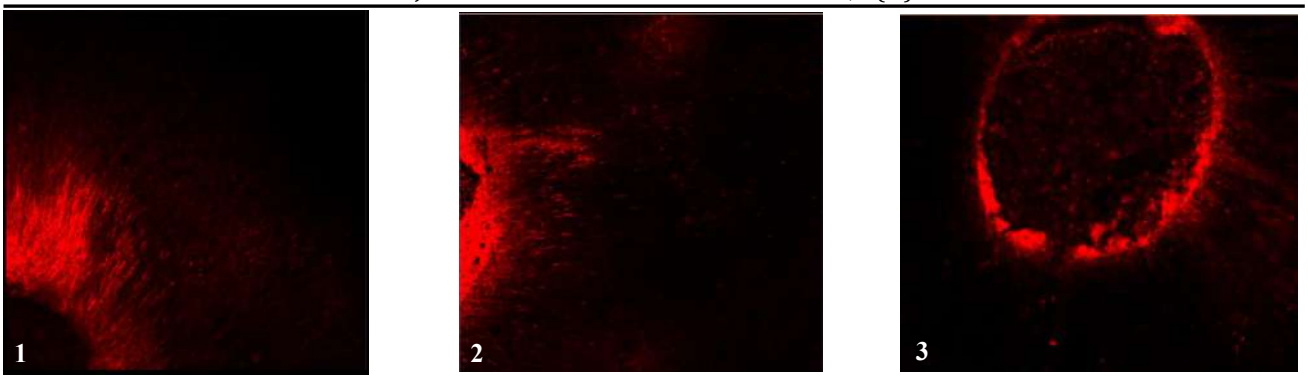

Figure 1-3: Conofocal Laser Microscope analysis of depth of penetration of Endosequence BC sealer. Where 1. at coronal level, 2. at middle level, and 3. at apical level .

Table 2: Intergroup Comparison of Depth of Penetration (µm) of the Sealers in the Coronal Zone.

Sealer group at coronal third	Groups	Mean Difference	Standard Error	Significance
Endosequence	MTA-Fillapex	279.78658	45.68794	0.000*
	AH-Plus	420.57308	45.68794	0.000*
	Roekoseal	447.64967	45.68794	0.000*
	Endomethasone	545.61067	45.68794	0.000*
MTA-Fillapex	AH-Plus	140.78650	45.68794	0.026*
	Roekoseal	167.86308	45.68794	0.005*
	Endomethasone	265.82408	45.68794	0.000*
AH-Plus	Roekoseal	27.07658	45.68794	0.976
	Endomethasone	125.03758	45.68794	0.061
Roekoseal	Endomethasone	97.96100	45.68794	0.217

* Significant differences were observed between the groups.

Table 3: Intergroup Comparison of Depth of Penetration (µm) of the Sealers in the Middle Zone.

Sealer group at middle third	Groups	Mean Difference	Standard Error	Significance
Endosequence	MTA-Fillapex	284.36708	26.72156	0.000*
	AH-Plus	379.11375	26.72156	0.000*
	Roekoseal	495.76958	26.72156	0.000*
MTA-Fillapex	Endomethasone	651.02842	26.72156	0.000*
	AH-Plus	94.74667	26.72156	0.007*
	Roekoseal	211.40250	26.72156	0.000*
	Endomethasone	366.66133	26.72156	0.000*
AH-Plus	Roekoseal	116.65583	26.72156	0.001*
	Endomethasone	271.91467	26.72156	0.000*
Roekoseal	Endomethasone	155.25883	26.72156	0.000*

* Significant differences were observed between the groups.

Table 4: Intergroup Comparison of Depth of Penetration (µm) of the Sealers in the Apical Zone.

Sealer group at coronal third	Groups	Mean Difference	Standard Error	Significance
Endosequence	MTA-Fillapex	238.57183	21.94659	0.000*
	AH-Plus	327.29342	21.94659	0.000*
	Roekoseal	342.22183	21.94659	0.000*
	Endomethasone	395.43150	21.94659	0.000*
MTA-Fillapex	AH-Plus	88.72158	21.94659	0.002*
	Roekoseal	103.65000	21.94659	0.000*
	Endomethasone	156.85967	21.94659	0.000*
AH-Plus	Roekoseal	14.92842	21.94659	0.960
	Endomethasone	68.13808	21.94659	0.024*
Roekoseal	Endomethasone	53.20967	21.94659	0.124

* Significant differences were observed between the groups.

In addition, the Endosequence BC root canal sealer exhibits a 0.2% expansion during the setting period. These characteristics also support the spread of sealer over the dentin walls of the root canal and filling of the lateral canals. All these features may contribute to the higher dentinal tubule penetration observed in the present study. This is in accordance with the literature reporting that tricalcium silicate–containing sealers penetrated into the tubules as deep as 2 mm due to the smaller particle size of BC Sealer [9] and also due to its high level of viscosity [10].

Penetration of MTA Fillapex is less when compared to Endosequence BC sealer as MTA Fillapex, a resin-based sealer has less than 20% MTA particles, and resin matrix shrinks 0.7% during setting. In contrast, the BC Sealer expands slightly (<0.1%), which may provide superiority for the latter [9,11]. However, MTA Fillapex has greater dentinal penetration than AH-Plus, Roeko Seal and Endomethasone. This greater penetration could be because of the presence of nano-particles, which enables a homogeneous mixture and a better flow of the sealer. MTA Fillapex is significantly more flowable, and this is attributed to the difference in composition and smaller particle size of the sealer [11-13].

The tubule penetration of resin-based sealers is not dependent on the hydraulic forces created during filling; instead, the sealer is drawn into the tubules by capillary action [1]. This may explain why AH Plus and Roekoseal, both with a longer setting time, exhibited significantly deeper penetration than Endomethasone. Endomethasone has the least penetration among all the sealers. Endomethasone contains both eugenol and paraformaldehyde, such as Endomethasone and N2, which were found to be the most toxic. Brodin et al. reported that Endomethasone could irreversibly inhibit the conduction of the action potential in the rat phrenic nerve [14].

In the present study, all the sealers exhibited the maximum penetration at the coronal third, followed by the middle third and least in the apical third. Various authors have demonstrated regional variation in the depth of tubular penetration [15-18]. Limitations of the present study include, temperature and humidity of the oral cavity are not simulated. Hence further ex-vivo and in vivo studies are needed.

5. Conclusion

From the present study, it can be concluded that the Endosequence BC sealer resulted in better penetration into the dentinal tubules. The maximum penetration of the five sealers was more in the coronal third followed by the middle third and least in the apical third.

Conflicts of interest: Authors declared no conflicts of interest.

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