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In Vitro Comparative Evaluation of Depth of Tubular Penetration of Three Different Root Canal Sealers Using CLSM

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Received: March 14, 2020; **Published:** September 24, 2020

Abstract

Aim: To evaluate and compare the penetrability of three different sealers AH plus, EndoREZ and Endosequence Bioceramic sealer into radicular dentinal tubules using a confocal laser scanning microscope.

Materials and Methods: 60 extracted, human, single rooted mandibular premolars were selected and decoronated at the cemento-enamel junction. All samples were instrumented to apical size F3 (#30), irrigated with 3% sodium hypochlorite and 17% ethylene-diaminetetraacetic acid. A final rinse will be performed by using 5 ml of distilled water. Samples were divided into 3 groups. All the sealers were mixed with Rhodamine B Isothiocyanate fluorescent dye and placed along the entire length of the canal and canals were obturated with cold lateral compaction technique. All samples were sectioned to obtain 3 segments and were examined with confocal laser scanning microscope.

Statistical Analysis Used: One way ANOVA and Tukey Post hoc Test.

Result and Conclusion: The maximum sealer penetration was seen in all sections of group 2 (Bioceramic group) while least penetration was seen in group 3 (EndoREZ group).

Keywords: *AH Plus; EndoREZ; Endosequence Bioceramic Sealer; CLSM; Tubule Penetration*

Introduction

Every aspect of endodontic treatment is imperative in obtaining a successful outcome. The main objective is to prevent reinfection of the canal by providing adequate seal against ingress of bacteria and their toxins and thus preventing reinfection. It is difficult to completely eliminate all microorganisms from the root canals even after adequate chemomechanical preparation. These remaining organisms may play a role in persistent periapical disease [1].

Root canal sealers are used along with a core filling material to attain an impervious seal between core material and root canal wall. It has the ability to fill voids and irregularities and to penetrate lateral canals, accessary canals and dentinal tubules, thus creating a union between the core material and the canal wall by filling residual spaces.

Thus, greater penetration of root canal sealers into the dentinal tubules may enhance the potential for successful outcome of endodontic therapy.

Hence, the ability of sealer to penetrate dentinal tubules effectively is one of the challenging factors influencing the choice for selection of sealer during obturation [2]. Ideally endodontic sealers should seal the canal laterally as well as apically and have good adaptation to the root canal dentin.

Variations in the physical and chemical properties of sealer cements also influence the depth of penetration [3]. Considering all above there is a need to compare the penetrability of different types of sealers used.

One of the upcoming biomaterial sealer i.e. Endosequence Bioceramic sealer (Brasseler, USA) fulfils many ideal requirements as an ideal sealer. Their features like osseo-conductivity, hydrophilicity, adhesiveness and chemical bonding to root canal walls appears to be an effective approach to eliminate the microspace on long term.

Purpose of the Study

Thus, the purpose of this study was to evaluate and compare the penetrability of three different sealers into radicular dentinal tubules using a confocal laser scanning microscope.

Materials and Methods

60 extracted, caries-free human, single-rooted mandibular premolars were selected. After cleaning, digital radiographs were taken to ensure the presence of a single root canal. The teeth were stored in normal saline until use.

All the teeth were decoronated at the cementoenamel junction (CEJ) by using a safe-sided diamond disk and working length was determined by inserting a 21-mm, #10 K file until just visible at the apical foramen. After 1 mm subtraction, this length was considered as final working length.

Instrumentation was performed using crown-down technique with Protaper rotary nickel-titanium instruments. The canals were prepared till rotary F3 (# 30) to estimated working length. During the entire preparation, alternate irrigation and recapitulation was done with 3% sodium hypochlorite and #10 K-file, respectively.

All canals were irrigated with 3 ml of 17% ethylenediaminetetraceticacid for 1 minute, followed by 3 ml of 3% sodium hypochlorite. A final rinse was performed by using 5 ml of distilled water to remove any remaining irrigating solution. The canals were dried and the entire lengths of roots were coated with 2 coats of nail varnish of different colours for different groups.

All the teeth were divided into 3 groups:

- Group 1: AH Plus sealer + Gutta percha (n = 20).
- Group 2: Endosequence Bioceramic (BC) sealer + Gutta percha (n =20).
- Group 3: EndoREZ + Resin coated Gutta percha (n =20).

Group 1: Paste A and Paste B were mixed in equal volume (1:1) on a mixing paper pad to obtain a homogenous consistency.

Group 2: The material is available in premixed calibrated syringes along with intracanal tips. It was dispensed on the mixing paper pad for coating the master cone with the mixture of sealer and Rhodamine B fluorescent dye.

Group 3: A two-part chemical material was mixed in an automix nozzle attached with a narrow Diameter syringe (Skini syringe) with a fine tipped cannula (NaviTip) provided by the manufacturer. Sealer was then dispensed on the mixing paper pad.

All sealers were manipulated according to the manufacturer's instructions. To facilitate fluorescence under Confocal Laser Microscopy, all the sealers were mixed with Rhodamine B Isothiocyanate fluorescent dye. A mixture of fluorescent dye and sealer was placed along the entire length of the canal with size # 25 Lentulo spiral @ 300 rpm.

Canals were obturated with cold lateral compaction technique. The teeth were sealed at coronal end using type-II glass ionomer cement and stored in an incubator at 37°C at 100% relative humidity for 48 hours.

Two markings were made at the level of the junction of coronal and middle thirds and middle and apical thirds and were sectioned perpendicular to long axis of teeth with the help of safe-sided diamond disc. This resulted in three equal-sized sections: coronal, middle and apical from each sample. All 180 sections thus obtained were grounded on a lathe machine so as to get thin transverse sections. Final finishing of sections was done with finishing stone.

Sections were then mounted onto slides and examined under 10x magnification of confocal laser scanning microscopy (CLSM). Obtained images were examined with the help of LSM image browser and maximum depth of sealer penetration for each section was evaluated in µm.

In those sections where the area of maximum sealer penetration was difficult to ascertain, 3 readings were taken and their average was considered as the maximum sealer penetration value for that particular section.

The obtained results were tabulated and statistically analysed using the One way ANOVA and Tukey post hoc test.

Results

All the teeth sections (coronal, middle and apical) of groups 1 - 3 exhibited various sealer penetration depths inside the dentinal tubules. The maximum sealer penetration was seen in all sections of group 2 (Bioceramic group) while least penetration was seen in group 3 (EndoREZ group). For Bioceramic group Maximum sealer penetration were seen with apical sections (mean 920.29 µm) followed by coronal (mean 918.07 µm) and middle (mean 893.70) sections. However, values are not statistically significant.

For other two groups i.e. AH Plus and EndoREZ, maximum penetration significantly were seen in coronal sections and least penetration were seen in apical sections. There was no significant difference between coronal and middle sections.

Groups	Coronal		Middle		Apical		P value	Significant Groups (Tukey Post hoc Test)
	Mean	SD	Mean	SD	Mean	SD		
EndoREZ	677.26	244.71	612.37	169.45	408.87	210.43	<.001	C & A, M&A
AH Plus	905.52	185.36	843.01	212.88	608.47	264.18	<.001	C & A, M&A
Bio Ceramic	918.07	251.25	893.79	183.4	920.29	266.62	0.92 NS	
P value (One way ANOVA)	<0.001		<0.001		<0.001			
Significant Groups (Tukey Post hoc Test)	EZ & AH Plus EZ & BC, AH & BC		EZ & AH Plus EZ&BC		EZ & AH Plus EZ & BC AH & BC			

Table 1: *Comparison of depth of penetration of three endodontic sealers by mean and SD.*

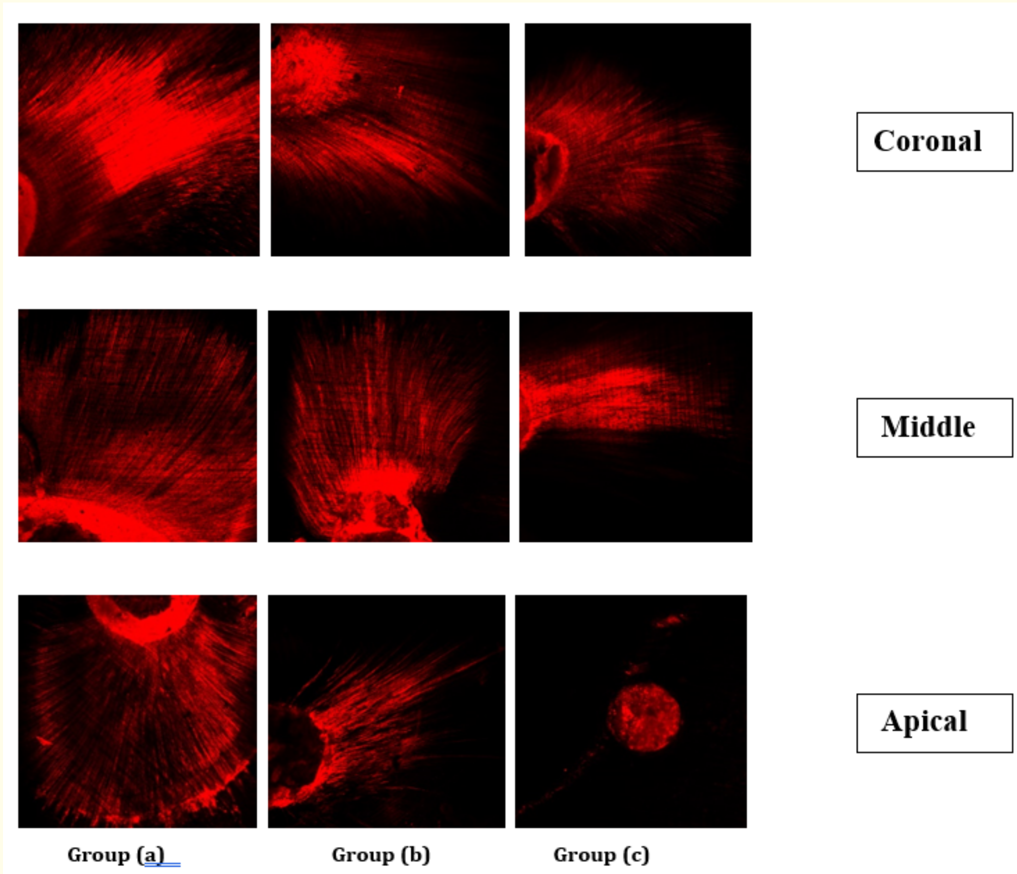

Figure: *Representative CLSM images of Bioceramic group (a), AH Plus group (b), EndoREZ group (c).*

Discussion and Conclusion

Endodontics has reached greater heights due to advancements in materials and techniques in the last few years with a predictably high success rate. One of the prime requisite for the success of endodontic treatment is three-dimensional sealing of the root canals thereby preventing the reinfection of the canal and thus preserving the health of the periapical tissues.

Inability to completely obliterate the irregularities of root canal spaces with the filling material and adequately sealing the apical foramen, accounts for nearly 60% of root canal failures [4-6] Several authors described that localized periodontal problems might be associated with necrotic and infected root canal ramifications, highlighting the importance of the capacity of the endodontic sealer to flow into these irregularities [7].

The use of endodontic sealers along with gutta percha became indispensable for proper sealing of the root canal system, because they penetrate the spaces that are not filled by gutta percha, thus making it difficult for resistant microorganisms to survive and prevents them or their products from reaching the apical region.

An ideal root canal sealer should have low viscosity, good wetting properties, insolubility against tissue fluids to promote improved sealing thus maintaining the bacteria inactive. It should have thin film thickness, low surface tension to flow into the irregularities. It should have sufficient setting time, good adhesion with canal walls and biocompatibility [8].

Different sealer formulations are available in the market and have been subjected to extensive research with respect to their mechanical and biological properties, reflecting that the appropriate selection of a sealer and its clinical performance may influence the clinical outcome.

In addition to various desirable properties of root canal sealers, their ability to penetrate into the dentinal tubules is considered as an important feature because it will increase the interface between obturating material and dentin [2]. This will improve the retention of obturating material inside the root canal due to mechanical locking [9]. It may also entomb any residual bacteria inside the tubules and may exert an antibacterial effect due to closer approximation of chemical components of sealers [10].

In this *in vitro* study, all the teeth samples were decoronated at the level of CEJ to negate discrepancy among coronal, middle and apical sections resulting from variation in root length of individual samples.

The teeth section made for imaging under confocal microscope were transverse sections because it allows for complete observation of all the dentin surrounding the canal [2].

For irrigants to serve their intended purpose, they should reach as close as possible to the working length. So, the complete irrigation protocol in this study was carried out using 30-gauge side-vented needles, which is known to be superior to open-ended irrigation needles regarding debris and irrigant extrusion [11].

In the present study, three different sealers AH Plus, Endosequence BC sealer and EndoREZ having different composition were compared and evaluated for dentinal tubular penetration.

Among the sealers used in this study, AH Plus is an epoxy-resin based sealer which comprises of two-paste system. It has many desirable properties like good handling property, good flow, dimensional stability, good sealing ability and good adhesion to dentin.

In this study, AH Plus sealer showed consistently greater sealer penetration depth into dentinal tubules than Endorez in all three sections of root. For both the group coronal sections showed greater penetration followed by middle and apical sections. The maximum penetration value in coronal sections of root for AH Plus is 905.52 μm and least penetration value in apical sections of Endorez is 677.26 μm.

The superior adaptation of AH Plus could be due to its ability to bond to root dentin chemically by reacting with any exposed amino groups in collagen to form covalent bonds between the epoxy resin and collagen.

EndoREZ is a urethane-dimethacrylate resin based sealer, non-etching and hydrophilic in nature and does not require the adjunctive use of a dentin adhesive [12]. A final rinse of EDTA solution was given followed by a rinse with distilled water, to eliminate the effect of the residual oxygen liberated from NaOCl on polymerization of resin sealers. The canal was dried with paper points for only 1 - 2 seconds, as the manufacturers of EndoREZ suggest that complete dehydration might hinder the penetration of the hydrophilic resins into the dentinal tubules.

Although EndoREZ is recommended for use with either conventional gutta-percha cone or with specific EndoREZ points (resin-coated gutta-percha), low bond strength to the dentinal wall was reported with conventional uncoated gutta-percha [12].

Endosequence Bioceramic (BC) sealer is a calcium silicate phosphate based sealer. There are two major advantages associated with the use of bioceramic materials as root canal sealers. Firstly, their biocompatibility prevents rejection by the surrounding tissues [13].

Secondly, bioceramic materials contain calcium phosphate which enhances the setting properties of bioceramics resulting in a chemical composition and crystalline structure similar to tooth and bone apatite materials, thereby improving sealer-to-root dentin bonding [14].

There are several other advantages such as improved sealing ability, antibacterial due to high alkalinity (pH 12.9), does not shrink upon setting, small particle size, ease of application, remarkable flowability, and an increase in strength of root following obturation [14,15].

The material is available in premixed calibrated syringes with intracanal tips to deliver the paste inside root canals, thus having advantage of saving time and improving convenience and delivery. It also eliminates the potential for heterogeneous consistency may occur during hand mixing.

It hardens only when exposed to a moist environment, as that produced by the dentinal tubules. Therefore, if any residual moisture remains in the canal after drying, it will not adversely affect the seal established by the Bioceramic sealer. This is also an advantage for teeth with root resorption and immature apices providing long term hermetic sealing of apical third, thus ensures conditions for hard tissue closure of root canal apical orifice in time [13].

The alkaline nature of Bioceramic sealer has been reported to denature dentinal collagen fibres, which then facilitated the penetration of sealers into the dentinal tubules [13].

In the present study, maximum sealer penetration depth into dentinal tubules of Bioceramic sealer was found to be consistently greater than AH Plus and EndoREZ sealer.

This finding is in agreement with the results obtained by Greer E McMichael., *et al*. (2016) who found higher range of percentage sealer penetration of BC sealer when compared with other sealers (QuickSet2, NeoMTA Plus, MTA Fillapex) regardless of obturation technique used. They stated that to achieve tubule penetration, the particle size of the material must be smaller than the tubule diameter; the larger the tubule, the deeper a particle can penetrate. It is reasonable to assume that because of the smaller particle size (< 1 μm) of BC sealer, they will be well suited for tubule penetration [16].

For visualization under confocal microscope, sealers were labelled with Rhodamine B dye. Since there is no specific methodology devised for incorporating dyes into dental materials in the literature, current study tried to maintain standardization for the amount of dye to be incorporated-for 10 parts of sealer, 1 part of dye powder was taken and mixed manually so that approximate concentration of 0.1% of dye is maintained. It has been investigated that this dye would not alter the properties of sealers [17,18].

For determining maximum sealer penetration depth, CLSM was used in the present study because it has several advantages over SEM [2,19,20]. CLSM model allows for a full cross-sectional observation. It does not require any special specimen processing, so tends to produce fewer artefacts as compared to SEM. Furthermore, CLSM is a non-destructive approach as it allows the reuse of the same specimen in another evaluation and also it can collect serial optical sections even from thick specimens [21].

Regional variation in the depth of tubular penetration has been demonstrated by a number of authors [22-24]. In this study, maximum sealer penetration was significantly greater in all the three root sections of BC sealer compared to other two groups. For the BC sealer group, maximum sealer penetration mean value were in apical root sections followed by coronal and middle sections, although the value is not statistically significant.

These findings were in agreement with the results obtained by Greer E McMichael., *et al*. (2016) who found similar tubular penetration at both the level (1-mm and 5-mm) with the tricalcium silicate containing sealers [16].

Bortolini., *et al*. (2010) found increased intratubular penetration in the cervical and apical sections of AH Plus sealer when compared with the Endorez sealer [25].

Amita Rouhani., *et al*. (2013) also found similar results. They evaluated the depth of dentinal tubular sealer penetration of AH Plus and Epiphany system in apical third of canal and found average penetration in this region [26].

Key Messages

To search best sealer with good percentage of penetration in dentinal tubules.

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Volume 19 Issue 10 October 2020