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An In Vitro Evaluation of Depth of Tubular Penetration of Ah plus and Endosequence Bioceramic Sealer: A Confocal Laser Scanning Microscopic Investigation

Dr.VandanaGade (MDS), Professor, Department of Conservative Dentistry and Endodontics, SwargiyaDadasahebKalmeghSmruti Dental College and Hospital, Nagpur.

Dr.Neelam Rahul (MDS), Assistant Professor, Department of Conservative Dentistry &Endodontics, V.Y.W.S. Dental College and Hospital, Amravati.

Dr.Ambar W. Raut(MDS) Associate Professor, Department of Conservative Dentistry &EndodonticsSwargiyaDadasahebKalmeghSmruti Dental College and Hospital, Nagpur

Dr.ReemaAsani, Post Graduate student, Department of Conservative Dentistry &EndodonticsSwargiyaDadasahebKalmeghSmruti Dental College and Hospital, Nagpur

Dr.JaykumarGade, (MDS) Professor, Department of Prosthetic Dentistry and Implantology, SwargiyaDadasahebKalmeghSmruti Dental College and Hospital, Nagpur

Dr.RakshaKusumbe, Post Graduate student, Department of Conservative Dentistry &EndodonticsSwargiyaDadasahebKalmeghSmruti Dental College and Hospital, Nagpur

ABSTRACT

OBJECTIVE: The present study investigated the depth of penetration of AH Plus and EndosequenceBioceramic sealer by a confocal laser scanning microscopic investigation.

METHOD:

20 Mandibular single rooted premolar sound teeth were selected. Samples were decoronated at cementoenamel junction to achieve a standardized root length of 14mm. Access opening and biomechanical preparation was done till #F3. Samples were divided into 2 groups for obturation as, Group 1-Endosequence Bioceramic sealer, Group 2- AH Plus sealer. The depth of sealer penetration into dentinal tubules was calculated using confocal laser scanning microscope. One way ANOVA and Tukeys test were applied for the pairwise comparison of the depth of sealer penetration between the groups.

RESULTS:

There was statistical significant difference between Group 1 and Group 2 at coronal andmiddle third of root however there was no statistical significant difference between Group 1 and Group 2 at apical third of root. Group 1 showed more depth of penetration than group 2.

CONCLUSION:

The depth and consistency of dentinal tubule penetration of sealer cements appears to be influenced by the chemical and physical characteristics of the materials. EndosequenceBioceramic sealers displayed deeper and more consistent penetration.

Keywords:AH Plus Sealer, Bioceramic Sealer, confocal laser scanning microscopic, Tubular penetration, EndosequenceBioceramicsealer

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INTRODUCTION:

Proper chemomechanical preparation is a must to accomplish a successful root canal treatment. However, a close alliance has been found between the root canal preparation and obturation[1].The trilogy of thorough canal debridement, potent disinfection, and obturation of the canal space is considered to be essential in order to achieve a successful root canal treatment [2]. The seepage of microorganisms and bacterial toxins into the endodontic system can be impeded with root canal obturation using "Hermetic Seal"[3.] A study suggested that approximately 60% of endodontic failure were accounted due to apical percolation of periradicular exudate into the root canal which is incompletely filled [4]. A three-dimensional obturation seals all "portals of exit" and forbids percolation and microleakage of periapical exudates into the root canal space, restricts reinfection, all of which generates an encouraging biological environment for healing [5].Historically, beginning with Bowman's introduction of the material into endodontic in 1867, gutta-percha, has been the material of preference for root canal obturation.[6] One of the drawbacks with using guttapercha is that it cannot hermetically seal the root canal irrespective of the root canal filling technique used, .This drawback can be attributed to non-adherent nature of guttapercha to canal wall.In order to overcome this limitation, sealer has to be used in conjugation with gutta-percha to procure a fluid-tight seal, and this also fill up the spaces which ispresent between the canal wall and the obturating material [7-9].Bioceramic-based sealers have been introduced in endodontics for the past thirty years, their ascend to prominence commensurate with thegrowingutilisation of bioceramic technology in the area of medicine and dentistry[10]. Based on the interaction of bioceramic material with the surrounding living tissue, bioceramic materials are classified into bioactive or bioinert materials [11].

Bioceramic materials as root canal sealers possess two major desirable characteristics. Firstly, beingbiocompatible it averts rejection by the neighbouring tissues [12]. Secondly, superior setting properties of bioceramics, which is due to addition of calcium phosphate, whichresults in a chemical composition and crystalline structureresembling to tooth and bone apatite materials[13], thereby,helping to ameliorate the sealer-to-root dentin bonding.

Therefore the present study was undertaken to compare and evaluate the depth of sealer penetration of newly introduced BC(Bioceramic)sealer that is,EndosequenceBioceramic sealer and AH-plus sealer into the dentinal tubules using Rhodamine B dye under confocal laser scanning microscopy. The null hypothesis tested was that there are no differences in the depth of sealer penetration between the sealers groups tested.

MATERIALS AND METHODS

The study was approved by the institutional review board and ethical committee (SDKS/STRG/Staff/endo1/2017)

20 extracted single rooted permanentmandibular premolar teeth with fully developed root apices and straight roots were selected. Teeth having cracks,resorptivedefects, caries, and fractures were excluded. After removal of the external debris, teeth were placed in 2.5% sodium hypochlorite solution for 2 hours and stored in normal saline.

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All samples were decoronatedatcementoenameljunction leaving a standardized length of 14 mm. Working lengths werecalculated 1mm short of the apical foramen. Each tooth was prepared using crown down technique to size 30, 0.06 taper using stainless steel hand files and ProTaper Universal Nickel titanium rotary instruments (DENTSPLY, Maillefer, Switzerland).

During instrumentation to maintain patency and improve irrigant penetration, recapitulation to the working length was accomplished after each rotary instrument series using a size 10 K-file. Irrigation of canal was done with 1 mL 2.5% NaOCl (Neelkanth Health Care (P.)LTD, India) after each instrument and it was performed using a 30-G side-vented irrigation needle and a syringe. During irrigation, the irrigation needle was moved up and down in the canal to within 1–2 mm of the working length. After instrumentation was completed the tooth were given final flush with 17% EDTA and 5% Sodium Hypochlorite to remove the smear layer followed by irrigation with 10ml of distilled water to remove remaining irrigant residue. The canals were dried with the help of sterile paper points (Dentsply, Malliefier).

All the samples were divided randomly into 2 groups of 10 teeth each for the obturation using F3 master cone with respective sealers as:

Group 1: EndosequenceBioceramic sealer(Brasseler, Savannah, USA) along with 6% gutta-percha points (Dentsply-Maillefer) of size 30.

Group 2: AH Plus sealer (Dentsply, De Trey Konstanz, Germany) along with 6% gutta-percha points (Dentsply-Maillefer) of size 30.

Manipulation of each sealer material was done according to manufacturers' instructions. For fluorescence under confocal laser scanning microscopy, 0.1% fluorescent rhodamine B isothiocyanate was mixed with sealer. The quality of root canal obturation wereassessed by radiographs. All specimens were stored in 100% relative humidity at 37°C for 24 hours to ensure complete setting of the root canal sealers.

After the resin had set completely, each tooth was sectioned perpendicular to its long axis in 1-mm-thick sections using a slow-speed handpiece at three different points measuring from the root apex 3, 6, and 9 mm.So three sections were obtained - coronal, middle and apical third of the samples.Samples mounting wasdone on glass slide and were examined under Confocal Laser Scanning microscope to evaluate the depth and percentage of sealer penetration into the dentinal tubules. The depth of sealer penetration was measured with the help of measuring tool in the software. (Figure 1,2,3,4,5,6)

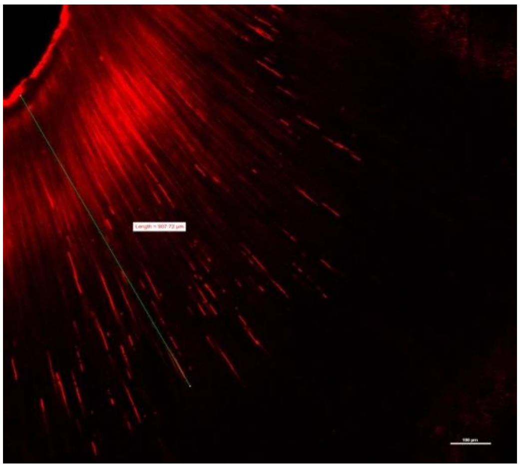

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Fig. 1 Confocal laser scanning microscope image showing sealer penetration in AH-Plus Sealer –Coronal

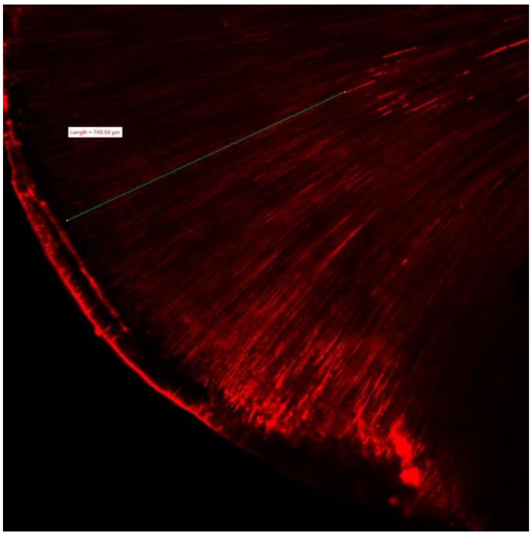

Fig. 2 Confocal laser scanning microscope image showing sealer penetration in AH plus Sealer – Middle

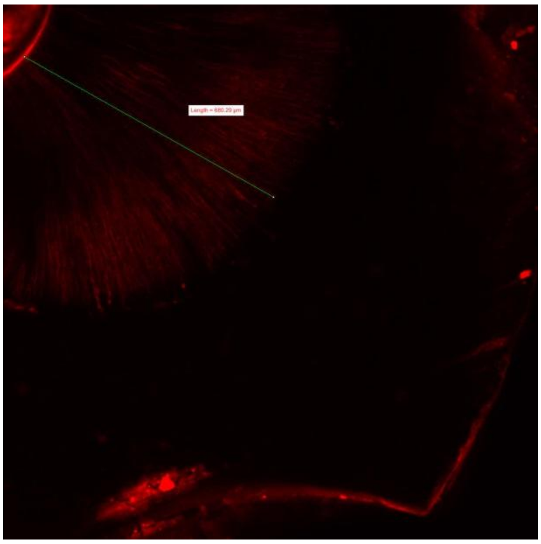

Fig. 3 Confocal laser scanning microscope image showing sealer penetration in AH plus Sealer – Apical

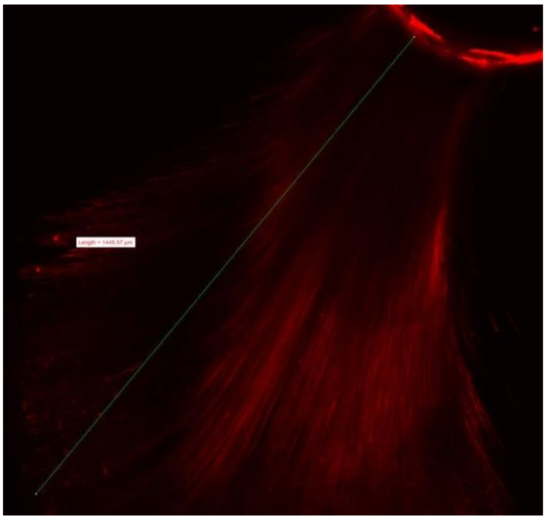

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Fig.4 Confocal laser scanning microscope image showing sealer penetration in BioCeramic Sealer- Coronal

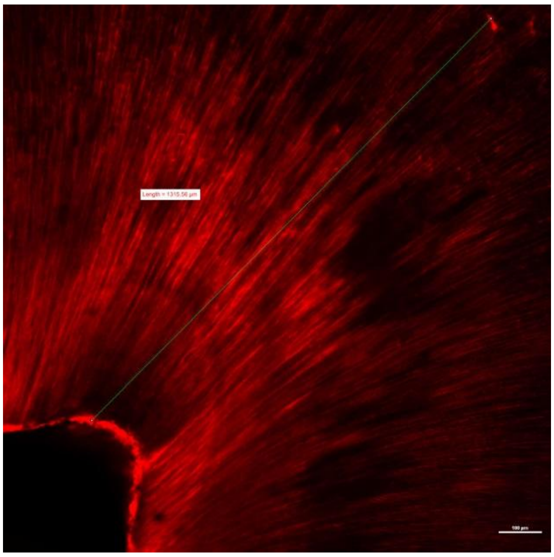

Fig. 5 Confocal laser scanning microscope image showing sealer penetration in BioCeramic Sealer –Middle

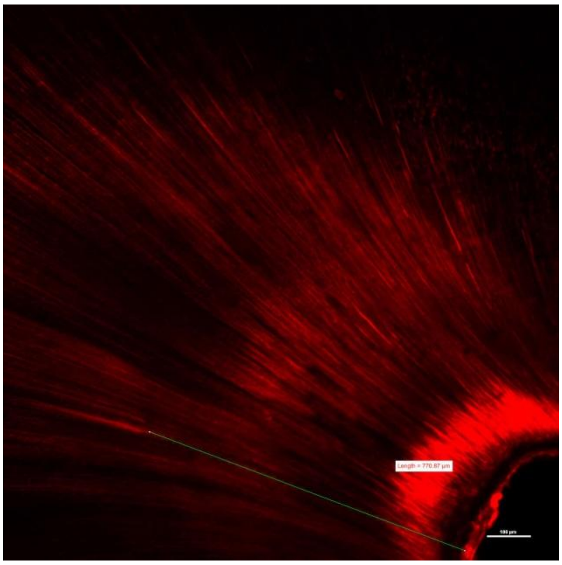

Fig. 6 Confocal laser scanning microscope image showing sealer penetration in BioCeramic Sealer –Apical

Statistical Analysis:

Statistical analysis was done by using descriptive and inferential statistics using student's unpaired t test, one way ANOVA and Tukeys test were applied for the pairwise comparison of the depth of sealer penetration between the groups and software used in the analysis were SPSS 24.0 version and GraphPad Prism 7.0 version and $p<0.05$ is considered as level of significance.

RESULTS

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Descriptive statistics for Depth of penetration (in micrometre) at Coronal, Middle and Apical site in group 1 and 2 is mentioned in Table 1. The graph showing the comparison of depth of sealer penetration is given in Figure 7. Table 2 shows the mean difference between the depth of penetration (in micrometre)in group 1 and 2.On pairwise comparison EndosequenceBioceramic sealer showed more sealer penetration when compared to AH Plus sealer at coronal, middle and apical thirds. There was statistical significant difference between Group 1 and Group 2 at coronal third (P value 0.007) and middle third (0.0001) of root. However, there was no statistical significant difference between Group 1 and Group 2 at apical third of root (P value 0.073).

Table 1:Descriptive statistics for Depth of penetration (in micrometre) at Coronal, Middle and Apical site in group 1 and 2

Groups		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Group 1 (BC Sealer)	Coronal	10	1424.23	527.15	166.69	767.91	2083.94
	Middle	10	1104.19	186.46	58.96	736.64	1320.30
	Apical	10	1235.82	217.59	68.80	854.52	1571.77
Group 2 (AH Plus Sealer)	Coronal	10	912.61	57.71	18.25	791.14	987.21
	Middle	10	807.54	68.38	21.62	644.08	879.21
	Apical	10	1095.07	85.15	26.92	1000.21	1245.32

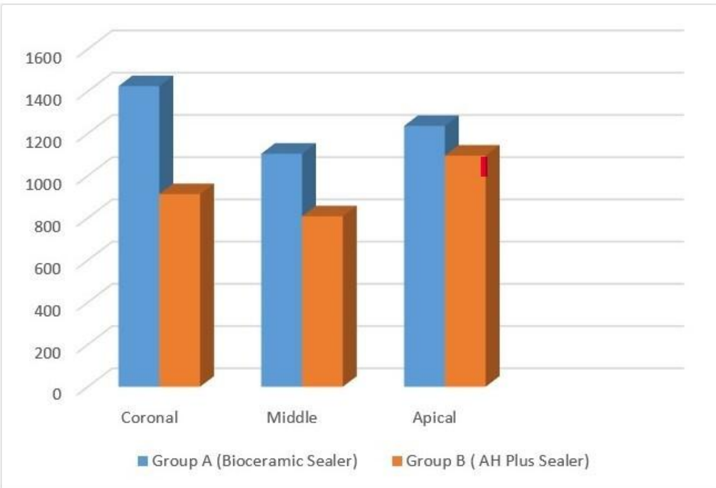

Fig. 7 The depth of sealer penetration into dentinal tubules at the coronal, middle, and apical sections.

Table 2:Comparison of depth of penetration (in micrometre) at Coronal, Middle and Apical site in group 1 and 2

Site	Mean Difference	SD of difference	t-value	p-value
Coronal	511.62	167.69	3.05	0.007

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Middle	296.65	62.80	4.72	0.0001
Apical	140.74	73.89	1.90	0.073

DISCUSSION

Flow allows the sealer to fill difficult-to-access areas, such as accessory canals, isthmus, narrow irregularities of the dentin and voids present between the master and accessory cones [14].In the present study, the depth of penetration of two resin-based root canal sealers (EndosequenceBioceramic sealer and AH Plus sealer) into the dentinal tubules was evaluated with the help of Confocal laser scanning microscope.

Confocal laser scanning microscopy (CLSM) aids to acquire a series of optical images that are recorded through the thickness of the dentin. In the present study, CLSM was preferred over Scanning Electron Microscope(SEM),because at magnification of as low as X50-X100 the former allows the detection of sealer penetration along the canal circumference of each sample through the use of fluorescent rhodamine– marked sealers. Consequently, with 1 or 2 CLSM pictures the percentage of adaptation of sealers to the root canal walls can be achieved effortlessly[6]. An additional advantage of using CLSM in segments is that the sealer can be visualised at several depths. It provides various advantages over conventional wide-field optical microscopy and SEM[15-18]. For the identification of sealers within the dentinal tubules rhodamine B (SigmaAldrich, St Louis, MO, USA) has been used as an indicator under CLSM [18] and as long as a small amount of dye (less than 0.2%) is mixed with the sealers physical properties of the sealers are not hampered[19].

In all the experimental groups on an average, greater penetration was seen at the cervical third, followed by the middle third, and least in the apical third. These results are similar to those of other various studies [20-21],onejustification for this result may be that the diameter and number dentinal tubules reduce apically in the root canal. Furthermore, certain areas in apical third are dentinal tubule free, and tissue-like cementum can contour the apical root canal wall, which will obstruct the tubules.Better removal of the smear layer in the coronal region can also be another possible reason for lesser sealer penetration in apical segment[22].

The existence of smear layer has shown to obstruct the sealers from penetrating the dentinal tubules. [(23)] Accordingly, the smear layer was removed by the sequential use of EDTA solution followed by 5.25% NaOCl, with the help of 30 gauge side vented needle, so as to reach in close proximity of the apex and yield maximum effect on the entire length of the canal wall.

In order to eliminate the effect of the residual oxygen liberated from NaOCl on polymerization of resin sealers, a final rinse of EDTA solution was given followed by a rinse with distilled water.

AH Plus is an epoxy-bis-phenol resin based sealer. This sealer contains adamantine and bonds to root canal. It has gained popularity among numerous types of sealer used today, due to its radiopacity, biocompatibility, availability and ease to use [(24)]

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Endosequence BC Sealer is a freshly introduced Calcium Silicate based BC sealer, described by its manufacturer as an, radiopaque, insoluble, aluminium-free material that mandates the presence of water to set and harden.[25,26] It is hydrophilic and biocompatible in nature and it expands on setting which results in forming of 'self seal'.

In the current study it was found that depth of dentinal tubule penetration was more in BC sealer than AH plus sealer in all sections of tooth. This difference in penetration of the root canal sealer into dentinal tubules can be attributed to the difference in particle size.Extremely small particle size (less than 2 µm) is responsible for deeper penetration of Endosequence BC sealer especially in smaller tubules at the apical root area; whereas, larger calcium tungstate particles with an average size of 8 mm and zirconium oxide particles with a size of 1.5 mm present in AH Plus,might not enter easily into the smaller tubules at the apical root area.Also, properties of Endosequence BC such as low initial viscosity level, hydrophilic nature and low contact angle,promote the sealer to spread effortlessly over the dentinal wall and flow into all aspects of the canal anatomy. Moreover, minimal or no shrinkage is exhibited by Endosequence BC in the setting phase In addition, 0.2% expansion is attained by the Endosequence BC root canal sealerduring the setting period. Also, the spread of sealer over the dentin walls of the root canal and filling of the lateral canals are supported by these characteristics. These features may have contributed to higher dentinal tubule penetration observed in the present study. This is in accordance with the literature reporting that due to the smaller particle size of BC Sealer and also due to its high level of viscositytricalcium silicate–containing sealers penetrated into the tubules as deep as 2 mm [27, 28]. However, further in vivo studies should be conducted.

CONCLUSION

Within the limitation of the experimental design and the test parameters, it can be concluded that the depth and consistency of dentinal tubule penetration of sealer cements is influenced by the physical and chemical characteristics. EndosequenceBioceramicsealerexhibited more consistent and deeper penetration than AH Plus sealer. The maximum penetration of the both sealers was more in the coronal third followed by the middle third and least in the apical third.

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