Original Article

Effect of final irrigant on depth of tubular penetration of resin-based root canal sealer and bioactive sealers using confocal laser scanning microscope

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ABSTRACT

Background: Deep dentin tubular penetration of the sealer provides a three dimensional sealing of root canal space

Aim: To evaluate the effect of final irrigant on depth of tubular penetration of resin based root canal sealer and bioactive sealers using Confocal Laser Scanning Microscope (CLSM)

Materials and Method: Ninety- six freshly extracted human mandibular first premolar teeth were decoronated at 15 mm from the apex. Cleaning and shaping procedure was accomplished using Hyflex CM rotary files till F3. The sample were divided into three groups(n=32) according to final irrgant used : Group A (17 % EDTA), Group B (QMix 2 in 1), Group C (Distilled water). The final irrigation in each respective group was performed with EndoVac system. The samples were further subdivided into 4 subgroups (n=8) according to the type of sealer used for obturation with 6% guttapercha cones - Subgroup I (AH Plus), Subgroup II (Gutta Flow Bioseal), Subgroup III (Endosequence BC), Subgroup IV (EndoSeal MTA). Two mm horizontal sections were obtained at 2 mm (apical sections), 5 mm (middle sections) and 7 mm (coronal sections) from the root apex using CLSM to evaluate the maximum depth and percentage of sealer penetration into the dentinal tubules by using Kruskal-Wallis test for overall analysis and a series of Mann-Whitney U tests for pairwise comparison. **Result:** Endosequence BC showed maximum depth of penetration and penetrated percentage perimeter, while Gutta Flow Bioseal showed least values. Q Mix 2 in 1 showed better penetration values than EDTA and distilled water. Conclusion: Irrigants, nature of sealer and level of root canal affected sealer penetratio.

Keywords: Confocal laser scanning microscope, endoseal MTA, endosequence BC, endovac, gutta flow bioseal, Q mix 2 in 1

INTRODUCTION

Sealers are the integral part of obturation process due to their ability to adhere to dentin and gutta-percha. Deeper tubular penetration depth of sealers provides superior sealing by entombing residual micro-organism deeply seated inside dentinal tubules.[1]

The effective sealer depth of penetration inside the dentinal tubules depends upon many factors such as presence/absence of dentinal permeability, root canal dimension, presence of water, and sealer's physical and chemical properties.[2,3] Furthermore, the depth of penetration of a sealer and irrigant is a compound effect of physical properties, namely, flow, surface tension, solubility, viscosity, chemical composition, and working and setting time.[4,5]

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Different types of sealers and obturation systems have been introduced in the field of Endodontics. Gutta-percha core material in conjunction with AH plus sealer has been thought of as the "gold standard" filling material.

Newer generation Bioactive sealers such as Endosequence BC, GuttaFlow Bioseal, Endoseal MTA are being engineered to enhance their ability to penetrate into dentinal tubules and bond to, rather than simply adhering to, both the dentin and core material surfaces.[3]

Confocal laser scanning microscope (CLSM) is a proven reliable tool to evaluate the tubular penetration depth of sealer and has several advantages over other microscopic studies as CLSM produces fewer artifacts, which aids in visualizing up to 10 µm below the surface of the specimen.[6] Furthermore, it works with high contrast points to identify the sealers within the dentinal tubules.[7]

To the best of our knowledge, no study has been done till date to compare the effect of final irrigant, i.e., Q Mix 2 in 1 on depth of tubular penetration of resin based root canal sealer (AH Plus) and bioactive sealer (Endosequence BC, GuttaFlow Bioseal, and Endoseal MTA). Therefore, the aim of the present study was to comparatively evaluate the effect of final irrigant on depth of tubular penetration and penetrated percentage perimeter of resin based root canal sealer (AH Plus) and bioactive sealers (Endosequence BC, GuttaFlow Bioseal, Endoseal MTA) using CLSM. The null hypothesis tested were first that there was no difference in the effect of 17% ethylenediaminetetraacetic acid (EDTA) and Q Mix 2 in 1, when used as a final irrigant on the tubular penetration of resin based and bioactive sealers second there was no difference in the depth of tubular penetration of resin based sealer and bioactive sealer and third there was no difference in the depth of tubular penetration at all levels after the use of different irrigants.

MATERIALS AND METHODS

Specimen selection

Clearance for this study was attained from institutional ethical committee. Ninety-six human mandibular first premolars with relatively straight roots and fully formed apices, freshly extracted for orthodontic reasons, exhibiting only one canal, free of any cracks, caries, and restoration were chosen. The sample size was decided on the basis of pilot study done with eight sample used per subgroup using Open Epi, Version 3. The samples used in the pilot study were discarded.

Sample preparation

The crowns were sectioned at 15 mm from the apex using diamond disk in slow speed hand piece. The teeth having canals patent to size <10 K or more than 20 K were discarded. Working length was established with the help of radiograph. The apex was sealed using sticky wax to prevent the extrusion of irrigation solution from the apex. Biomechanical preparation of the canals was done using rotary Hyflex CM till no. 30 (0.06% taper). Canals were irrigated between the files with 2 ml of 5% NaOCl and recapitulation with no. 10 K-file was done between each instrument.

Grouping of samples

The specimens were randomly divided into three experimental groups according to the type of final irrigation regimen used to remove smear layer. The groups were as follows:

- Group I (n = 32) Initial rinse with 5 ml of 5% NaOCl (Qualigens Fine Chemicals, Mumbai, India) for 2 min followed by saline flush and finally 2 min rinse with freshly prepared 5 ml 17% EDTA (Central Drug House Pvt. Ltd., New Delhi)
- Group II (n = 32): Initial rinse with 5 ml of 5% NaOCl for 2 min, followed by saline flush and final rinse of 5 ml of Q Mix 2 in 1(Dentsply Tulsa Dental, USA) for 2 min
- Group III (n = 32): Initial rinse with 5 ml of 5% NaOCl for 2 min, followed by final rinse of 5 ml of distilled water (Cero Distilled water, Mumbai).

The final irrigation in all the groups was done with Endovac irrigation device (Dental Kerr Sybron Endo, USA) as per manufacturer's instruction.

Subgrouping of samples

Samples of all the groups were further subdivided randomly into four Subgroups based on the type of sealer and obturation material used.

- Subgroup A (n = 8): Obturated with AH Plus sealear (DentsplyMalliefer, Ballaigues, Switzerland)
- Subgroup B (n = 8): Obturated with ROEKO GuttaFlowBioseal (Coltene- Whaledent, Switzerland
- Subgroup C (n = 8): Obturated with Endosequence BC sealer (Brasseler, USA)
- Subgroup D (n = 8): Obturated with Endoseal MTA sealer (Maruchi, wonju, Korea).

Sealer was mixed with (0.1%) of rhodamine dye in all the subgroups and was applied with the help of lentulospiral and obturated with single cone technique using 0.06/30 GP cones.

All the specimens were stored in 100% relative humidity at 37°C for 24 h to simulate the oral conditions, to provide a

uniform environment during setting and to provide enough time for complete polymerization of sealers.

Confocal laser scanning microscope examination

Two millimeter horizontal sections were obtained at 2 mm (apical sections), 5 mm (middle sections), and 7 mm (coronal sections) from the root apex using diamond disc. They were then embedded in 70%, 80%, 96%, and 100% ethyl alcohol bath each for 30 s for preserving the dimensions and morphology.

The horizontal dentin segments were examined and analyzed under ×10 lens of confocal microscope (Olympus Fluoview FV 1000) (Pennsylvania, USA). The respective absorption and emission wave lengths for the Rhodamine B were 540 nm and 590 nm. Ruler tool LAS-AF software was used to quantize the depth of sealer penetration and recorded at 4 standardized points. The canal wall served as the starting point, and sealer penetration into dentinal tubules was measured to a maximum depth of 1000 μm. All the data points were averaged to obtain a single measure for each section. To calculate the percentage of sealer penetration around the root canal, first each image was imported into software and the circumference of root canal measured using its ruler tool. Next, areas in the canal walls in which the sealer penetration took place inside the dentinal tubules were outlined and measured using the similar methodology. Finally, the percentage of root canal sealer penetration in that section was established. Depth and percentage of sealer penetration were analyzed, by doing nonparametric Kruskal–Wallis test for overall analysis, and a series of Mann–Whitney *U*-tests for pair wise comparison using statistical package of social sciences (SPSS) statistics version 21.0 (Illinos, Chicago, USA) and Epi-info version 3.0. $P < 0.05$ was considered statistically significant.[8]

RESULTS

Mean sealer penetration and penetrated percentage perimeter was maximum in Endosequence BC, followed by AH Plus, Endoseal MTA and least in GuttaFlow Bioseal at all the root levels. Q MIX 2 in 1 when used as final irrigant showed maximum mean sealer penetration and penetrated percentage perimeter followed by EDTA irrespective of type of sealer and root level. Coronal third showed maximum mean sealer penetration and penetrated percentage perimeter and minimum with apical third irrespective of type of sealer and final irrigant.

The difference between all the groups and subgroups was statistically significant at all the level.

DISCUSSION

Depth of dentinal tubular penetration of a sealer is one of the important factors to achieve a three dimensional impermeable seal of root canal space. The results of this study showed that depth of penetration of sealers is influenced by various characteristics of sealer, type of final irrigant used and level of root. Therefore, all the null hypothesis were rejected. In the present study, EndoSequence BC Sealer showed significantly highest mean dentinal tubular penetration and maximum mean penetrated percentage perimeter area.[9] (Table-1,2) This might be due to its good flow rate (23.1 mm and 26.96 mm), particle size (<2 μ), hydrophilic nature and low contact angle that allows the sealer to spread easily over the canal providing adaptation and good hermetic seal through mechanical interlocking.[10] It has setting time of 2.7 hrs which gives it sufficient time to penetrate the tubules.[9] Moreover, it shows 0.2% expansion as it utilizes moisture from the dentinal tubules to complete the setting reaction.[9] Alkaline nature of by-products of bioceramic sealers have been reported to denature the dentinal collagen fibers, which also facilitates the penetration of sealers into the dentinal tubules.[11] The findings of our study are in accordance with the study of Akcay *et al*. who evaluated penetration of various sealers and found that iRoot SP exhibited a significantly higher penetration area than AH plus and bioactive sealers.[9] However, contradictory study by Bharath *et al*. reported that AH plus has better penetration in comparison to Endosequence BC sealer. The reason for this difference might be due to the difference in method of the assessment of penetration.[12]

AH Plus showed significantly better dentin tubular penetration than Endoseal MTA and GuttaflowBioseal.[13] (Table 1,2) This might be attributed to its creep capacity with good flow rate (21.2 mm),[14] reduced film thickness (26 μm),[15] small particle size of 20–25 μm. In addition, expansion of 0.1% makes it adhere to dentin.[16] Its long setting time of 11.5 ± 1.5 h increases the mechanical interlocking between sealer and root dentin.[16] The wettability of AH Plus sealer on the root surface dentin was found to be better than the other two sealers due its lower contact angle which aided in better tubular penetration.[15] Also, superior adaptation of AH Plus to root dentine is attributed to its ability to bond to root dentine chemically by reacting with exposed amino group in collagen and form covalent bond between epoxy resin and collagen.[17]

EndoSeal MTA showed inferior penetrability as compared to AH Plus because of presence of more voids in Endoseal MTA sealer.[17] (Table 1,2) The superior penetrability of

Table 1: Intragroup comparison of depth of penetration of sealers at various levels

Depth of penetration of sealers	Mean±SD			
	Sub Group I AH plus	Sub Group II GuttaFlow bioseal	Sub Group III Endosequence BC	Sub Group IV Endoseal MTA
Group A 17% EDTA				
C	448.2±47.14	275.58±63.52	574.95±36.43	373.98±24.91
M	237.24±27.28	183.52±41.07	288.8±54.43	206.94±61.41
A	70.54±33.95	40.61±35.64	90.16±60.47	53.44±15.21
Group B QMIX 2 in 1				
C	500.77±91.37	309.96±121.99	650.39±32.27	454.48±17.46
M	273.78±14.17	215.87±33.12	351.55±32.35	246.57±28.87
A	87.59±16.23	40.71±30.16	110.85±15.49	62.67±21.15
Group C distilled water				
C	223.53±68.28	146.64±31.41	305.45±100.23	192.44±39
M	123.53±73.96	62.89±26.28	159.03±67.44	73.62±57.61
A	28.35±8.88	17.06±7.92	36.51±11.72	21.51±5.89

P<0.05 was considered statistically significant. SD: Standard deviation; EDTA: Ethylenediaminetetraacetic acid

Table 2: Intra subgroup comparison of various levels with respect to penetrated percentage perimeter of sealers in different groups

Penetration percentage perimeter	Mean±SD			
	Sub group I AH plus	Sub group II Guttaflow bioseal	Sub group III Endosequence BC	Sub Group IV Endoseal MTA
Group A 17% EDTA				
C	66.80±6.43	55.39±3.00	71.89±12.55	60.56±7.30
M	61.51±3.80	50.96±5.69	66.98±8.18	55.66±5.43
A	55.66±4.19	44.64±2.94	59.06±2.56	49.42±2.61
Group B QMIX 2 IN 1				
C	73.30±5.81	62.87±7.42	81.20±4.63	66.83±6.58
M	68.05±4.14	57.84±6.93	75.13±3.82	61.72±7.08
A	60.69±5.55	51.59±10.98	68.49±5.21	55.59±4.61
Group C distilled water				
C	58.17±4.16	46.61±2.35	63.55±7.60	52.38±4.85
M	53.92±3.10	41.53±3.12	58.87±4.88	48.29±3.50
A	47.17±4.16	35.99±2.41	51.51±4.09	43.14±2.93

P<0.05 was considered statistically significant. SD: Standard deviation; EDTA: Ethylenediaminetetraacetic acid

Endoseal MTA as compared to GuttaFlow Bioseal might be due to reduced film thickness of Endoseal MTA (15 μm)[18] as compared to GuttaFlow Bioseal (35.4 mm)[9] and flow rate(21 mm).[9] Also, GuttaFlow Bioseal has large particle size (28–30 μm) and poor wetting ability.[19] Moreover, because of the presence of silicone in GuttaFlow Bioseal, production of high surface tension makes the sealer more difficult to spread resulting in lower penetration.[19] Less setting time of 17.4 min makes the penetration of sealer inside the dentinal tubules difficult.[9,20]

Q Mix 2 in 1 showed better depth of penetration and mean percentage perimeter than the other two groups for all the type of sealers. (Table1,2). Although the smear layer removal capability of both 17% EDTA and Q Mix 2 in 1 has been found to be similar,[21] Q Mix 2 in 1 is composed of 17% EDTA, CHX and a surfactant which consequently enhances the demineralization of radicular dentin due to the chelating effect of 17% EDTA, while disinfecting at the same time. The presence of surfactant lowers the surface tension of the solution and increases the wettability and thus enhances penetrability.[22] Moreover, CHX present in the constitution of Q Mix 2 in 1 has also been shown to increase surface energy of dentine and decrease the contact angle of root canal sealer thereby improving its wettability.[22,23] Inferior penetration with EDTA might be due to the fact that EDTA decreases surface energy.[23]

Minimal dentinal penetration was seen in distilled water group in comparison with the other two irrigant groups. This might be due to lack of any cleaning and chemical effect on smear layer.[24]

Apical third least penetration and penetrated percentage area perimeter of all the resin sealers as compared to middle third and coronal third as apical dentin displays less tubule density,[3] sclerotic dentin with some areas completely devoid of tubules[3] and ineffective smear layer removal techniques,

all of which can hinder the penetration of irrigating solutions and root canal sealers. Also, greater compressive forces during obturation at coronal and middle third might be another contributing factor.[25] Truncer reported contradictory results to this study with no significant difference of sealer penetration and penetrated percentage perimeter between coronal and middle third.[26] This might be due to efficient cleaning of the root canal by Endovac (negative pressure irrigation) as the system used for final irrigation in our study as compared to their study where final irrigation was done by conventional needle (positive pressure irrigation). Endovac provides effective irrigation as it holds irrigant into the canal and removes it by negative pressure at working length.[4]

CONCLUSION

Within the limitations of the present study, it can be concluded that type of irrigant, nature of sealer and level of root canal affected sealer penetration. Therefore, the sealers should be strictly manipulated and placed according to the manufacturer's instruction along with following appropriate and effective irrigation regime and technique. Endosequence BC showed the maximum depth of dentin tubular penetration among all the sealers used in the study. Q Mix 2 in 1 was the most effective final irrigating solution compared to EDTA and Distilled water.

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Conflicts of interest
There are no conflicts of interest.

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