Comparison of Root Canal Sealer Penetration and Distribution by Novel Reciprocating NITI Spreader with Sonic, Ultrasonic and Rotary Methods: A Confocal Laser Scanning Microscopic Study.

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ABSTRACT

Background: A sealer is required during obturation to fill space between gutta-percha and canal walls. It provides an impervious seal, fills the irregularities and minor discrepancies between the root canal wall and core filling material, and assists in microbial control. Aim: To evaluate the penetration depth and percentage area of a root canal sealer placed by five different techniques using confocal laser scanning microscope (CLSM). In our study we compared ultrasonic, endoactivator (sonic device), lentulospiral (rotary) and master gutta-percha with NiTi spreader in reciprocating hand piece for sealer placement using AH Plus sealer. **Methods**: Thirty extracted then decoronated and standardised mandibular premolars were prepared by Protaper rotary files up to master apical file F3. Samples were randomly divided into five groups for sealer placement; Group 1, Ultrasonic file; Group 2, Endoactivator; Group 3, Reciprocating hand piece with NiTi spreader; Group 4, Rotary Lentulospiral; Group 5, Master apical gutta-percha .Two root sections from apical and coronal third were analysed for sealer distribution and depth of sealer penetration by confocal microscope, using the ruler tool of the IOB software (Olympus). **Results**: Results showed that maximum depth and percentage of sealer penetration is shown by Group 1; ultrasonic file and least by Group 5; master apical gutta-percha both at apical as well as coronal levels. **Conclusion**: Sealer penetration is influenced by placement method and new method of using reciprocating NiTi spreader was comparable to ultrasonic method which showed best sealer penetration and distribution.

Keywords: Confocal laser scanning microscopy, Endoactivator, Lentulospiral, Sealer placement techniques, UltIrasonics.

INTRODUCTION

Among the various materials used for obturation of root canals gutta-percha is most commonly used core filling material. Although it can be reasonably adapted to the root canal walls, but the canal irregularities, space between core material and the size of the dentinal tubules necessitate the use of a root canal sealer to assist not only in filling irregular spaces, but also to enhance the seal during compaction and to penetrate into small, normally inaccessible, areas like dentinal tubules. Root canal sealers are used in conjunction with a core-filling material to attain an impervious seal between the core material and root canal wall.^[1] A few investigators did not observe any significant correlation between degree of micro leakage and sealer penetration into dentinal tubules.^[2] But majority of studies suggested that mechanical interlocking of sealer inside dentinal tubules following smear layer removal improve dislocation resistance of root fillings and decrease microleakage.^[3-5] It was observed that the smear layer obstructed the penetration of sealers into the tubules.^[6,7] Currently, ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCI) are

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used to remove the smear layer and improve sealer penetration.^[8] Bacteria have been shown to penetrate 150 to 400 μ m into dentinal tubules. The ability of the sealers to penetrate into the dentinal tubules may be especially beneficial to control or kill bacteria that may be located there.^[9]

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Less and inadequate sealer placement results in spaces and voids between core material and dentinal walls which results in micro-leakage and hence endodontic treatment failure. While as excess results in extrusion into periradicular area and delays or prevents healing.^[10]

Several techniques for sealer placement have been described in literature including lentulospiral, file or reamer, master gutta-percha cone and ultrasonic instruments. Newer methods of sealer placement include sonic and ultra-sonic activation.^[11,12] At present, no single technique has yet been proven to be completely satisfactory and no study has evaluated effect of reciprocating motions on sealer placement. The aim of this study was to test the dentinal tubule penetration and percentage distribution of AH Plus sealer placed by five different techniques and evaluated by Confocal Laser Scanning (CFLS) microscope.

MATERIALS AND METHODS

Thirty extracted human mandibular premolar teeth with single canals, closed apices, straight roots and no caries or resorption were used in this study. The teeth were stored in 10% formalin solution until they were used for the study. Teeth were decoronated with diamond disc at cementoenamel junction under continuous water cooling to obtain a standardized root length of 14 mm. A #10 K-file (SybronEndo, USA) was introduced into the canal until visualised at the apical foramen. This length was recorded, and the working length was established by subtracting 1mm from the recorded length. All canals were instrumented to working length using Protaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) to master apical file F3. 2 mL of 5.0% NaOCl was used for irrigation between each instrument with a final rinse of 5mL 17% EDTA for 1min followed by 5mL of distilled water. Canals were dried with absorbent paper points. Specimens were randomly divided into 5 experimental groups (n=6) according to the mode of sealer activation. Group 1: Ultrasonic activation group

Size 15 ultrasonic K-file (Piezotec Files, Satelec, France) was attached to a piezoelectric ultrasonic handpiece (woodpecker) and used at medium power for the activation of the sealer. Because the ultrasonic oscillates in a single plane, the file was activated for 20 seconds with 2-3 mm back and forth movements in the buccolingual direction and another 20 seconds in the mesiodistal direction of the root canal, 2mm short of working length as a standard procedure. All the samples in the group were then obturated with Protaper F3 gutta-percha cones and coronal orifices sealed with provisional restorative material.

Group 2: Sonic activation group

Endoactivator (Dentsply, Maillefer) was used for the sonic activation of the root canal sealer. Small (#15/.02) polymer tip was attached to the device and stopper placed at 2 mm short of the working length. Device was activated at medium speed, moved in short 2-3 mm vertical strokes for a total time period of 40 seconds. Samples were then obturated as in group 1.

Group 3: Reciprocating NiTi spreader group.

An NSK TEP 10:1 gear reduction handpiece (Nakanishi Inc., Japan) with 60° twist mounted on a micro motor hand piece was used with a #25/0.04 NiTi spreader for sealer placement. Sealer coated spreader was rotated for 40-seconds inside the canal, 2mm short of the apex. Samples were then obturated as in group 1.

Group 4: Rotary Lentulospiral group.

In this group size 25 lentulospiral (Dentsply Maillefer, Ballaigues, Switzerland) was used for 40-seconds keeping the instrument 3 mm from the canal apices with the handpiece running at 300 rpm to distribute the sealer placed into canal. Samples were then obturated as in group 1.

Group 5: Master apical gutta-percha group.

In this group size master apical gutta percha was used to place the sealer into root canals. Samples were obturated as in other groups.

All the specimens were then stored in a humidifier with 100% humidity and temperature maintained at 37°C for seven days.

AH Plus sealer was mixed on a mixing pad until a homogeneous consistency was obtained. To facilitate fluorescence under confocal microscopy, the resin sealer was labelled with fluorescent Rhodamine - B dye (Himedia Laboratories, Mumbai, India) to an approximate concentration of 0.1%. The dye–sealer mixture was placed along the entire length of the root canals in each of the groups.

After one week, the roots were horizontally sectioned into 2mm thick slices with the help of diamond discs. One slice each from apical and coronal part of roots was analysed for sealer penetration into the dentinal tubules, and the

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interfacial adaptation (gaps) on an inverted Nikon A1+ confocal laser scanning microscope (Nikon Corporation, Japan) by the similar method described by Guimarães et al.^[13] The respective absorption and emission wavelengths for the Rhodamine - B were set to 540 and 590 nm, respectively. Then, the images were recorded using the fluorescent mode to a size of 512×512 pixels.

Analysis of all images was performed with the Image J V1.46r software (National Institutes of Health, Bethesda, MD) [Figure 1]. The total circumference of the canal was obtained first. Then, segments of sealer penetration into the dentinal tubules and inter-facial adaptation (gaps) of the total circumference were measured, and the values were converted into percentages.

Statistical analysis: Statistical software SPSS (version 20.0) and Graph Pad (Prism 5.00) were used to carry out the statistical analysis of data. Continuous variables were summarized in the form of means and standard deviations. Analysis of variance test (ANOVA) was employed for inter group analysis of data and for multiple comparisons, 'Least Significant Difference' test was applied. A P-value of less than 0.05 was considered statistically significant. All P-values were two tailed.



Figure 1: Photograph showing image analysis using Image J V1.46v Software (National Institute of Health, Bethesda, MD).

RESULTS

The mean and standard deviation of sealer penetration depth and percentage of sealer penetration are presented in [Table 1]. Ultrasonic activation of sealer (group 1) showed maximum depth of penetration both at apical as well as coronal level with highest at coronal level [Figure 2]. On comparing the percentage of sealer penetration at apical and coronal level, significant differences were recorded in between the Groups 1&3, 1&4, 2&5 at coronal level and 1&5 at both coronal and apical levels. On comparing the percentage of sealer distribution at coronal and apical levels, insignificant differences exist in Group 2&3 and 4&5 at both levels. Ultrasonic group showed maximum penetration, followed by sonic group and reciprocating group. On comparing different groups for sealer distribution, ultrasonic showed maximum distribution followed by sonic and reciprocating spreader with insignificant difference between the later. Master apical guttapercha showed least penetration as well as percentage sealer distribution.



ure 2

Figure 2: Photographs of Apical(A) and Coronal (B) segments showing sealer penetration and distribution in different groups: 1 – Ultrasonic group, 2 – Sonic group, 3 – Reciprocating NiTi group, 4 – Lentulospiral group, and 5 – Master apical guttapercha group.



Mean and standard deviation of depth and percentage of sealer penetration of various groups

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	Table 1: Mean and standard deviation of depth and percentage of sealer penetration of various groups.								
G		Depth of sealer penetration(µm)				Percentage of sealer penetration			
	Groups	Apical		Coronal		Apical		Coronal	
		Mean	SD	Mean	SD	Mean	SD	Mean	Г

	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group 1	248.2	140.74	471.2	202.51	74.8	11.15	83.5	9.21
Group 2	211.6	63.49	357.5	91.73	56.1	4.21	70.9	3.57
Group 3	143.7	33.32	240.2	68.94	54.0	4.51	67.2	4.38
Group 4	128.9	35.28	195.3	26.08	37.1	7.81	49.3	6.01
Group 5	107.2	41.44	147.5	28.16	29.9	2.48	48.0	5.64
P-value\$	0.015*		< 0.001*		< 0.001*		< 0.001*	

 Table 2: Multiple comparison among various groups based on depth of sealer penetration and percentage of sealer penetration at different levels

	Depth of sealer penetration				Percentage of sealer penetration			
Comparison	Apical		Coronal		Apical		Coronal	
	P-value@	Sig.	P-value@	Sig.	P-value@	Sig.	P-value@	Sig.
1 vs 2	0.913	NS	0.360	NS	0.001	S	0.011	S
1 vs 3	0.142	NS	0.007	S	< 0.001	S	0.001	S
1 vs 4	0.072	NS	0.001	S	< 0.001	S	< 0.001	S
1 vs 5	0.024	S	< 0.001	S	< 0.001	S	< 0.001	S
2 vs 3	0.526	NS	0.331	NS	0.983	NS	0.831	NS
2 vs 4	0.334	NS	0.089	NS	< 0.001	S	< 0.001	S
2 vs 5	0.143	NS	0.016	S	< 0.001	S	< 0.001	S
3 vs 4	0.997	NS	0.945	NS	0.002	S	< 0.001	S
3 vs 5	0.914	NS	0.559	NS	< 0.001	S	< 0.001	S
4 vs 5	0.986	NS	0.933	NS	0.374	NS	0.995	NS

DISCUSSION

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It is desirable to bond core material to dentin walls by means of a sealer. Hoen et al suggested that the sealer placement into the root canal system should be done in a manner that completely covers the dentinal walls.^[14] The penetration of the root canal sealers into the dentine tubules is desirable because it will improve the retention of the material, decrease micro leakage and may exert antibacterial effect.^[5,9] The penetration ability of the sealers depends on several factors including: smear layer removal, number and size of the dentine tubules, flow properties of the root canal sealer and filling technique.^[15]

The present study was undertaken to evaluate and compare the effect of four different sealer placement techniques with a novel reciprocating motion handpiece using NiTi spreader for sealer placement. Most of the studies that evaluated the sealer penetration and distribution employed traditional high-vacuum scanning electron microscopy. SEM has been used by a number of investigators to evaluate the penetration of sealer cements into dentinal tubules,^[5,7,16,17] & digital microscope.^[18,19] However, in the present study, the use of the CLSM model allowed for a full cross-sectional observation, which clearly showed the amount of labelled sealer inside the dentin. Confocal microscopy allowed visualization of the sealers within the dentinal tubules without resorting to specimen preparation techniques that would have caused artefacts.^[20] Scanning confocal microscopy offers improved rejection of out-of-focus noise and provides greater resolution than conventional imaging, yielding greatly enhanced images of biological structures. Therefore new studies use $\ensuremath{\text{CLSM}}.^{[6,21]}$

The chosen sealer was AH Plus, nowadays strongly recommended for its low solubility, long-term dimensional stability, relatively long working time, adhesive properties, and excellent physicochemical and biological properties.^[11] Resin sealers are known to have adequate flow and deeper penetration owing to their thin film structure. The thin film can penetrate greater when lateral condensation obturation technique is used.^[11] Kokkas et al also found that the epoxy resin sealer AH-Plus displayed deeper penetration than the zinc oxide– eugenol based sealer Roth 811.^[22]

The use of fluorescent dyes in microscopy is a powerful investigative tool. By far, Rhodamine B is the most frequently used fluorochrome for different applications. This compound is excited using green light (540 nm) and emits red in color (590 nm). We used Rhodamine B as it is effective in very low concentration, fairly labile, moves freely across the bonded interface, and is easily detected microscopically with appropriate filters.^[23]

The results of this study indicate that all five methods of sealer placement may not consistently and completely cover dentin walls. Although sealer was present in the majority of the areas examined, apical area demonstrated significantly less sealer coverage than coronal level. In all groups, the percentage of sealer penetration into dentinal tubules in the coronal was significantly greater than in the apical except for sonic and reciprocating spreader and between rotary and master apical gutta percha. The greater penetration of the sealer at the coronal thirds might be related to the greater lateral compaction during obturation and the limited action of EDTA at the apex of the root canal as verified by other researchers.^[24] This could be because of the fact that a superior removal of the smear layer in the coronal and middle levels and the ineffective delivery of irrigant to the apical region of the canal occurs.^[25] Another factor may be that the apical level contains less tubules, and when present, the diameter is smaller or they are more frequently closed.^[26] Overall ultrasonic group showed better percentage of sealer penetration than the Endoactivator group. Significantly better percentage of sealer penetration and depth of sealer penetration observed in ultrasonic group, substantiate the findings of previous studies.^[12,14,24,27] The ultrasonic energy apparently propels the relatively viscous sealer along the length of the file to the appropriate depth and laterally into numerous canal aberrations. Kahn et al found lentulospiral and the Max-i-Probe Delivery System to be the most effective methods of sealer placement along the canal walls, followed by ultrasonic and sonic files.^[28] But in this study the internal aspects of the sealer-coated canal was examined using a stereomicroscope. It considered the sealer distribution along the dentinal walls and did not check the sealer penetration into the dentinal tubules.

CONCLUSION

Within the limitations of this study, it can be concluded that ultrasonic activation results in better sealer penetration than the sonic activation group and reciprocating spreader group. Whereas, sonic activation and reciprocating spreader application showed better distribution and adaptation than the lentulospiral application and master apical gutta percha both at apical and coronal levels.

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