ORIGINAL RESEARCH

Comparative Evaluation of the Depth of Penetration and Persistence of Sealer Residues in Retreated Dentinal Tubules

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Abstract

Aim: To compare the depth of penetration and persistence of sealer residues in obturated canals with three commercially available root canal sealers using confocal laser scanning microscopy (CLSM).

Methodology: Thirty single-rooted human premolars were selected and decoronated with diamond disks to standardize the root length at 14 mm. Access cavity and working length were determined; following which biomechanical preparation was carried out using rotary files in a crown-down manner. Copious irrigation was carried out during recapitulation so as to effectively debride the canal. Subsequently, the canals were dried and obturated using lateral condensation technique with gutta-percha (GP) sticks, coated with three categories of dye-incorporated sealers, that is, AH Plus, Sealapex, and BioRoot RCS. The teeth were coronally sealed and allowed to set for 2 weeks. All samples were reattended, sectioned 6 mm from the apex, and evaluated by confocal laser scanning microscope.

Results: AH Plus sealer showed the highest depth of penetration followed by BioRoot RCS and Sealapex, respectively.

Conclusion: The depth of penetration of sealers plays a pivotal role in the outcome of endodontic treatment. It is virtually impossible to remove the sealer residue of the contracted dentin tubules. However, complete removal is not an essential factor in follow-up treatment in endodontics.

Clinical significance: The study provides insights into selection of appropriate sealer to achieve optimal penetration and retrievability clinically.

Keywords: AH Plus, BioRoot RCS, Confocal laser scanning microscopy, Depth of penetration, Sealapex.

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Introduction

The intent of root canal fillings is to avert reinfection of the disinfected canal space. This is accomplished primarily by ensuring a suitable barrier against bacterial invasion and the toxins that accompany it.[1] Chemomechanical preparation of root canals has fundamental importance for successful clinical therapy.[2] Root filling materials provide a mechanical barrier for the isolation of necrotic tissue or bacteria responsible for the persistence of periapical inflammation or postoperative pain, by eliminating all avenues of leakage from the oral cavity and the periradicular tissues into the root canal system by creating a fluid-tight seal.[3,4]

An important parameter in the evaluation of new sealers is the depth of penetration into the root dentin.[5,6] This increases the interface between the material and dentin, and mechanical interlocks improve the sealing and confinement of the material.[7] Sealers are presently categorized as zinc oxide eugenol sealers, sealers including calcium hydroxide, resin-based, glass ionomer-based, silicone-based, and bioceramic sealers, based on their chemical components.[8] The sealers used in our study are AH Plus sealer (resin-based sealer), BioRoot RCS sealer (tricalcium silicate-based sealer), and Sealapex (non-eugenol calcium hydroxide-based sealer) which differ in their material characteristics such as flowability, film thickness, and setting time.

Nonsurgical retreatment is the principal treatment of choice to manage post-treatment disease. Retreatment entails removing existing obturation material from the root canal system in order to clean it and create an environment conducive to periradicular healing. The obturation material must be completely removed to improve the chances of success. The sealer/dentin interface can be evaluated using a stereomicroscope, scanning electron microscopy (SEM), transmission electron microscopy, and CLSM, among other techniques. Confocal laser scanning microscopy has several advantages, including the ability to use undecalcified or hard tissue specimens without the need for a special surface coating; and exclusion of artifacts by using fluorescent rhodamine dyes to provide accurate information at low magnification.[7]

Major causes of post-treatment failures are persistent or secondary infections. Nonsurgical endodontic retreatment is indicated in such scenarios where initial procedures have failed and it can be rectified by improving root canal disinfection, debridement, and placing suitable root filling material with a sealer.[9] The primary goal of retreatment is to re-establish health of the periapical tissues.[10] To achieve this goal complete removal of filling material from inadequately instrumented and obturated canals is imperative. Retreatment procedure is time-consuming and challenging, hence this procedure has a major clinical impact as the instruments used for retreatment should reach the entire canal system for promoting

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better cleaning and disinfection.[9] Root canal retreatment is regarded as a predictable solution for cases with post-treatment disease. Once the existing root filling material is removed, apical patency and an adequate working length will be re-established. And the revised cleaning, shaping, and obturation procedures are associated with a positive outcome in most retreatment cases.[10] Several methods have been used to remove root canal filling material and regain apical patency; use of hand files, rotary systems specifically developed for retreatment, and new reciprocating motion approach.[11] Regaining apical patency is considered to be an essential factor for a positive outcome following nonsurgical endodontic retreatment, because of the potential of this critical apical region to harbor a higher concentration of debris and bacteria. In most studies conducted to assess filling material removal from root canal walls using tomography, radiography, or tooth sectioning, the apical zone was the one with the least cleaning results.[6]

BioRoot RCS sealers has not been extensively studied/compared with the existing sealers such as AH Plus and Sealpex. Hence, this study aims to compare and evaluate three categories of commercially available root canal sealers based on their depth of penetration and persistence of sealer residues in root canals obturated with GP, using CLSM.

Methodology

Thirty single-rooted human premolars, extracted as part of orthodontic management, were collected on appropriate consent from the institutional ethics committee (Protocol No.: YEC2/195). Sample size, that is, 10 in each group was calculated considering a margin of error of 5% and a confidence level of 95%. The collected teeth were cleaned immediately after extraction by removing all attached hard and soft tissues and immersing them in 250 mL of 5.25% sodium hypochlorite (NaOCl) for 24 hours. The teeth were then stored at room temperature in a container with a lid containing 0.9% sterile saline until further processing. The crowns of the teeth were then decoronated with a diamond disk under water coolant until they reached a specified root length of 14 mm. All prepared teeth were again held in 0.9% sterile saline at room temperature until the testing period.

Evaluation

For the root canal treatment, X-Smart Endomotor (Dentsply Maillefer, Ballaigues, Switzerland) and ProTaper Gold rotary file system (Dentsply Maillefer, Ballaigues, Switzerland) with the crown-down technique were used. A #10-K file was used to establish the working length at 0.5 mm from the apical foramen. The root canals were then instrumented with ProTaper Gold until an F3 (30/09) instrument. The speed and torque values were set according to the manufacturer's recommendations. Recapitulation and irrigation with 1 mL of 2.5% NaOCl were done and activated with EndoActivator (Dentsply) so as to efficiently abolish the smear layer. The irrigation protocol culminated with 3 mL of distilled water, prior to which the canals were irrigated for 3 minutes with 3 mL of 2.5% NaOCl and 17% ethylenediaminetetraacetic acid. Finally, the canals were dried with paper points and the specimens were randomly divided into three groups. The sealers were blended according to the directions provided by the manufacturer. Rhodamine B fluorescent dye (Sigma-Aldrich, St. Louis, Missouri, USA) was added to the sealers in a 0.1% proportion. The sealers were placed inside the root canals using a lentulo spiral. F3-sized single cones were used to obturate the canals of each group coated with the respective sealers marked with Rhodamine B dye. A round carbide bur was used in a slow-speed handpiece without water to shear off excess cone in each root at the cemento-enamel junction level. There were no additional cones utilized. The access cavities were sealed with temporary restorative material (Cavit G, 3M ESPE, St. Paul, Minnesota, USA). To completely cure the sealant, the teeth were protected at 100% humidity at 37°C for 14 days.

Division of Groups

- Group I: root canals sealed with AH Plus and filled with GP cone.
- Group II: root canals sealed with BioRoot RCS and filled with GP cone.
- Group III: root canals sealed with Sealapex and filled with GP cone.

Retreatment

The GP from coronal, middle, and apical thirds were removed using ProTaper Universal retreatment files, D1, D2, and D3, respectively. Irrigation with 3.25% NaOCl between filings was done. Each sample was sectioned horizontally 6 mm from the apex into 2 mm thick slices using a diamond disk with continuous water flow. The surface was next polished under running water with sandpaper number 600 to remove any debris leftover from the cutting procedure.

Image Analysis Using Confocal Laser Scanning Microscope

Samples were analyzed with a Zeiss Pascal laser scanning microscope (Carl Zeiss, Göttingen, Germany) having an argon mixed gas laser as its light source with the greatest wavelength of excitation being 543 nm. Confocal laser scanning microscopy images were documented in fluorescent mode. The detected light was conducted through a 560-nm long-pass filter. Images were recorded at 10× numerical aperture 0.3 and 40× aperture 1.25, 0.7 zoom, and oil immersion. The volume of the 10× images documented was 1302 m^2, and the resolution was 1736 pixels. The volume of the 40× images documented was 83.356 m^2, and the resolution was 2048 pixels. For each 10× sample, a uniform fluorescent ring was examined around the canal surface to show the distribution of the sealers. Using a linear measurement, the highest depth of penetration of the root canal sealers was reported for each sample in that group. Penetration of the sealer into the dentin tubules was measured and recorded to the maximum depth of each tooth segment in each group.

Statistical Analysis

All the data were collected and analyzed using SPSS version 23.0 and ImageJ software. The level of significance was set at $p < 0.05$. Frequencies and percentages were used for describing categorical variable. One-way analysis of variance (ANOVA) was used for comparison of three groups (Table 1). Tukey's honestly significant difference (HSD) was used for within-group comparison (Table 2). Kruskal–Wallis test was used for comparison of mean percentage of residual sealer for three groups (Table 3).

The depth of sealer penetration into the dentinal tubules was deemed an important prerequisite of a favorable sealer in this study. Confocal laser scanning microscopy was used along with Rhodamine B fluorescent dye to visualize the specimens. Depth of penetration was measured in microns (μm) from the wall of the root canal up to the maximum depth the sealer penetrated into the dentine (Fig. 1).

Results

Comparison of the sealer penetration depth (µm) of three groups using ANOVA revealed statistically significant results. Group I (AH Plus sealer) showed the longest penetration depth of 1213.53 µm followed by group II (BioRoot RCS Sealer) and group III (Sealapex Sealer) with a mean value of 959.93 µm and 869.29 µm, respectively. To justify the results, Figure 1A (AH Plus sealer) shows the largest dentin penetration followed by Figure 1B (BioRoot RCS sealer) and Figure 1C (Sealapex sealer), respectively. Further, Tukey's HSD test showed that there were statistically significant differences within the three groups ($p < 0.05$). Mean penetration depth (µm) of the Sealapex group with AH Plus group showed significant differences. So did the AH Plus group with BioRoot RCS group. However, there were no significant differences between Sealapex with BioRoot RCS groups.

Table 1: Comparison of sealer penetration depth (µm) using ANOVA

Sealers	*Mean (standard deviation)*	*F*	*p-value*	*95% confidence interval*
Sealapex	869.29 (242.35)	9.473	0.001*	683.01–1055.58
AH Plus	1213.53 (75.81)			1155.25–1271.81
BioRoot RCS	959.93 (223.52)			835.37–1084.49

*$p < 0.05$ is considered as significant

Table 2: Multiple comparison of sealer penetration depth (µm) within groups using Tukey's HSD

Sealers	*p-value**	*95% confidence interval*
Sealapex with AH Plus	0.001	–548.97 to –139.49
Sealapex with BioRoot RCS	0.52	–295.38 to 114.10
AH Plus with BioRoot RCS	0.013	48.85–458.34

*$p < 0.05$ is considered as significant

Table 3: Mean % of residual sealer among three groups

Sealapex	*AH Plus*	*BioRoot RCS*
47.45%	76.42%	55.72%

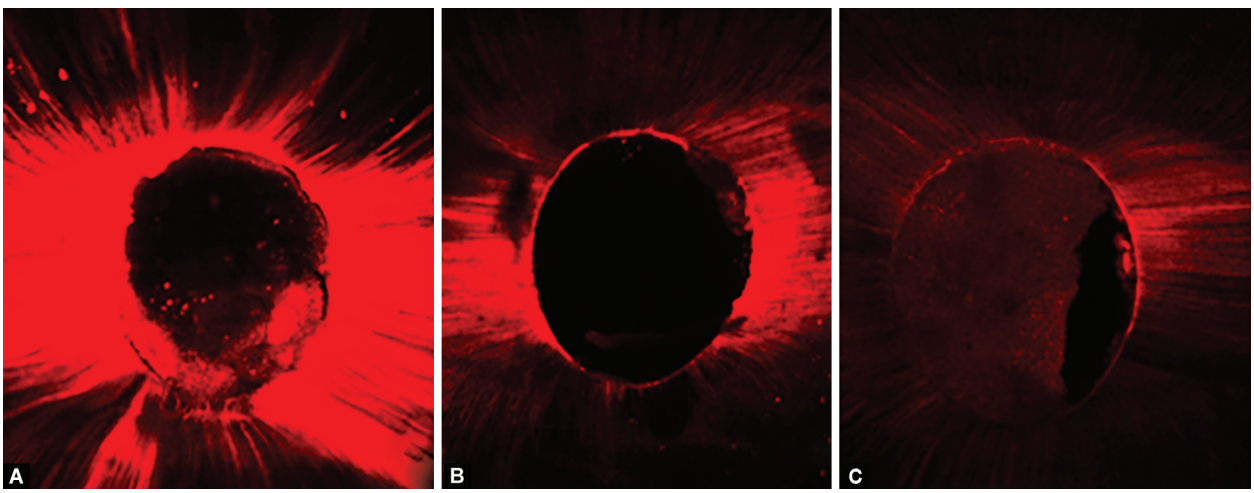
Kruskal–Wallis test revealed that although there were differences in the residual sealer remnants in retreated dentinal tubules among the three sealer groups, that is, BioRoot RCS showed better retrievability than AH Plus and Sealapex. However, they were not statistically significant as the sealers could not be completely retrieved.

Discussion

One of the major concerns for the clinicians is the level of safety of the instruments during root filling removal procedure. So, the instruments should have the ability to remove filling material effectively,[11] at the same time with less fracture. When used for retreatment, nickel–titanium rotary systems were effective in removing filling material faster than hand files. Rotary and reciprocating techniques are considered time-saving and it causes less fatigue to the clinician as well as less discomfort to the patient.[12] Statistical analysis confirmed that with regard to time taken; reciprocating instrumentation was more efficient than rotary instruments in extracting the obturating material swiftly and restoring apical patency, therefore in this study ProTaper Universal retreatment system (D1, D2, and D3) was adopted. On the contrary, they must be used with caution as they remove more dentin which could adversely weaken the root subjecting it to radicular fractures or perforations than manual systems.[13] However, studies regarding retreatment have invariably reported that none of the systems effectively remove all remnants from the root canal space.[12] Therefore, retreatment procedure remains a challenge to endodontists even with the aid of magnification and ultrasonics.

Many new sealers have been introduced in the market. Interestingly, an increasing use of bioactive materials in endodontics is noted, for a wide variety of applications ranging from pulp capping to apexification, including root canal sealing. However, the retreatability of some of these sealers is still unknown. Various approaches have been expressed for extracting obturated GP, that is, rotary instruments, manual hand files, solvents, and combinations thereof. However, several researches have proved that regardless of the technique, nature of instrument (i.e., manual or rotary system), and the application of solvents, it is inaccessible to abolish the obturating material from root canal walls and isthmus[14]; this is in agreement with the present study.

Penetration of a root canal sealer into dentinal tubules is a desirable property because it can provide a mechanical interlocking between the sealer and root canal dentin. Tubule penetration of a



Figs 1A to C: Confocal laser scanning images depicting the sealer penetration. (A) AH Plus sealer; (B) BioRoot RCS sealer; (C) Sealapex sealer

material depends on the diameter of dentinal tubules and physical and chemical properties of the sealer, such as solubility, viscosity, and surface tension. In radicular dentin, the dentinal tubules are wide enough to allow for penetration of endodontic sealers. The depth of sealer penetration differs at the cervical, middle, and apical levels of the root, according to studies.[15] This study focused on single-rooted teeth based on previous studies and the fact that we wanted to demonstrate differences in less complex anatomy. It is a possibility that more complex anatomy like mesial roots of mandibular molars could demonstrate even more significant differences between the sealer groups.[16] The sealers penetrate the dentinal tubules due to capillary pressure and that justifies why AH Plus with longer setting time of 8 hours showed deeper penetration than BioRoot RCS and Sealapex, that is, setting time of 4 hours and 45 minutes, respectively. Secondly, Dash et al. proved AH Plus exhibits less film thickness and better flow than Sealapex which is in accordance with our study.[17] Many researches have been conducted on AH Plus owing it to be a top-notch sealer. However, it proved efficacious against calcium hydroxide-based/resin-based sealers[2,4,5] and gave varying results when compared to BioCeramic sealers regarding its depth of penetration and retrievability[18]; as constant evolution is taking place with BioCeramic sealers. Kim et al. proved AH Plus showed better dentin penetration than BioCeramic sealers; however their retreatment retrievability was the same.[8] This was in favor with our study regarding the depth of penetration; however BioRoot RCS (BioCeramic sealer) showed better retrievability than AH Plus.

The presence of root canal sealers within dentinal tubules has been validated using an array of microscopic approaches, including light microscopy, SEM, and CLSM. Confocal laser scanning microscopy was employed instead of SEM in this investigation because it provides fluorescent detection of sealer penetration along the canal circumference of each sample. Confocal laser scanning microscopy displays the sealer penetration within the dentinal tubules and increased sealer visualization at various depths by forming high contrast spots.[5] It has various advantages over conventional widefield optical microscopy and SEM, including the capacity to regulate the depth of field, reduce background information distant from the focus plane, and build several optical sections, even from thick specimens.

The sealer must be labeled with a fluorescent dye when utilizing CLSM for evaluation. Rhodamine B dye was chosen as an indication in this study because it enables the detection of sealers within the dentinal tubules and has no effect on the sealers' physical qualities when only a little amount of dye (less than 0.2%) is mixed with the sealers. The Rhodamine B dye (powder form) obtained for the study was mixed with the sealer, that is, 0.1% or approximately 3 grains; which is unlikely to affect the setting characteristics of the sealer.[18] Literature indicates that the retrievability of various types of root canal sealers from retreated dentinal tubules found no significant differences among the different sealers, or between the techniques used. No group showed complete removal of the filling material, with greatest leftover in the apical third.[8,19,20]

The prime purpose of sealer application is to achieve a uniform obturation and a fluid-tight seal at the apical foramen. To prevent ingress of bacteria coronally, an intact coronal seal is essential.[21] The complete retrievability of the sealers was not possible due to the varying depths of sealer penetration in our study, that is, AH Plus was the most difficult to retrieve in comparison to BioRoot RCS and Sealapex. Sealer permeability into the dentinal tubules proved to be efficacious during endodontic treatment than in retreatment cases[13,16]; concluding that total removal of the root canal sealer is not an essential factor in endodontic retreatment, and not all sealer materials have negative effects during retreatment.

There are certain limitations of the study. The study is an *in vitro* analysis and the results may not be directly extrapolated to clinical oral conditions. The experiment was performed using three commercial brands of sealers. The results may vary with other brands not tested. Future studies must focus on studying brands other than the ones used in our experiment to establish the standard for direct comparison. *In vivo* long-term studies may be beneficial in obtaining direct clinical relevance.

Conclusion

The intention of root canal treatment is to completely eliminate the root canal system from pathogenic organisms, and seal the space with GP. To prevent bacteria from entering the channels between the GP and the root dentine, root canal sealers are inserted in the canals between the GP and the root dentine. The sealing ability of the sealers is considered an integral part in the outcome of root canal treatment. Therefore, within the limitations of this study, AH Plus sealer showed the highest depth of penetration followed by BioRoot RCS and Sealapex sealers, respectively. On the contrary, AH Plus was difficult to retrieve followed by BioRoot RCS and Sealapex, respectively. Ideal root canal sealers must have good penetration depths but easily retrievable at the same time. Novel BioRoot RCS sealer may be a good balance between the two.

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