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Original Research

An *In Vitro* Scanning Electron Microscopy Study to Evaluate the Dentinal Tubular Penetration Depth of Three Root Canal Sealers

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***Abstract*:**

Background: It is difficult to identify a potential sealant with penetration depth until practically analyzed. A scanning electron microscopy (SEM) overview could better describe the internal picture of the materials sealed to the tooth surface on *in vitro* experimentation since it being a vital procedure for successful obturation. Hence, this study was undertaken to compare the penetration depth of three root canal sealers most commonly available *viz.,* AH Plus® (Dentsply, New Delhi, India), Tubli-Seal™ (Kerr Dental, New Delhi, India), and Apexit® Plus (Ivoclar Vivadent, New Delhi, India) with different compositions using SEM.

Materials and Methods: A total of 30 single-rooted mandibular premolars decoronated and the canal preparation done by step back technique was used for this study. 17% of ethylenediaminetetraacetic acid (EDTA) used as final flush. Prepared specimens were divided into three groups of 10 teeth each, and different sealers were used for each group (zinc oxide eugenol-based - Tubli-Seal™, calcium hydroxide-based - Apexit® Plus, and resin-based sealer - AH Plus®). After obturation, teeth were split longitudinally and viewed under SEM.

Results: There was no statistically significant difference among the means of measured depth of penetration of AH Plus® and Apexit® Plus sealer. However, Tubli-Seal™ values projected statistically significant differences in comparison to AH Plus® and Apexit® Plus sealer. Means of measured depth of penetration of different sealers were subjected to one-way ANOVA and Duncan's multiple range test with a statistical significance at < 0.001.

Conclusion: Zinc oxide eugenol-based sealer (Tubli-Seal™) shows less depth of penetration as compared to the calcium hydroxide-based sealer (Apexit® Plus) and resin-based sealer (AH Plus®).

Key Words: Canal sealer, electron microscopy, root canal, root canal filling materials, sealants

Introduction

The root canal therapy success lay with the instrumentation, disinfection, and three-dimensional obturation of root canal system. Obturation plays a vital role of all the factors contributing to the success of therapy. Sealers have become an integral part of obturation process which improves the quality of obturation. The ability of root canal sealers to adhere to dentin and gutta-percha results in superior sealing ability, which in turn reduces microleakage and improves the quality of obturation.[1] Deeper the penetration of the sealer into the dentinal tubule better the sealing achieved. Thereby the retention of material is enhanced by mechanical locking.

The smear layer prevents penetration of root canal sealer into the dentinal tubules.[2-5] Thus, removing the smear layer would increase the chances of sealer penetration into the dentinal tubules. The penetration of the sealer into the dentinal tubule is of increasing clinical importance since there is no chemical adhesion between dentin and different types of sealers. It has been suggested that a mechanical interlock for lack of chemo-adhesion through dentinal tubular penetration may be the solution. However, penetration mainly depends on the rheological properties and setting time of the sealer material used for obturation.

A large variety of sealer materials are available within the fraternity with all proposed best properties of sealing. It becomes difficult to identify a potential sealant with penetration depth until practically analyzed. A scanning electron microscopy (SEM) overview could better describe the internal picture of the materials sealed to the tooth surface on *in vitro* experimentation.

Hence, this study was undertaken to compare the penetration depth of three root canal sealers most commonly available *viz.*, AH Plus® (Dentsply, New Delhi, India), Tubli-Seal™ (Kerr Dental, New Delhi, India), and Apexit® Plus (Ivoclar Vivadent, New Delhi, India) with different compositions using SEM.

Materials and Methods

About 30 caries free single-rooted human mandibular premolars freshly extracted for the therapeutic purpose were used for this study. Teeth with similar root canal anatomy were selected with the help of radiograph. Those specimens selected were stored in saline at room temperature. De-coronated at the cement-enamel junction using diamond discs to facilitate instrumentation. Those specimens in which #20 K file was binding at the apex and just visible at the apical foramen were only selected, and the rest got replaced with new specimens.

After removal of the remnant pulp tissue with a barbed broach, a #20 K-file (Dentsply, Maillefer, New Delhi, India) was inserted into each root canal until it could be seen through the apical foramen. A rubber stopper was then adjusted. The working length was established by reducing from this length by 0.5 mm. The apical third of the external surface of all the roots was sealed with sticky wax to prevent irrigants from passing through the apical foramen.

Canal preparation was done by step back technique using stainless steel K-files (Dentsply, Maillefer, New Delhi, India) and apex enlarged to file size #35. Inter-instrument irrigation was done using 3% NaOCL (Prime Dental, Mumbai, India). At the end of the preparation, all the canals were irrigated with 3 ml of 17% ethylenediaminetetraacetic acid (EDTA) (Dentwash, Prime Dental, Mumbai, India) for 3 min followed 3 ml of 1% NaOCl (Prime Dental, Mumbai, India) solution for 3 min. Then, the specimens were randomly divided into three groups of 10 each (Groups A-C).

Using a calibrated insulin syringe, 0.25 ml of sealer was coated onto hand lentulo spiral (Dentsply, New Delhi, India). The sealer with lentulo spiral applied manually, 10 rotations for each application in an anti-clockwise direction. AH Plus® sealer was used for group A, Tubli-Seal™ sealer was applied for Group B and Apexit® Plus sealer was applied to Group C. Master cone with a thin film of sealer was gently seated to the established working length. Cold lateral condensation was done in each canal with the help of endodontic finger spreaders. Accessory cones were used to fill the remaining part of the canal.

Obturated specimens were stored in an incubator at 37°C and 100% humidity for 15 days to allow the sealer to set. All the roots were sectioned longitudinally, after making a groove with diamond discs and using a chisel and mallet. The sectioned specimens were then mounted onto aluminum stabs, coded and then coated with a thin layer of gold film.

The specimens were then viewed through a SEM (Figures 1-3) the focus of observation was the interface between the dentin and the obturation. Three different sites on the middle third of the root were observed and the maximum penetration depth of the sealer into the dentinal tubules was measured at these sites and tabulated. Tabulated data were statistically analyzed by one-way Analysis of Variance (ANOVA) and Duncan's multiple range test with the help of SPSS software version 16 (IBM Inc., Chicago, USA). Energy dispersive spectroscopic analysis was done in the selected areas to confirm the sealer in the canal and also with sealer inside the dentinal tubules.

Results

The measured depth of penetration of different sealers was compared using one-way ANOVA. Duncan's multiple range test was performed along with ANOVA with statistical significance at <0.001. Duncan's multiple range test showed that there is no statistically significant difference in groups given by superscript b. There is statistically significant difference between groups given by superscript a and b (Table 1 and Graph 1).

Means of measured depth of penetration of different sealers were subjected to one-way ANOVA and Duncan's multiple

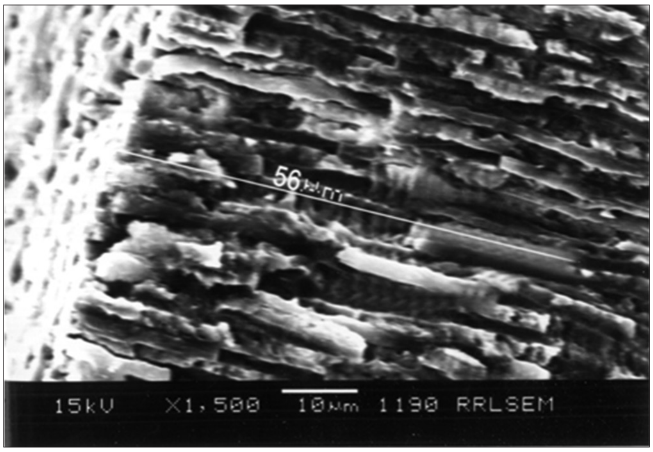


Figure 1: Scanning electron photomicrograph of AH Plus® penetration in the dentinal tubule.

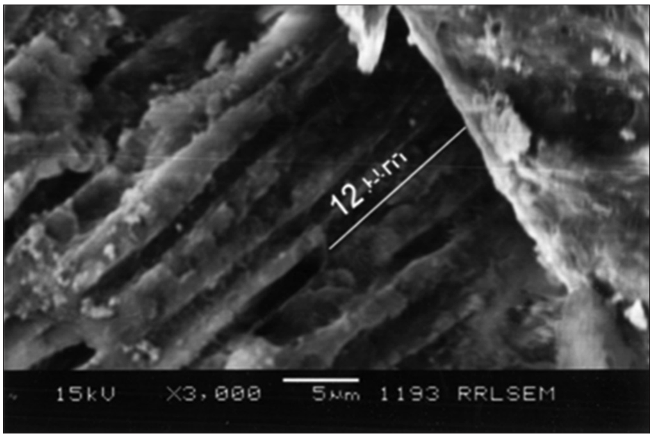


Figure 2: Scanning electron photomicrograph of Apexit® Plus penetration in the dentinal tubule.

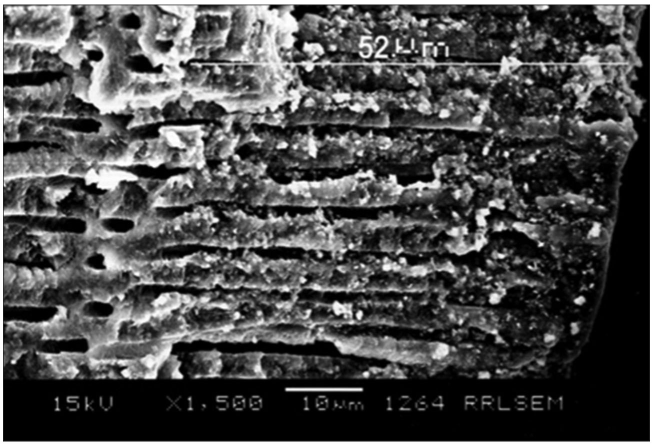
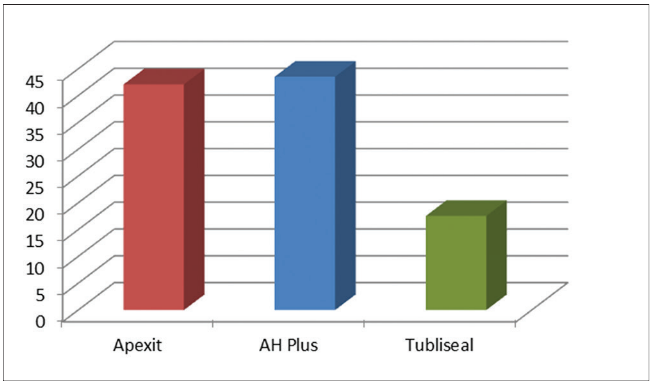


Figure 3: Scanning electron photomicrograph of Tubli-Seal™ penetration in the dentinal.

Table 1: One-way ANOVA comparing different materials dentinal tubular penetration depths.

Material	Mean±SD	*F* value	*P* value
AH Plus	43.53^{b}±7.0408	289.182	<0.001
Tubli-seal	17.56^{a}±4.1041		
Apexit	42.09^{b}±5.7518		

[a,b]Means with same superscript do not significantly differ each other (Duncan's multiple range test), SD: Standard deviation



Graph 1: Mean comparing different materials penetration depths.

range test. Means calculated for Tubli-Seal™ were the least as compared to the other two groups. The standard deviation did not vary much among the three groups defining adequate dispersion of the data along the central value. There was no statistically significant difference among the means of measured depth of penetration of AH Plus® and Apexit® Plus sealer. However, Tubli-Seal™ values projected statistically significant differences in comparison to AH Plus® and Apexit® Plus sealer.

Discussion

Biomechanical preparation is significant for successful endodontic treatment outcome.[6-9] However, this does not negate the importance of the quality of the obturation in which the sealer has a role to play. The primary objective of obturation is to create a fluid impervious seal over the entire length of the root canal system. Gutta-percha alone will not seal the canal, as it has no adherence to the dentin thus a sealer is required to fill the space between the gutta-percha and the canal wall.

Sealers can be grouped based on their primary constituents, such as zinc oxide eugenol, calcium hydroxide, and resins. In this study, we have used a zinc oxide eugenol-based sealer (Tubli-Seal™), a calcium hydroxide-based sealer (Apexit® Plus), and an epoxy resin-based sealer (AH Plus®).

Zinc oxide eugenol-based sealers have been traditionally the most common employed sealers. They have served as a benchmark with which other sealers are in endodontics, as they reasonably meet most of the Grossman requirements.[10] Tubli-Seal™ is a paste-paste system that consists of a base paste that is primarily composed of zinc oxide with bismuth oxide and an accelerator paste that consists of oleoresins and eugenol. The antimicrobial property of Tubli-Seal™ is due to the action of eugenol, which is a phenolic compound that acts on microorganisms using protein denaturation.

Anders *et al.* found that for calcium hydroxide sealer to be an effective antimicrobial agent, it should maintain a pH level <12.5.[11] As the calcium hydroxide sealer sets, the pH declines to approximately 9.14, causing it to lose effectiveness.

Epoxy resin-based sealers were introduced because of its advantages such as radio-opacity, low solubility and less shrinkage. AH Plus® is an improved epoxy resin-based sealer.

As chemical adhesion between dentin and sealers cannot be achieved, it has been suggested that sealer plugs inside the dentinal tubules provide mechanical interlock, thus improving the retention of the filling material to the root canal walls.

Smear layer can be removed with the help of a chelating agent. EDTA, which reacts with calcium ions in dentine and forms soluble calcium chelates. EDTA decalcifies dentin to a depth of 20-30 µm in 5 min and the optimal working time in the root canal is of 15 min and no more chelating action could be expected after this period.[12-14]

The flow of sealer and low surface tension are desirable factors affecting the depth of penetration. Materials complying with these requirements are capable of wetting the root canal walls thus providing well-adapted fillings. It is also important for the material to set slowly and to flow far as long as possible.[15-18]

Group A (AH Plus®) is having a mean penetration depth of 43.53 µm (ranging from 35 µm to 61 µm). When comparing Group A (AH Plus®) with Group C (Apexit® Plus), even though Group A there is little higher value than the Group C (Apexit® Plus). There is no statistically significant difference between each other. A study by Kaplan *et al.* found that the flow of the epoxy resin-based sealer increases with time compared to calcium hydroxide-based sealer, which has maximum initial flow and there is no difference in flow with time.[19] Compared to Group B (Tubli-Seal™), Group A (AH Plus®) is having a greater depth of penetration, this may be attributed to the slow setting of Group A (AH Plus®) which is 4 h compared to Group B (Tubli-Seal™).

Group B (Tubli-Seal™) is having a mean penetration depth of 17.56 µm (Ranging from 12 µm-22 µm). The depth of penetration of Group B is less when compared to other groups. The value obtained is having a significant difference between other groups statistically. The depth of penetration of the Group B sealer (Tubli-Seal™) may be attributed to the fast setting of this sealer compared to the other sealers used in this study. The setting reaction is also very sensitive to moisture.

Group C (Apexit® Plus) is having a mean penetration depth of 42.09 μm (32 μm-54 μm). There is no statistically significant difference between Group A and Group C. There is a statistically significant difference between Group B and Group C. This may be attributed to working time of Apexit® Plus compared to Tubli-Seal™.

According to our study, zinc oxide eugenol-based sealer Tubli-Seal™ shows less depth of penetration compared to the calcium hydroxide-based sealer Apexit® Plus and resin-based sealer AH Plus®. It may be attributed to the fast setting reaction and less flow of Zinc oxide eugenol-based sealer. Sealers Apexit® Plus and AH Plus® showed an adequate penetration to the dentinal tubules.

Conclusion

Thus, it can be concluded that:

1. After the removal of the sealer, there is a considerable depth of penetration of all the three sealers into the dentinal tubules.
2. Apexit® Plus and AH Plus® shows no significant difference in the depth of penetration into the dentinal tubules.
3. Apexit® Plus and AH Plus® shows increased the depth of penetration into the dentinal tubules when compared to Tubli-Seal™.

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