

**Comparative analysis of the penetration of epoxy resin based sealer into dentinal tubules after calcium hydroxide and triple antibiotic paste dressing: An in-vitro study.**

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**Type of Publication:** Case Report

**Conflicts of Interest:** Nil

**Abstract**

**Context:** The penetration of sealer into dentinal tubules is considered to be an important criterion as sealers perform various biological functions for successful endodontic treatment.

**Aim:** To assess penetration of Epoxy resin-based sealer into dentinal tubules after Calcium hydroxide and Triple antibiotic paste dressing using Confocal laser scanning microscope.

**Settings and Design:** Quality improvement analysis

**Material and Methods:** After decoronating maxillary first molars (n=45) palatal roots were prepared using wave one gold rotary files upto 40 0.08. Obturation was done using AH Plus sealer and Rhodamine b Dye. Later palatal roots were randomized into 3 groups control

group (n=15), calcium hydroxide group and Triple antibiotic paste All root specimens were sectioned at 3, 5 and 9 mm from apex and analysed under CLSM.

Statistical analysis used: Data were analysed using ANOVA and Tukey’s post hoc test

**Results:** Control group exhibited better AH Plus sealer penetration as compared to experimental groups at coronal and middle third root levels.

**Conclusions:** On Confocal Laser Scanning Microscopic analysis control group samples exhibited better AH Plus sealer penetration as compared to experimental groups as root canal dressing residues decreased dentinal tubular penetration of tested epoxy resin-based sealer.

**Keywords:** AH Plus Sealer, CLSM, Palatal Roots, Penetration Depth.

## Introduction

A primary goal of successful endodontic therapy depends on complete obturation of the root canal<sup>1</sup>. It is well established that the sealer cement is an extremely important component of the root canal filling in order to achieve three-dimensional obturation of the root canal space. Current endodontic therapy utilizes a combination of gutta-percha cones and a sealer cement. The main function of root canal sealer is sealing off voids, multiple foramina, forming a bond between the core of the filling material and the root canal wall, and acting as a lubricant while facilitating the placement of the filling core material and entombing any remaining bacteria<sup>2-3</sup>.

Endodontic sealers are categorized based on composition, as follows: zinc oxide eugenol-based sealer, epoxy resin-based sealer, glass ionomer-based sealer, silicone-based sealer, tricalcium silicate-based sealer, and methacrylate-based resin sealer systems<sup>4</sup>. Epoxy resin-based sealers, such as AH 26 and AH Plus are composed of low molecular weight epoxy resins and amines and set by addition reaction between epoxide groups attached to epoxy resins and amines to form polymer<sup>5</sup>. In 1920 Hermann introduced, calcium hydroxide Ca (OH)<sub>2</sub> which has been widely used in endodontics<sup>13</sup>. Various biological properties of Ca (OH)<sub>2</sub>, such as antimicrobial activity, inhibition of tooth resorption, and hard tissue formation, have been investigated, and its wide use in root canal treatment has been associated with periradicular healing<sup>6</sup>.

There are many modes of delivery for calcium hydroxide into root canals however pastes with oily vehicles showed larger zones of inhibition than those with aqueous or viscous vehicles. The Presence of intracanal medicament Ca (OH)<sub>2</sub> on dentinal walls has the ability to compromise endodontic treatment if not removed

completely<sup>7</sup>. Persistence of CH residues may interfere with sealing ability of endodontic sealers<sup>21</sup>, and affect the adhesion of endodontic sealers to the canal walls<sup>8</sup>.

Triple antibiotic paste (TAP) was largely developed by Hoshino and colleagues<sup>9</sup>, introduced especially for the regeneration and revascularization protocol and the treatment of open apex teeth with necrotic pulp. Triple antibiotic paste is combination of Ciprofloxacin, Metronidazole, Minocycline in ratio of (1:1:1)<sup>27</sup>, and is mainly effective against gram-negative, gram-positive, and anaerobic odontogenic microorganisms<sup>10</sup>. There are different cleaning protocols available in literature for medicament and smear layer removal for better sealer penetration into dentinal tubules.

Thus aim of this study was primarily to assess penetration depth and penetration area of epoxy resin based sealers after calcium hydroxide and Triple antibiotic paste dressings using Rhodamine B dye using Confocal laser scanning microscope (CLSM).

## Material and Method

Preparation of samples – A total of 45 intact palatal roots of maxillary molars without caries, well developed and closed apices were selected. Teeth with curved roots, teeth with root resorption, instrumented canals, and calcified canals were excluded from the study. All the selected teeth were cleaned, polished with pumice and stored in distilled water. The crowns were decoronated close to the cemento-enamel junction with a diamond disc mounted in straight hand piece under constant water cooling. The palatal roots of maxillary molars were standardised to a length of 12 mm and were explored with a #10 K-file until the foramen for confirmation of patency. The root canals were instrumented with a Wave One Large file (40/ 0.08) using motor in reciprocating motion. Then, Irrigation was done with 5 mL of 5.25%

sodium hypochlorite between each instrumentation, and final irrigation was done with 10 mL of 5.25% sodium hypochlorite of 2 cycles for 30 seconds each, followed by 5 mL of EDTA 17% for 3 min and 10 mL of distilled water. The root canals were dried using wave one large paper points and then randomly distributed into three groups (n=15) each.

Group 1 – Calcium hydroxide group

Group 2 – Triple antibiotic Paste group

Group 3 – Control Group

In group 1 Root canals were filled with a paste of calcium hydroxide and propylene glycol by using a lentulospiral carrier and were compacted with a finger plugger. The roots were coronally sealed with temporary cement and kept at 37°C and 100% humidity for 15 days. In group 2 Root canals were filled with a paste of Triple antibiotic Paste (Ciprofloxacin, Minocycline and metronidazole) in 1:1:1 ratio by using a lentulospiral carrier and were compacted with a finger plugger. The roots were coronally sealed with temporary cement and kept at 37°C and 100% humidity for 15 days. In group 3 control group no root canal dressing was given. In experimental groups root canal dressing was removed and obturation was done. The AH Plus sealer was manipulated according to the manufacturer's instructions and was weighed using an analytical precision balance scale. Rhodamine B powder was weighed and mixed with the sealer to a proportion of 1: 100 (w/w). Then, the root canals were obturated with rhodamine-sealer mixture and Wave One Large gutta-percha cone by using the single cone technique. Then obturation was cut at the entrance of the root canal by using a thermal condenser followed by cold vertical compaction. The root canal entrance was cleaned with 70% alcohol and sealed with temporary cement. After obturation, the

roots were kept at 37°C and 100% humidity for 7 days before being transversely sectioned with a Diamond disc at 9, 6 and 3 mm from the apex. The resulting sections were polished and then observed at 4x magnification using confocal laser scanning microscope at 10 µm from the surface, with an absorption wavelength of 540 nm and emission of 590 nm. Image J Software (Version 1.53e; National Institute of Health, USA) was used to analyse confocal microscopic images.

### **Statistical analysis**

The statistical analysis was done using Statistical Package for the Social Sciences (SPSS for Windows, Version 19.0). Descriptive statistics were calculated as mean and standard deviation. Prior to analysis, the normality testing of data was done using Shapiro-Wilk test which showed that the data were normally distributed ( $P>0.05$ ). Thereafter, the comparison of study parameters among the study groups was done using Analysis of Variance (ANOVA). In case of statistical significance, Tukey's post-hoc test was used for multiple comparisons. The level of significance for the present study was fixed at a p-value of less than 0.05.

### **Results**

At 3 mm root level there was statistically significant difference between control group and calcium hydroxide group ( $p<0.001$ ) with mean penetration depth and penetration area in control group significantly higher than in calcium hydroxide and Triple antibiotic paste group. At 6 mm root level there was a statistically significant difference in penetration area between control group and calcium hydroxide group ( $P<0.001$ ). The mean penetration area in control group was significantly higher than that in calcium hydroxide group.

There was a statistically significant difference in penetration depth between control group and triple

antibiotic paste group ( $P=0.003$ ). The mean penetration depth in control group was significantly higher than that in triple antibiotic paste group. There was a statistically significant difference in penetration area and penetration depth between control group, calcium hydroxide group and Triple antibiotic paste groups ( $P<0.001$ ). The mean penetration area and depth in control group was significantly higher than that in both other groups.

### Discussion

A primary goal of successful endodontic therapy depends on complete obturation of the root canal<sup>1</sup>. Current endodontic therapy utilizes a combination of gutta-percha cones and a sealer cement. The gutta-percha cone blocks the apical foramen while the sealer cement is intended to seal around the gutta-percha point to prevent leakage and fill the canal space<sup>2</sup>. If the sealer does not perform its function, microleakage may cause non-surgical root canal treatment failure via clinically undetectable passage of bacteria, fluids, molecules or ions between the tooth and restorative material<sup>11</sup>.

Epoxy resin-based AH Plus sealer was used in this study as it has excellent physical properties such as longer setting time, low solubility and interfacial adaptation and also are related to covalent bonds between epoxide rings and the exposed amino groups in the collagen network<sup>12</sup>. Sonu, K. R. et al. compared sealer penetration depth with and without smear layer removal AH Plus sealer showed greater sealer penetration, this was due to the sealer integrity as well as the property of sealer being drawn into the tubules by capillary action<sup>13</sup>.

Calcium hydroxide used in this study as intracanal medicament it dissociates into calcium and hydroxyl ions on contact with an aqueous solution. There are different types of vehicles used in carrying Calcium hydroxide in root canals based on water soluble, oil

based and viscous substances based as different vehicles have different rate of action. Safavi & Nakayama<sup>14</sup> studied effects of glycerine and propylene glycol vehicles on the pH of Ca (OH)<sub>2</sub> preparations. A range of 10–30% for a glycerine/water mixture and 10–40% for a propylene glycol/water mixture resulted in the greatest conductivity.

Triple antibiotic paste (TAP) was largely developed by Hoshino and colleagues<sup>9</sup>, introduced especially for the regeneration and revascularization protocol. Fereshte et al. evaluated that TAP as intracanal medicament can not be completely removed from root canals and it changes chemical structure of root canal dentin. The smear layer plays an important role in root canal therapy because it affects the adaptation of filling materials to the root canal walls. Smear layer affects the penetration ability of the filling materials into the dentinal tubules because this might be of clinical importance.

In the current study, penetration area and penetration depth of AH plus sealer in the coronal thirds at 9mm level was significantly greater than the apical third. These findings were in association with study by Carrigen PJ et al.<sup>15</sup> as greater diameter and number of the dentinal tubules coronally, lesser tubule density apically. At middle third (6 mm) level sealer penetration was deeper than at apical level Saunders et al. and Vassiliadis et al. reported similar findings. These investigators explained that the diameter of canal orifices at the middle third of the root are larger than in apical third enough to get a clean surface after treatment with a chelating agent for removal of smear layer and dressings.

In experimental groups, there was no statistically significant difference in penetration area and penetration depth ( $P=0.253$ ) of resin-based sealer at apical third (3 mm) level these attributes to difficulty in removing all

the Ca (OH)<sub>2</sub> and Triple antibiotic paste dressing especially from the apical third of the root canal.

### Conclusion

Within Limitations of present study, it can be concluded that on Confocal Laser Scanning Microscopic analysis control group samples exhibited better AH Plus sealer penetration as compared to experimental groups as root canal dressing residues decreased dentinal tubular penetration of tested epoxy resin-based sealer.

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**Legend Tables and Figures**

Table 1: Carrigan PJ, Morse DR, Furst ML, Sinai IH. A scanning electron microscopic

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		P value
					Lower Bound	Upper Bound	
Control	5	434.6000	39.20842	17.53454	385.9163	483.2837	0.007*
Calcium Hydroxide	5	353.6000	40.50062	18.11243	303.3118	403.8882	
Triple Antibiotic Paste	5	420.6000	21.67487	9.69330	393.6871	447.5129	
Total	15	402.9333	48.79471	12.59874	375.9117	429.9549	

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Comparison of penetration depth at 9 mm

\*Statistically significant (P<0.05, Tukey’s post-hoc test)

Table 2: Comparison of penetration area at 9 mm

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		P value
					Lower Bound	Upper Bound	
Control	5	588.0000	39.17269	17.51856	539.3607	636.6393	<0.001*
Calcium Hydroxide	5	327.3600	33.12171	14.81248	286.2340	368.4860	
Triple Antibiotic Paste	5	453.4000	45.96520	20.55626	396.3267	510.4733	
Total	15	456.2533	116.14999	29.98980	391.9316	520.5751	

\*Statistically significant (P<0.05, Tukey’s post-hoc test)

Confocal microscopic Images

Triple antibiotic Paste Group

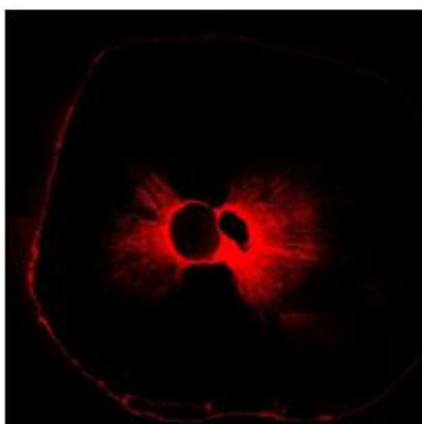


Figure 1: 3 mm from root apex

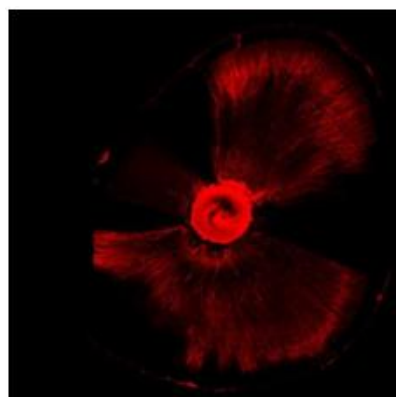


Figure 2 : 6 mm from root apex

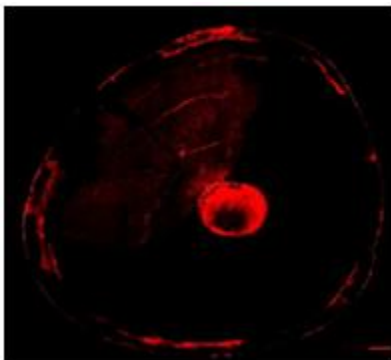


Figure 3: 9 mm from root apex