“Effect of 2% chlorhexidine final rinse on dentinal tubule penetration of root canal sealers- an in vitro study”

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Abstract:  
Introduction: Chemomechanical preparation reduces the bacterial load but does not result in complete disinfection of the root canal system. Irrigation is a critical compliment to instrumentation and 2% Chlorhexidine has shown a great promise in recent times as an endodontic irrigant. Penetration of root canal sealer into the dentinal tubules is considered a desirable feature for entombing residual bacteria. Making smear-free dentin more wettable with the help of tensioactive agents may further increase sealer penetration. Studies have shown that smear layer removal and final rinse with 2% chlorhexidine favoured the wettability of sealers. So, this study is done to determine the effect of 2% chlorhexidine as final rinse on dentinal tubule penetration of two different endodontic sealers: TubliSeal™ (zinc oxide eugenol- based) and AH Plus® (epoxy resin- based) with the aid of Confocal laser scanning microscope.  
Materials and Method: 80 extracted, human, single- rooted mandibular premolars were decoronated at the level of CEJ. Working length was determined and instrumentation done to apical #30 (0.06 taper). Irrigation was done with 5 mL of 3% NaOCl solution between instruments. 2% chlorhexidine was used as the final irrigant in half of the samples. Teeth were obturated using sealers labelled with 0.1% Rhodamine B dye, sectioned into coronal, middle and apical sections and examined under confocal laser scanning microscope.  
Results and Conclusion: AH Plus® showed more penetration depths than TubliSeal™. Maximum mean sealer penetration depth was seen in middle sections, followed by coronal root sections and least in apical sections.  
Keywords: 2% Chlorhexidine, Smear layer, Root canal sealers, Sealer penetration, Wettability, Confocal laser scanning microscope.

Introduction  
The main objective of endodontic treatment is complete elimination of microorganisms from the root canal system and prevention of recontamination. Irrigation is a critical component because irrigants can reach areas inaccessible to instruments.  
Along with sodium hypochlorite, 2% Chlorhexidine is another irrigant used which is equally antimicrobial at similar concentrations [1] and has the unique property of substantivity [2]. After cleaning and shaping, root canal sealers are used in conjunction with an inert core filling material to attain an impervious fluid- tight, three-dimensional obturation. Among the various ideal requisites of an endodontic sealer, its penetration into dentinal tubules is considered to be a desirable property. It increases the interface between obturating material and dentin and mechanical retention of obturation. Another significant advantage is the potential antibacterial effect and entombment of viable bacteria within dentinal tubules.  
However, the sealer penetration inside dentinal tubules is affected by presence of smear layer. It is a 1-2 µm thick amorphous structure created as a result of endodontic instrumentation and covers the prepared root canal walls occluding the dentinal tubules. It is recommended to use EDTA and NaOCl for effective removal of organic and inorganic components of the smear layer [3].  
Removal of smear layer leaves the dentinal tubules patent. Physical properties of these patent tubules are similar to that of a capillary tube. Lowering the surface tension of a fluid increases the fluid flow into, and out of, a capillary tube. So, the percentage and depth of sealer penetration in dentinal tubules also acts as an indicator of degree of smear layer removal [4].  
Making smear-free dentin more wettable with the help of tensioactive agents/ surfactants may further increase sealer penetration. Smear layer removal and final rinse with 2% chlorhexidine also favoured the wettability of sealers [5]. So, this study is done to determine the effect of 2% chlorhexidine as
Effect of 2% chlorhexidine final rinse on dentinal tubule penetration of root canal sealers - an in vitro study

Materials and Method

80 extracted, human, single-rooted mandibular premolars were selected. Teeth were visually inspected to be free of caries, cracks or restorations. The presence of single root canal in all the teeth was confirmed by taking digital radiographs.

After cleaning, teeth were stored in normal saline. They were then decoronated at the level of CEJ using diamond disc. Working length was determined by passing a #10 K-flex file (SybronEndo, USA) in the root canal until just visible at the apical foramen. 1 mm was subtracted from this length and the resultant length was considered as final working length.

Instrumentation was done using MTwo® rotary nickel–titanium instruments (VDW, Germany) to apical #30 (0.06 taper) according to manufacturer’s instructions. Irrigation was done with 5 mL of 3% NaOCl solution (Neelkanth healthcare, India) between each file using 30-gauge side-vented needle. Apical patency was maintained by passing #10 K-flex file through the apical foramen between files.

For smear layer removal, all canals were irrigated with 5 mL of 17% EDTA solution (Dent Wash, Prime dental products, India) for 1 minute. It was followed by irrigation with 5 ml distilled water to prevent further action of irrigants. In half of the teeth samples, 5 ml of 2% Chlorhexidine (Chlor X, Prevest DenPro, India) was used as the final irrigant. Then the teeth were divided into four groups (n=20) as listed in table 1:

<table>
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<tr>
<th>Group</th>
<th>Final irrigant</th>
<th>Sealer used</th>
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<tr>
<td>I</td>
<td>2% Chlorhexidine</td>
<td>TubliSeal™</td>
</tr>
<tr>
<td>II</td>
<td>2% Chlorhexidine</td>
<td>AH Plus®</td>
</tr>
<tr>
<td>III</td>
<td>Distilled water</td>
<td>TubliSeal™</td>
</tr>
<tr>
<td>IV</td>
<td>Distilled water</td>
<td>AH Plus®</td>
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External surfaces of teeth were coated with 2 layers of nail varnish. Both TubliSeal™ and AH Plus® sealers were mixed according to manufacturer’s instructions. During mixing of both the sealers, few grains of Rhodamine B dye (LOBA Chemie, India) were added to aid in confocal microscopy. The mixed sealers were applied to root canal walls using #30 lentulospiral @ 300 rpm.

Obturation was done using #30 (0.06 taper) master gutta percha cone and accessory gutta percha cones using lateral compaction technique. Teeth were sealed at coronal ends using temporary restorative material and stored in incubator at 37°C and 100% relative humidity for 48 hours to allow setting of sealers.

The samples were then sectioned perpendicular to long axis of teeth with the help of diamond disc into 3 equal-sized sections: coronal, middle and apical. All sections thus obtained were ground on a lathe machine so as to get thin sections. Final finishing of sections was done over finishing stone.

Sections were then mounted onto slides and examined under 10x magnification of confocal laser scanning microscope (LSM 510 META, ZEISS, Germany). Images obtained from confocal microscope were examined with the help of LSM image browser and maximum depth of sealer penetration for each section was evaluated in µm. In those sections where the area of maximum sealer penetration was difficult to ascertain, 3 readings were taken and their average was calculated.
The obtained results were tabulated and statistically analyzed using the unpaired t-test.

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**Fig. 1 Representative confocal images**

**Results**

All the sections (coronal, middle and apical) of groups I-IV exhibited various maximum sealer penetration depths inside dentinal tubules. Maximum sealer penetration depths were seen with middle sections (mean 789.72 µm) followed by coronal (mean 670.72 µm) and apical (mean 625.91 µm) sections.
Discussion

Successful endodontic treatment depends on accurate diagnosis, proper cleaning and shaping followed by three-dimensional obturation resulting in a hermetic seal. Root canal sealer forms an integral part of obturation. AH Plus® is an epoxy-resin based sealer which comprises of two-paste system. TubliSeal™ is a zinc oxide eugenol-based sealer. Sealers should have good flow to penetrate inside dentinal tubules and exert antibacterial effect. Both the sealers used in this study have these properties [6]. It is also important that the percentage of sealer-dentin interface and the percentage of tubule penetration by the sealer be as great as possible in all the cases, whether previously infected or not [7]. The teeth sections made for imaging under confocal microscope were transverse sections because it allows complete observation of all the dentin surrounding the canal [8]. On the other hand, with longitudinal sections, there are chances to miss areas of deep sealer penetration.

The complete irrigation protocol in this study was carried out using 30-gauge side-vented needles. As the apical preparation size was #30 for this study, 30 gauge needles with an external diameter of 0.32 mm [9] would have adequately reached near canal terminus. In the present study, maximum penetration depth of AH Plus sealer was found to be consistently greater than TubliSeal, irrespective of the final irrigant used and section of tooth under examination. This may be attributed to better flow characteristics of AH Plus sealer. For visualization under confocal microscope, both the sealers were labelled with Rhodamine B dye. Studies [10, 11] have shown that Rhodamine B dye does not bring about any changes in the physicochemical properties of root canal sealers.

Many factors may influence the percentage and maximum depth of sealer penetration [11, 12]. These include the effectiveness of smear layer removal, the obturation technique, physical and chemical properties of sealer, number and diameter of open dentinal tubules, presence/absence of water, the anatomy of root canal system and the contact angle formed between sealer and root dentine.

Among these, the contact angle between sealer and root dentin and wettability of the root canal sealer are very important factors. Wettability can be expressed in terms of contact angle which is formed between the drop of a liquid and the plane surface of the solid. This measurement shows the tendency of a liquid to spread on a solid surface [13]. Contact angle has an inverse relationship with the surface free energy.

Wettability is strongly dependent on chemical composition of solid surface, its roughness, hydration state and could suffer influence from tubule density [14]. EDTA improves the wettability of sealers.
by removing smear layer which increases the roughness of dentin surface [15]. An increase in roughness leads to a decrease in contact angle. de Assis et al[5] found that 2% chlorhexidine final irrigation decreased the contact angle and increased the wettability of AH Plus sealer, both in the presence as well as absence of smear layer. The authors attributed this finding to the presence of surface surfactant in its composition which increases the dentin surface energy, and hence, its wettability [16].

So, this study was designed to investigate whether 2% chlorhexidine final irrigation, owing to its property of increasing the wettability of root canal sealer, could influence the dentinal tubule penetration of TubliSeal and AH Plus sealers in smear-free dentin. In this study, final irrigation with 2% chlorhexidine resulted in decreased tubule penetration depth of TubliSeal™ sealer, especially in coronal and middle sections. This may be due to the ability of 2% Chlorhexidine to diffuse into tubular structure of dentin, not allowing the root canal sealer to effectively penetrate inside dentinal tubules[17].

In apical sections, however, 2% Chlorhexidine final rinse did not significantly reduce penetration of TubliSeal™ possibly due to its inability to reach apical region of root canal and the presence of less number of dentinal tubules in the apical root region. On the contrary, the penetration depth of AH Plus sealer was unaffected by the final irrigant used. Better flow of this sealer may be responsible for such findings. In this study, maximum sealer penetration was seen in the middle root sections. This finding is in agreement with the results obtained by Vassiliadis et al [18]. They explained that in this area, the diameter of dentinal tubule orifices is large enough and the greatest forces would be expected to have been applied during the lateral compaction of gutta percha. Confocal Laser Scanning Microscope was used in the present study. It allows for a full cross-sectional observation, which clearly shows the amount of labelled sealer inside the dentine. CLSM does not require any special specimen processing, so it tends to produce fewer artefacts as compared to SEM. Also, the observations can be made under normal environmental conditions. CLSM also has the advantage that it can collect serial optical sections even from thick specimens [19]. Furthermore, CLSM is a non-destructive approach as it allows the reuse of the same specimen in another evaluation. Hence, it is a suitable experimental feature when the final purpose is to determine the total amount of sealer penetrated into dentinal tubules.

**Conclusion**

Within the parameters and limitations of this study, it can be stated that final irrigation with 2% chlorhexidine reduced maximum penetration depth of TubliSeal™ sealer in smear-free dentin with significant reduction at coronal and middle levels. However, final irrigation with 2% chlorhexidine did not significantly affect the maximum penetration depth of AH Plus® sealer.

Among the root canal sealers, AH Plus® consistently displayed significantly higher maximum sealer penetration depths than TubliSeal™ irrespective of the root level i.e. coronal, middle or apical. Maximum mean sealer penetration depth was seen in middle sections, followed by coronal root sections and least mean maximum penetration depth was seen with apical sections.

**Acknowledgement**

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**References:**