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Research Article

A FLUOROMICROSCOPIC EVALUATION OF DENTINAL TUBULE PENETRATION OF AH PLUS AND MTA FILLAPEX ROOT CANAL SEALERS: AN IN VITRO STUDY

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ABSTRACT

Root canal therapy has several components involved in order to guarantee success of the treatment. It broadly involves adequate caries removal and access along with three dimensional shaping and disinfection of the complete anatomy of the root canal space, along with a hermetic seal of the space. To obtain a 3D hermetic seal, the filling material needs to be of optimum properties.
The aim of this in vitro study was to evaluate dentinal tubule penetration of different endodontic sealers after irrigating the riot canals with/without EDTA.

INTRODUCTION

Pulpal and endodontic problems are primarily related to microorganisms or their by-products in the root canal system.[1]The bacteria present in the infected root canal system are mainly obligate anaerobes with similar numbers of facultative anaerobes. These bacteria may form a complex biofilm, and may penetrate into the dentinal tubules.[2]

The main objectives of root canal therapy are the complete elimination of microor- ganisms from the root canal system and the prevention of recontamination. Complete root canal disinfection is mandatory for the successful outcome of endodontic treatment. complete disinfection of root canal system can be achieved thru biomechanical, chemomechanical techniques, and various irrigation devices which ensure complete removal of debris from the most complex part of root canal, especially apical third which includes many ramifications such as isthmuses, deltas, and lateral canals.[3]

Subsequent to sufficient chemomechanical preparation, obturation with a biocompatible material is another important objective of root canal treatment and it eliminates all avenues of leakage from the oral cavity and the peri-radicular tissues into the root canal system by creating a fluid-tight seal.[3] The root-canal system is usually obturated with gutta-percha combined with a sealer. Gutta-percha serves as the core- filling material, whereas the sealer acts as a binding agent and lubricant. Sealer cements create a union between the core material and the canal wall by filling any residual spaces.[6]

Different types of sealers are regularly used in endodontic treatment. They should provide an excellent seal when set, dimensional stability, a slow setting time to ensure sufficient working time, insolubility to tissue fluids, adequate adhesion with the canal walls, and biocompatibility.[7] In an ideal future, these materials would be able to fill out dentinal tubules, bind intimately to organic and inorganic surfaces of dentine, destroy or neutralise micro-organisms and their byproducts, induce new cementum formation and strengthen the system of root canals. Unfortunately, all current sealers are unable to fulfil these purposes.[8]

Penetration of sealer cements into dentinal tubules is influenced by a number of factors including smear layer removal, dentine permeability and filling technique.[8, 10] Variations in the physical and chemical properties of sealer cements also influence the depth of penetration.[8, 10]Variations in the physical and chemical properties of sealer cements influence the depth of penetration. Therefore, it is important to compare the penetrability of various sealers that are used in routine clinical practice. The aim of this study was to evaluate and compare the penetrability of 2 different sealers, a resin based sealer (AH Plus), and an MTA based sealer (MTA Fillapex).

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MATERIALS AND METHODS

60 human mandibular premolars, extracted for orthodontic reasons were used in this study. Only sound, non-carious single rooted mandibular premolars were included in the study. All teeth were stored in physiological saline at 4°C until use. The teeth were decoronated at the cementoenamel junction (CEJ) by using a safe-sided diamond disk, to have 14mm of standardized root length. The canals were accessed, pulp tissue was extirpated and working length was determined by inserting a 25-mm, #15 K-flexile file (Mani Inc.) until just visible at the apical foramen. 1 mm was subtracted to obtain the final working length. Instrumentation was done by using crown-down technique with ProTaper Next rotary nickel-titanium instruments (Dentsply Maillefer). A standardized method of root canal preparation and obturation was undertaken by a single operator to reduce variations in the final results.

The canals were prepared to apical size of X2 to working length. During canal instrumentation, RC Help was used as a lubricant with each single instrument. Irrigation was done between files with 5 mL of 2.5% sodium hypochlorite and 17% EDTA (Prime Dental Product). During instrumentation, apical patency was maintained by passing #10 K-file (Mani) through the apical foramen.

The samples thus obtained were divided into 2 groups (n=30).

Group A: AH Plus
Group B: MTA Fillapex

5 mL of normal saline was used to remove any remaining irrigating solution from the samples. The canals were dried with sterile absorbent paper points (Dentsply Maillefer).

Each experimental sealer was manipulated according to the manufacturer's instructions. To facilitate study under stereomicroscope, the sealers were labeled with the fluorescent rhodamine B dye to an approximate concentration of 0.1% to provide fluorescence and allow microscopic assessment. The prepared teeth were filled by using single cone technique. Size 25 gutta-percha cone of 6% taper was checked for tug back. The canal walls were coated with the experimental sealers in each group with the help of master gutta-percha cone. The cone was re-coated with sealer and seated in the canal. The cone was seared off at the level of the orifice and lightly condensed with a plugger. Coronal end of canals were sealed with Cavit G temporary restoration cement. All samples were stored for 2 weeks at 37° C in 100% humidity to allow the sealers to set. Samples were stored in a humidity chamber at room temperature for the remainder of the study.

After 2 weeks each specimen was sectioned horizontally at 3, 6 and 9 mm from the apex, using a low-speed diamond disc. Three slices per root were obtained, resulting in a total of 180 slices. Each slice was observed at 4x in a high-resolution fluromicroscope with RGB fluorescence filter for penetration of sealers in the dentinal tubules. The images obtained were saved and analyzed using the ImageJ software. Images were calibrated using the reference set scale. The values obtained in pixels, were then converted to millimeters.

To obtain the values of the sealer penetration, each sample image was outlined using the lasso tool and the inner circumference of the canal lumen was measured. Next, areas along the canal walls in which sealer penetrated into dentinal tubules were measured circumferentially using the same method. The values of canal circumference were subtracted from the values of dentinal penetration of the sealer.

Statistical analysis

The data obtained were subjected to statistical analysis. Pairwise comparison of sealers was done by using post-hoc Tukey's test. Section wise comparison of dentinal penetration of the sealers was analyzed by performing one-way ANOVA.

RESULTS

Table 1 Descriptive Statistics

Treatment	N	Mean	Std. Deviation
A(AH PLUS)	30	2.032	1.373
B (MTA FILLAPEX)	30	0.980	0.906
Total	60	1.505	1.221

From descriptive statistics (Table 1), it is clear that Group A shows maximum penetration (mean=2.032) than Group B (0.980). There is significant difference in the mean values of penetration of AH Plus and MTA Fillapex. Hence, we can conclude that dentinal penetration of AH Plus is better than MTA Fillapex.

Pairwise Comparison of Sealers for Dentinal Penetration

Table 2 Post hoc Tukey's test

Multiple Comparisons							
Dependent Variable: Penetration							
	(I) Sealer	(J) Sealer	Mean (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Tukey HSD	Group B	Group A	-0.716	0.206	0.002	-1.204	-0.228

	Sealer	N	Subset 1	2
	Group B	60	1.025	
	Group A	60		1.741
	Sig.		0.339	0.101

Table 2(Post hoc Tukey's test) shows that means of group A and group B differ significantly. Mean of AH Plus is significantly higher than that of MTA Fillapex. Hence, we can conclude that, MTA Fillapex performed poorly than AH Plus.

Section wise comparison of Sealer Penetration (Coronal, Middle and Apical thirds)

Table 3 One Way Anova

Descriptives								
Penetration								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Coronal	60	1.741	1.403	0.181	1.378	2.103	0.1	5.683
Middle	60	1.315	1.026	0.132	1.050	1.580	0.1	4.01
Apical	60	1.025	0.932	0.120	0.784	1.265	0.062	3.88
Total	180	1.360	1.170	0.087	1.188	1.532	0.062	5.683

ANOVA Penetration	Sum of Squares	df	Mean Square	F	Sig.
Bet.Groups	15.565	2	7.783	5.997	0.003
Within Groups	229.694	177	1.298		
Total	245.259	179			

In Table 3, as the p-value < 0.05, significant difference is observed in the means of the three sections. There is significant difference between means of Coronal (1.741) and Apical sections (1.025). Mean of Coronal section is significantly more than mean of Apical section. Hence, we concluded that the sealer penetration is more in the coronal third, and minimum in the apical third of the canal.

DISCUSSION

The main objective of a root canal filling is to seal the root canal system to prevent reinfection.[23]The potential for bacteria to colonise dentinal tubules is well established.[29] Bacterial penetration into dentinal tubules may reach 200-2000 microns.[15]Chemo-mechanical preparation is considered the most important step in the management of the infected root canal system; however, it is difficult to completely eliminate organisms from the canal space. Bacteria can persist in areas such as lateral canals and dentinal tubules, as these areas may provide protection from the disinfecting actions of irrigants and medicaments. These remaining bacteria may play a role in persistent peri-apical disease. However, a number of studies have shown that most teeth with apical periodontitis will heal despite having a positive bacterial culture at the time of root filling. Root canal filling eliminates all avenues of leakage from the oral cavity and the peri-radicular tissues into the root canal system by creating a fluid tight seal [3, 31]; and it seals the spaces and any irritants that cannot be fully removed during cleaning and shaping procedures within the root canal system. Because of this bacterial entombment the bacteria remaining within the root canal space are rendered harmless, as they are deprived of essential nutrients and space which is required for growth and proliferation.[3, 4, 21]

Root canal filling is associated with a hard core, like gutta-percha, and a sealer, to better adapt the root canal filling material and complete the seal of the root canal filling effectively. The sealer can fill the irregularities of the root canal wall and the dentinal tubules, which cannot be filled by gutta-percha. Sealer penetration into the tubules could affect the seal of the root filling, because an increase of the contact surface between filling material and dentin is related to an improvement of the sealability.[23]

The penetration of sealer cements into dentinal tubules is considered to be a 'desirable outcome'[19] for a number of reasons: it will increase the interface between material and dentine thus improving the sealing ability and retention of the material may be improved by mechanical locking. Sealer cements within dentinal tubules may also entomb any residual bacteria within the tubules and the chemical components of sealer cements may exert an antibacterial effect that will be enhanced by closer approximation to the bacteria. The antibacterial effect, also enables avoidance of the colonisation of residual bacteria and reinfection of the root canal.[9]

Penetration of sealer cements into dentinal tubules is influenced by a number of factors including filling technique[8], dentine permeability[10] and smear layer removal.[32] Variations in the physical and chemical properties of sealer cements also influence the depth of penetration.[33] For example, the flow of the sealer, which is one of the main physical factors influencing the tubular penetration. Flow reflects the ability to penetrate into small irregularities and ramifications of the root canal system and dentinal tubules and enter un-instrumented accessory root canal anatomy.[18]

The smear layer may be considered deleterious because it prevents irrigants, medicaments, and filling materials from penetrating into the dentinal tubules, or even contacting the canal wall.[14,11, 37]The removal of smear layer allows the root canal sealer to contact the canal wall and completely penetrate the dentinal walls. This ensures a tight seal, thereby reducing the amount of micro-leakage.[17] The influence of smear layer removal on the penetration of sealer cements has been investigated by a number of authors with general agreement that smear layer removal results in deeper and more consistent sealer penetration. Removal of the smear layer of the root canal walls is considered to be fundamental to allow sealer penetration into dentinal tubules irrespective of the root canal sealer used.[10, 19, 32, 33] EDTA is most commonly used to remove the smear layer. The combination of NaOCI and EDTA used alternately completely removes the smear layer from the instrumented canal wall that is a smoothly planed surface with patent tubular orifices. In addition to efficiently removing the smear layer, the combination regimen of NaOCI and EDTA may also be capable of preventing the material comprising the smear layer from becoming packed into the dentinal tubules.[11, 12, 13, 37] Moon *et.al* observed that the use of EDTA before canal obturation resulted in significantly better sealer penetration at both coronal and apical levels.[22]

The ability of any one particular sealer cement to penetrate dentinal tubules consistently and effectively will be one of many factors influencing the choice of material for filling. It is therefore important to compare the penetrability of different types of cements used. It is also important to validate the results from in vitro studies with findings from clinical cases. To date, only one study is conducted, in vivo by Vassiliadis et al. and they reported sealer penetration of dentinal tubules.[16, 21] Sealer penetration both in terms of depth and the percentage are important for a successful root canal treatment. Several test methods have been used to evaluate the sealing ability of obturated root canals, like Linear measurement of tracer dye or isotope, Fluid filtration models, Bacterial leakage models, Electro-chemical models, Spectrophotometry and SEM. However a single conclusive method, technique, or material over any other has still not been reached. The variety of evaluative methodologies and their assessment parameters are major reasons for such disagreement.[41]

The present study was focused on evaluating the ability of 2 different root canal sealers; AH Plus MTA Fillapex, to penetrate into the dentinal tubules of root canals. The study also compared the penetration depth of these sealers in coronal, middle and apical thirds of the root canal.

In the present study, Rhodamine B was used instead of other histological stains, because of the difficulty of incorporating the dye into some sealer types and the potential for such stains to interfere with the physical properties, handling characteristics and setting times of the sealer cements.[21] Rhodamine B is the most frequently used fluorochrome for different applications. This compound is excited using green light (540 nm) and emits red in colour (590 nm). Rhodamine B is effective in very low concentration, fairly labile, moves freely across the bonded interface, and is easily detected microscopically with appropriate filters. The compound is also stable under various pH conditions.[43]

For microscopic study, each specimen was sectioned horizontally (transverse sections) at 3, 6 and 9 mm from the

apex, using a low-speed diamond disc. Three slices per root were obtained, resulting in a total of 180 slices. Longitudinal sectioning techniques were employed in older studies. The disadvantage of this orientation is that it does not allow for complete observation of all of the dentine surrounding the canal and there is potential to miss areas of deep penetration. Weis et al. analyzing transverse sections reported that sealer penetration was the deepest and the most consistent in the buccal and lingual directions. They reported sealer penetration into the outer third of the dentine wall in these areas.[18] This was consistent with the penetration depth reported Mammotil and Messer's study, which also used transverse sections.[21]

Each slice was observed at 4x magnification in a high-resolution microscope with fluorescence filter for penetration of sealers in the dentinal tubules. The images obtained were saved and analyzed using the ImageJ software, which is capable of calculating area and pixel value statistics of user-defined selections and intensity- thresholded objects. Images were calibrated using the reference set scale. The value thus obtained in pixels, was then converted to microns. To obtain the values of the sealer penetration, each image was outlined using the lasso tool and the circumference of the canal was measured. Next, areas along the canal walls in which sealer penetrated into dentinal tubules to any distance were measured using the same method. The outlined distances were subtracted by the canal circumference to calculate the total depth of any canal wall sealer penetration in that section.

The results showed that, AH Plus showed the maximum depth of penetration in the dentinal tubules, than MTA Fillapex sealers. Both sealers showed more penetration in the dentinal tubules in coronal third, than the the middle and apical third of the canal.

AH Plus is characterized by very good mechanical properties, high radio opacity, little polymerization shrinkage, low solubility, and, not least, a high degree of stability on storage. It has been designed to be slightly thixotropic, for optimal flow behavior. Literature shows enough evidence that AH Plus has good adhesion to the canal walls and sealing ability.[20, 23]

In our study, AH Plus (Group A) shows the best results. The maximum penetration observed with AH Plus could be because of its physical properties such as flow, surface tension, solubility, viscosity, chemical composition, and working and setting time. 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.[26] Tubule penetration of resin-based sealers is not dependent on the hydraulic forces created during filling; instead, the sealer is drawn into the tubules by capillary action.[21, 44, 45]

The better sealing ability of AH Plus can be explained by the fact that this epoxy resin sealer is considered contraction-free during setting reactions, which is responsible for its appropriate interfacial adaptation. AH Plus forms an intimate contact with dentin, remains micro-mechanically retained, reinforces the tooth structure and prevents recontamination.[46]
It has been suggested that tubular penetration and adhesion to dentin/adaptation to root canal wall go hand in hand. So adaptation and adhesion are a direct indication of depth of penetration of the sealer. AH Plus, being a resin sealer, exhibits good adhesive properties. It has ability to react with exposed amino groups in collagen in order to form covalent bonds among the resin and the collagen[47] which allows increased surface contact between sealer and dentin and hence deeper penetration in the dentinal tubules.[48]

K. R. Sonu, T. N. Girish *et.al*. investigated the dentinal tubule penetration of mineral trioxide aggregate (MTA) Fillapex ® (Angelus, Londria, PR, Brazil), GuttaFlow 2 sealer (Coltene/Whaledent) with standard sealer AH Plus (Dentsply Detrey, Konstanz, Germany) in instrumented root canals obturated by using cold lateral compaction techniques in either the presence or absence of the smear layer. They found that AH plus showed deeper penetration in the dentinal tubules.[49]
Pablo Andres Amoroso-Silva et. al analysed the quality of obturation and physical properties of AH Plus sealer and MTA Fillapex. As far as flow properties and depth of penetration in dentinal tubules is concerned, they observed that AH Plus sealer exhibited greater depth of penetration.[50]

Bouillaguet S, Shaw L *et.al* evaluated the long-term sealing ability of four contemporary endodontic sealers [Pulp Canal Sealer (PCS), AH-Plus, GuttaFlow and Epiphany] using a fluid filtration technique. The values obtained for AH Plus sealer showed greater sealer penetration.[51]The results of our study are in accordance with above mentioned studies.

MTA Fillapex is an endodontic sealer based on MTA. It is presented in dual syringes with auto-mix tips or tubes. Being a versatile and bioactive material, MTA has been an asset in endodontics. Hence, we have selected this as one of the experimental sealers. In the present study MTA Fillapex sealer showed minimum penetration as compared to AH Plus and Sealapex. This could be because of physical properties like flow, viscosity, particle size, solubility etc. MTA Fillapex has smaller particles.[53] Some studies reported significantly lesser flow.[50, 52] MTA Fillapex has higher solubility as compared to AH Plus sealer.[28] MTA Fillapex might not have penetrated as deep as AH Plus due to it's lack of hydrophilic characteristics.[54]

MTA Fillapex contains resinous components (salicylate, diluting and natural resin). Orstavik et al. reported that sealers containing salicylate in its com- position showed initial volumetric shrinkage during the setting reaction, increasing the contraction factor. The contraction during setting may lead to dis-adaptation of the sealer to the canal walls, leading to adhesion failure. This could be the reason for less penetration of MTA Fillapex in the dentinal tubules.[55]

The chelating effect of the EDTA demineralises and removes the inorganic component of the smear layer. In addition to efficiently removing the smear layer, the combo-regimen of NaOCI and EDTA may also be capable of preventing the material comprising the smear layer from becoming packed into the dentinal tubules.[11]

The number and diameter of the dentinal tubules varies considerably in coronal, middle and apical third of the root canal. Taking into consideration this fact, this study also evaluated the depth of penetration of experimental sealers in coronal, middle and apical third of the samples. It was observed that all three experimental sealers, showed deeper penetration in coronal third, followed by middle third. In apical third, all the sealers penetrated less. The penetration of these sealers was not affected by the use of EDTA. The statistical analysis revealed significant difference in the values of coronal and apical thirds of the samples, whereas the values

of penetration of coronal and middle thirds, and, middle and apical thirds did not show statistically significant difference.
This could be because of the fact that the apical root canal contains less tubules, and when present, the diameter is smaller or they are more often closed.[56] Dentin tubules are smaller at the apex and larger toward the crown. The apical portion of roots shows a pronounced variation in structure. Primary dentinal tubules are irregular in direction and density; some areas are devoid of tubules. Also, cementum-like tissue can line the apical root canal wall, occluding any tubules.[57] The poorer dentinal tubule penetration in the apical thirds can be explained by the ineffective delivery of irrigant to this region of the canal, the smaller diameter and reduced number of dentinal tubules in this third, and its greater more tubular sclerosis.[58] The effectiveness of smear layer removal techniques is also reduced closer to the apex.[59] Another explanation for deeper penetration of all the sealers in coronal third is application of greater compressive(vertical) forces during obturation.[8]

Balguerie *et.al*. investigated, in vitro, the tubular adaptation and penetration depth and the adaptation to the root canal walls in the apical, middle, and coronal third of the root canal of 5 different sealers used in combination with softened gutta-percha cones. The sealer penetration depth in the apical root canal was less because of the different properties of the apical root canal.[23]

Ackay *et.al* conducted an in vitro study. The aim of this study was to use a laser scanning confocal microscope in order to assess the dentinal tubules penetration of various sealers after the application of different final irrigation techniques. Statistically significant differences were determined at each root canal third (coronal > middle > apical; $P < 0.001$).[42]

Saurabh S. Chandra *et.al* in their in vitro study evaluated the depth of penetration of 4 different endodontic resin sealers into the radicular dentinal tubules with the aid of confocal microscopy. The results showed that the maximum penetration was in the coronal third, followed by middle third and least at the apical third.[45] The results of our study are in agreement with the above mentioned and some other studies.

Thus, from this in vitro study we can conclude that amongst the sealers tested, AH Plus showed maximum depth of dentinal penetration. MTA Fillapex showed minimum penetration than AH Plus. All the sealers showed maximum penetration in coronal third, followed by middle third and least in apical third of the canal. We recommend further studies to be planned and research should be focused on the properties of sealers that improve their dentinal penetration.

CONCLUSIONS

Within the parameters and limitations of this in-vitro study the following conclusions were drawn

- Statistically significant difference in the depth of sealer penetration was noted between Group A(AH Plus) and Group B(MTA Fillapex) at all three levels (cervical, middle and apical).
- The penetration depth of both sealers at the coronal third of root was significantly more than apical third.

Hence, we conclude that, amongst the experimental sealers, AH Plus shows maximum penetration followed by MTA Fillapex. For both sealers, penetration was maximum in coronal third than middle and apical third of the canal.

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