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RESEARCH ARTICLE

"COMPARATIVE EVALUATION OF DENTINAL PENETRATION OF THREE DIFFERENT ENDODONTIC SEALERS"- SCANNING ELECTRON MICROSCOPIC STUDY

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ABSTRACT

Aim and Objectives: The aim of this study was to compare the depth of penetration of 3 different root canal sealers into the dentinal tubules, Apexit plus, AH Plus, Roekoseal, Using Scanning Electron Microscopy.
Materials and Methods: Thirty freshly extracted single rooted premolar teeth were taken and coronal portions were cut with a diamond disc and the root canal length standardized at 14mm.Then, the teeth were instrumented by using the protaper technique to a size of the F2 insrument at the working length. After preparation, thirty samples were randomly divided into 3 groups of 10 each, Group I (Roekoseal), Group II (AH plus), Group III (Apexit plus) and obturation done with lateral compaction technique. Then the samples were examined under scanning electron microscope and the results are obtained.
Results: the mean value of depth of penetration of three root canal sealers at the apical third of the roots are Group-III(Apexit plus)167.80±0.84,Group II (AH plus)155.60±0.89 and Group I (Roekoseal) 116.00±0.71. Group III and Group II showed higher penetration depth values compared to Group I.**Conclusion:** Apexit plus showed deeper sealer penetration into the dentinal tubules than AH plus and Roekoseal sealer.

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INTRODUCTION

Successful endodontic treatment requires three dimensional cleaning, shaping and obturation of the root canal systems with a biologically compatible filling material to provide a fluid tight seal from the apical segment of the canal to the dentinocementum junction in order to prevent reinfection. However, it is difficult or even impossible to eliminate all organisms from the canal space. Bacteria may 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 periapical disease (Kuçi *et al.,* 2014). Prior to the obturation phase, the clinician must establish the proper shape and size of the root canal. Today's clinicians have a number of methods, materials and technologically advanced instruments at their disposal to achieve these goals. Among various obturation techniques cold lateral compaction stands as a practical and reliable method for the effective obturation of the root canal system (Mamootil *et al.,* 2007). Gutta percha is considered as a "gold standard" filling material (Peng *et al.,* 20074). Though it has many desirable properties yet it solely fails to provide an effective three dimensional seal. To overcome this insufficiency, endodontic sealers are used in conjuntion with gutta percha which acts as a binding agent between the gutta percha and the canal wall and fills anatomical spaces the primary filling material has failed to reach. Most commonly used root canal sealers are zinc oxide eugenol, calcium hydroxide sealer, glass ionomer sealers, resin sealers, silicone sealers, bioceramic sealer, etc.

According to Orstavik (Patil *et al.,* 2013), sealers play an important role in sealing the root canal system with entombment of remaining microorganisms and filling of inaccessible areas of the prepared canal. Newer generation sealers are being engineered to improve their ability to penetrate into dentinal tubules or bond to both the dentin and core material surfaces.

Apexit plus is a calcium hydroxide based sealer. It has excellent tissue tolerance, long working time, radiopaque, non shrinking and excellent flow properties, can be used even if the canal morphology is unfavorable (Orstavik, 2005).

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AH Plus is a widely used epoxy resin based sealer and posses positive handling characteristics and superior physical properties. Previous studies showed that the epoxy resin based root canal sealer AH plus is cytocompatible, biocompatible, has good tissue tolerance, long term dimensional stability and good sealing ability (Aida, 1992).

Roekoseal is a silicone based sealer. It combines exceptional material properties such as excellent flowability, mechanical adhesion to canal wall due to expansion, no shrinkage, highly radiopaque and biocompatibility with practical handling (Kouvas *et al,* 1998).

The ability of a sealer to penetrate into the dentinal tubules plays an important role in the prevention of the reinfection of root canal systems through dentinal tubules. Penetration of root canal sealer into dentinal tubules is influenced by a number of factors including smear layer removal, dentine permeability and filling technique. Variations in the physical and chemical properties of root canal sealer also influence the depth of penetration (Wu *et al.,* 2000). The penetration of root canal sealers into the dentinal tubules consistently and effectively will be one of the key factor in choosing root canal sealer for the obturation technique. So we have done a study on comparison of depth of penetration of 3 root canal sealers (1) Apexit plus, (2) AH Plus,(3) Roekoseal, using Scanning Electron Microscopy.

MATERIALS AND METHODS

Thirty single rooted human mandibular premolar teeth were used in this study. After extraction all teeth were stored in physiological saline solution until use. The crowns were removed at the cemento enamel junction with a high speed fissure bur under water cooling. The pulp tissues were removed using standardized broaches.

Root canal preparation and filling

Radiographs were exposed from facial and proximal views to ensure the presence of single canal. A #10 k file was introduced into the canal and advanced until it was just visible at the apex and then retracted 1 mm to establish the working length. A glide path was verified or established using k files #10 to 25.All the roots were instrumented till F2 protaper file. Irrigation procedures were accomplished by using 2ml 1.0% sodium hypochlorite for each file used. Apical patency was maintained by passing a size 10 k file though the apical foramen between files. To remove the smear layer, all canals were irrigated with 3ml 17% ethylene diamine tetra acetic acid over 2 minutes followed by 2 ml 1.0% sodium hypochlorite over 1 minute. A final rinse of 5ml distilled water was used to remove any remnant of the irrigating solutions. The canals were dried using paper points. After preparation, the roots were randomly divided into 3 groups according to the sealer used;

- Group 1-Roekoseal,
- Group 2-AH Plus,
- Group 3-Apexit Plus

The sealers were mixed according to their manufacturer’s instructions. Sealer was placed into the canals using a size 25 lentulo spiral (Dentsply) in a slow speed handpiece introduced to approximately 2 mm short of the working length.

After sealer placement a size F2 gutta percha cone coated with the sealer was seated to the working length. The remaining canal space filled by lateral condensation with fine accessory gutta percha cones using finger spreaders. Excess gutta percaha was removed by using a heated plugger and vertical compaction was performed at the orifice level. The filling materials were then allowed to set for 48 hours.

Sectioning and Scanning electron microscopic analysis of the roots

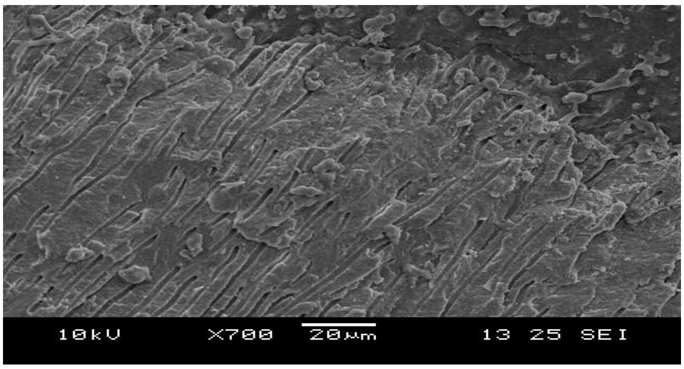
Each root was split into two by placing parallel longitudinal grooves on the full length of buccal and lingual surfaces, which did not penetrate the root canal. The roots were then split into two halves using chisel and mallet. The root segments were examined under scanning electron microscope by mounting the samples on an aluminum stub, coated with gold atoms. Considering the canal wall as the starting point the maximum and minimum depth of sealer penetration in dentinal tubules was measured (microns) at apical third of root.

Statistical analysis

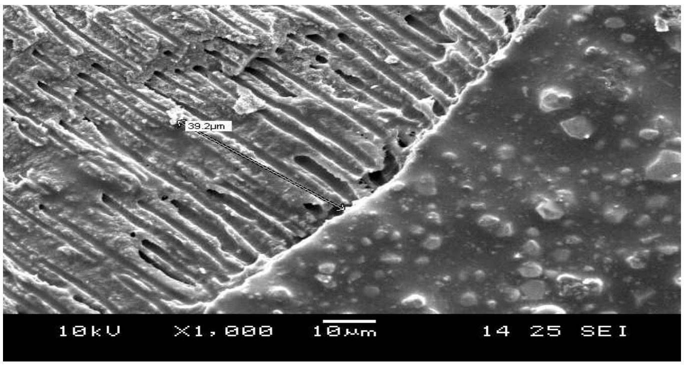
Statistical analysis was performed for comparing the values among the group and within the group using one way ANOVA and post hoc test.

RESULTS

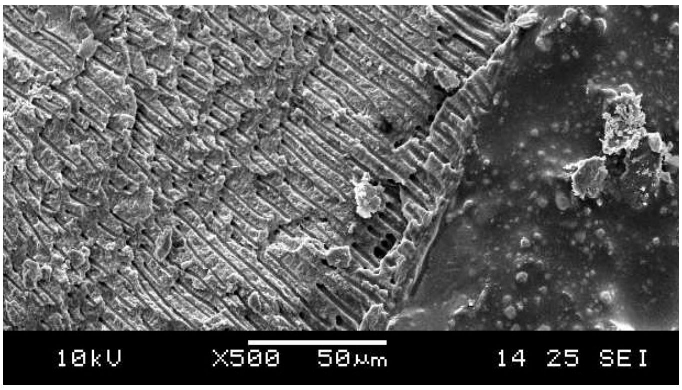
Tubular penetration was observed in all the groups. All the data were collected, the mean depth of tubular penetration of sealers and standard deviation in different experimental groups were presented in Table 1. When groups were compared statistically, significant differences were found between Groups I, II, and III ($P < 0.05$), indicating that Apexit Plus showed the greatest sealer penetration.



Group 1. Depth of sealer penetration



Group 2. Depth of sealer penetration



Group 3. Depth of sealer penetration

Table 1. Showing the Mean, Standard Deviation and F- Value of Dentinal penetration of three different Endodontic Sealers

Groups	N	Mean	Std. Deviation	F-Value	P-Value
Group – I Roekoseal	10	116.00	0.71		
Group – II AH Plus	10	155.60	0.89	5500.30	0.001 (S)
Group – III Apexit Plus	10	167.80	0.84		
Total	30	146.47	22.90		

DISCUSSION

Successful root canal therapy consists of thorough cleaning and shaping and requires complete obturation of the root canal system with an inert filling material (Vertucci, 2005). Root canal obturation is defined and charecterized as "the three dimensional filling of the entire root canal system as close to the cement dentinal junction as possible with minimal amounts of root canals sealer, which have been demonstrated to be biologically compatible, are used in conjunction with the core filling material to establish an adequate seal". The preliminary objectives of obturation are development of a fluid-tight seal at the apical foramen i.e hermetic apical seal, prevent reinfection and promotes favourable environment for healing (Salz *et al.,* 2009).

In general, a root filling is composed of two materials; a solid core material and a sealer. The most commonly used core material is gutta percha, which can be placed into the root canal in a cold or a warm state (Skinner *et al.,* 1987). Gutta-percha has been universally accepted as the 'gold standard' root filling material, and the material against which most others are compared. It is used in a number of forms in practice, with various filling techniques, and associated with different types of sealers (Eric Balguerie *et al.,* 2011). Sealers are used to attain an impervious seal between the core material and root canal walls. They can be grouped according to their basic components, such as zinc oxide eugenol, calcium hydroxide, resins, glass ionomers, iodoform or silicone. Ideally, these material should seal the canal laterally and apically and have good adaptation to root canal dentine (Patel *et al.,* 2007). Accoding to Grossman, the ideal requirements of root canal sealers as follows; It should be biocompatible, easy insertion into and removal from root canal, viscosity while handling, good adhesion to root canal walls, satisfactory handling time, promotion of a three dimentional sealing, dimensional stablity, good flowing, good radiopacity, lack of colour change, insolubility to tissue fluids and saliva, solubility to common solvents when necessary, impermeability and anti-microbial activity.

The main functions of root canal sealers include, it lubricates and aids the seating of the master gutta percha cone, acts as a binding agent between the gutta percha and the canal wall and fills anatomical spaces the primary filling material has failed to reach. Root canal sealers, although used only as adjunctive materials in the obturation of root canal systems, have been shown to influence the outcome of root canal treatment (Mamootil and Messer, 2007). The use of sealer cement in conjunction with a core filling material is recommended with most obturating techniques. Gutta percha has no bonding properties to dentin regardless of the filling technique employed (Mamootil and Messer, 2007). The penetration of sealer cements into dentinal tubules is considered to be a desirable outcome 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 (Mamootil and Messer, 2007).

Sealers based on epoxy resins afford very good physical properties and ensure adequate biological performance. Excellent apical sealing has been found with epoxy resin based sealers (Sevimay and Kalayci, 2005). By far the most successful of resin based sealers has been the AH series. The protype was developed more than 60 years ago by Andre Schroeder in Switzerland and is a bis phenol resin using methanamine for polymerization. As methenamine (also known as urotrpin) gives off some formaldehyde during the setting reaction, substitutes were sought and found in a mixture of amines that could affect polymerization without the formation of formaldehyde. AH plus is the result of this product development (Orstavik, 2005). Studies showed that the epoxy resin based root canal sealer AH plus is cytocompatible, biocompatible and has good tissue tolerance, long term dimensional stability and good sealing ability (Sevimay and Kalayci, 2005).

According to the manufacturer's description, AH plus is a two component paste root canal sealer, posses advantageous properties similar to that of AH26, as it preserves the chemistry of the epoxy amines. The material does not release formaldehyde, which interferes negatively with the biocompatibility of AH26. Therefore, AH plus has been continuously used in comparative studies of physicochemical, biological and antimicrobial properties (Versiani *et al.,* 2006).

Apexit plus is a newer calcium hydroxide based sealer. It has two component materials, which sets by complex formation. For this complex formation, three components calcium hydroxide, salicylate and water are needed. It differs from Apexit in that it is supplied in a more convenient form and has a more hydrophylic formulation. Consequently, the material is more reliable if used in thicker layers. It is a radiopaque, non shrinking root canal sealer. It has excellent tissue tolerance, long working time and excellent flow properties, can be used even if the canal morphology is unfavorable (Apexit Plus, 2007).

Roekoseal is a silicone based sealer. It is considered as initial form of guttaflow, was removed more easily from the canals than a resin based sealer (Kosti *et al.,* 2006). A gutta precha containing silicone sealer expands slightly and thus leakage was reported to be less than for AH26 with gutta percha over a

period of 12 months[21]. It combines exceptional material properties such as excellent flowability, mechanical adhesion to canal wall due to expansion, no shrinkage, highly radiopaque and biocompatibility with practical handling.
The penetration of sealer into dentinal tubules may be biologically beneficial, because laboratory studies have shown that endodontic sealers can exert antibacterial effect against bacteria in infected dentinal tubules (Ronald Ordinlo-Zapata, 2009). Bacterial penetration into dentinal tubules may reach 100-1000µm and it can be enhanced by the absence of smear layer. Many species seen in the infection of the root canal have the propensity to penetrate deeply into the dentinal tubules, such as facultative and anaerobic species, even close to the dentinal cementum junction (Ronald Ordinlo-Zapata, 2009). Penetration of sealer cements into dentinal tubules is influenced by a number of factors including smear layer removal, dentine permeability and filling technique. 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 sealer used (Mamootil and Messer, 2007).

Lateral compaction of gutta percha and a root canal sealer is one of the most widely used obturation technique (Ronald Ordinlo-Zapata, 2009). Cold lateral condensation is probably the most commonly taught and practiced filling technique worldwide and is regarded as the benchmark against which others must be evaluated. The method is generic, encompassing a range of approaches in terms of master cone design and adaptation, spreader and accessory cone selection, choice of sealer and spreader application.

Lateral condensation works by vertical loading of the wedge shaped spreader to move materials vertically and laterally. The greater the taper of the spreader, the greater the lateral component of force. Vertical loads in the range of 1-3 kg have been reported as typical and adequate to deform gutta percha without undue risks to the tooth. Although the technique is described as cold lateral condensation, heat is always applied to sever the root filling at or below canal orifice level and compact it apically with a cold plugger. This may soften and consolidate material several mm into the canal and improve seal (John whitworth, 2005). One of the criticisms against the lateral compaction technique has been the lack of a 3 dimensional fill by the presence of a small amount of gutta percha filled area compared with vertical compaction techniques, especially in oval canals or irregular canals (Wu *et al.,* 2001). However, studies in which the lateral compaction was evaluated shows contradictory results about the ability of sealers to penetrate into dentinal tubules.

A further consideration would be how the sealer was placed in the canal and the degree of canal wall coverage achieved with the technique of placement. There are multiple methods of placement advocated, ranging from paper points to a file, a lentulo, a root canal spreader, ultrasonics, and master gutta percha cone. Laboratory studies have shown that distribution of sealer in canal wall is not affected by the method of sealer placement (Weinman and Wilcox, 1991). However, Stamos et al showed that ultrasonic sealer placement improves the incidence to fill accessory channels (Stamos, 1995). However, depth of sealer penetration could be explained exclusively by the physical properties of the root canal sealer, because a statistical difference was shown in this study.

The analysis of the dentin/sealer interface allows the determination of which materials and filling techniques could obturate the root canals with less gaps and voids. Several microscopy techniques are currently used to evaluate the sealer/dentin interface, including stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) (Marciano *et al.,* 2010). Scanning electron microscopy offers a number of advantages where the images produced using SEM allow highly detailed observation of the dentinal tubules and the integrity and the surface appearance of the sealer cement (Chandra *et al.,* 2012). The adaptation of the sealer cement to the tubule can be seen in detail at high magnification. It allows the observation of sealer cement within the tubules at distant sites from the canal wall where the density of the tubule is less (Ayad *et al.,* 2010). It also allows accurate measurement of penetration depths.

With these factors in mind, this study was conducted to evaluate the dentinal tubule penetration of Apexit plus, AH plus and Roekoseal in instrumented root canals obturated by using cold lateral compaction techniques in the absence of smear layer at the apical one third of root by using scanning Electron Microscopy.

The results of our study showed, the mean value of depth of penetration of three root canal sealers at the apical third of the roots are Group-III(Apexit plus)167.80±0.84, Group II (AH plus)155.60±0.89 and Group I(Roekoseal) 116.00±0.71. Group III and Group II showed higher penetration depth values compared to Group I. This can be attributed to its specific composition, However, Group III showed more penetration into dentinal tubules compared to Group II at the apical third of root. The difference was found to be statistically significant ($p < 0.05$). The results of the present study confirm the findings of other studies that suggest that the penetration of sealer cements may be a function of their chemical and physical characteristics (Oksan *et al.,* 1993).

The values obtained for Group III 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 (Bouillaguet *et al.,* 2007; Iqbal *et al.,* 2001). Group I showed less sealer penetration at the apical third of root when compared to Group II and Group III. The difference was found to be statistically significant ($p < 0.05$). The presence of silicone in the polydimethylsiloxane based Roekoseal sealer, which possibly produces high surface tension forces, thus making its spreading on dentin difficult (Tummala *et al.,* 2012). Besides,its film thickness is higher than that AH plus and Apexit plus, this makes Roekoseal penetrate lesser into dentinal tubules.

The sealer penetration depth in the dentinal tubules depends on many factors like smear layer removal, dentin permeability (the number and the diameter of tubules), root canal dimension and physical and chemical properties of the sealer. The flow is one of the main chemical/physical factors to influence the tubular penetration and is defined as the ability of a sealer to penetrate in irregularities, lateral canals or dentinal tubules of the root canal system (Ana Paula Meirelles Vidotto, 2011). 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. In the present study, 17% ethylene diamine tetra acetic acid and a final rinse of sodium hypochlorite was used, as recommended by other authors. Kokkas et al reported that the successive application of EDTA and sodium hypochlorite removed the smear layer completely and allowed all sealers to penetrate into the dentinal tubules, although to varying depths (Andreas *et al.,* 2014).

The persistence of microorganisms in dentinal tubules, lateral canals and apical ramifications after root canal treatment has been reported. If the filling provides a good seal, it will only impair the exit of bacteria entrapped in the root canal system (Morgental *et al.,* 2011). However, to eradicate the remaining microorganisms, the antimicrobial activity and optimal penetration of the sealer could play an important role. In the present study, the depth of penetration of root canal sealers into dentinal tubules using the lateral compaction technique is influenced by the type of sealer and by the root canal level. Further research into the physical and chemical properties of the root canal sealers is mandatory to establish the specific factors that affect the penetration depth.

Conclusion

Based on this in-vitro study, it is concluded that

- Apexit plus showed deeper sealer penetration into the dentinal tubules than AH plus and Roekoseal sealer.
- AH plus sealer showed deeper penetration than Roekoseal sealer but lesser penetration than Apexit plus.
- Roekoseal sealer showed shallow sealer penetration into dentinal tubules compared to Apexit plus and AH plus sealer.

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