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COMPARATIVE EVALUATION OF THE ADAPTATION OF TWO CALCIUM SILICATE-BASED ENDODONTIC SEALERS WITH A CONVENTIONAL RESIN-BASED SEALER TO DENTINAL WALLS: AN IN VITRO SCANNING ELECTRON MICROSCOPIC STUDY

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ABSTRACT

Aim of the study: The aim of this study was to compare the adaptation of two calcium silicate-based root canal sealers with a resin-based sealer to root canal walls at the middle and apical levels using scanning electron microscope.

Material and methods: Twenty-six single-canalled lower premolars were instrumented and randomly divided into three groups according to the sealer used with gutta-percha (n=8); AH plus, Bioroot RCS, and Endoseal MTA. Two random specimens served as the blank control group to assess the smear layer removal from the dentinal walls. Teeth were sectioned at middle and apical levels and gap width was evaluated using scanning electron microscope. Data were statistically analyzed with significance level set at $p \leq 0.05$.

Results: At the apical and mid-root levels, the highest mean values were found in Endoseal MTA group followed by Bioroot RCS group while the lowest mean value was found in AH Plus group with statistically significant difference between all groups. Statistically significant differences were found between Endoseal MTA and each of Bioroot RCS and AH Plus groups. No statistically significant difference was found between Bioroot RCS and AH Plus groups.

Conclusions: AH Plus and Bioroot RCS showed statistically better results than Endoseal MTA regarding adaptation to the root canal walls.

KEYWORDS: AH Plus, Bioroot RCS, Endoseal MTA, Adaptation, Scanning Electron Microscope.

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INTRODUCTION

Poorly filled spaces in root canals could be the cause of bacterial growth. It was reported that 58% of root canal treatment failures were due to incomplete obturation[1]. Thereby, a three-dimensional canal space filling with a biocompatible material is the ultimate goal to avoid bacterial leakage. Since gutta-percha, as a core filling material, does not directly bond to the canal walls, a root canal sealer is mandatory. Endodontic sealers ensure sealing the canal system by filling the anatomical irregularities, ramifications and dentinal tubules, hence enhancing the adaptation of root filling at the dentin material interface[2]. The ability of root canal sealers to adhere to the core material and to the dentin, adds an advantage in sealing ability and reduction of leakage[3].

Epoxy resin-based root canal sealer AH Plus (Dentsply, Germany) has been used as the gold standard for comparison with other sealers. It offers the advantages of reduced solubility, micro-retention to the root dentin and tight apical seal. Its toxicity when freshly mixed and inability to bond to gutta-percha however remain problems[4].

In the continuous attempt to improve the performance of root canal sealers, new calcium silicate-based sealers have been developed. Bioroot RCS (Septodont, Louisville, USA), is a water-based bioceramic sealer that showed excellent biocompatibility in fresh and set states[5]. It is supplied in powder and liquid form; the powder is compsed of tricalcium silicate, povidone and zirconium oxide; the liquid is an aqueous solution of of calcium chloride and polycarboxylate. When contacting the physiologic solution, this sealer releases calcium and forms an interfacial calcium phosphate (apatite) layer, developing a chemical bond with the dentinal walls and presumes to enhance its adaptability[6,7].

Endoseal MTA (Maruchi, Wonju, South Korea), is another calcium silicate-MTA-based endodontic sealer, containing calcium silicates, calcium aluminates, calcium aluminoferrite, and calcium sulfates. It is a paste-type, premixed root canal sealer based on pozzolan cement that has superior physical and biological properties of MTA. It is preloaded in a syringe allowing its direct application into the root canal. According to the manufacturer, its advantages include; fast setting time, antibacterial effect, biocompatibility, adequate flow, excellent film thickness, and also hard tissue formation stimulation[8].

Good adaptation between sealer and root canal wall not only decreases the chance of microleakage, but also increases the fracture strength of the root[9,10,11]. Adaptation of sealers to canal walls and marginal gaps can be evaluated with scanning electron microscope (SEM), observing the defects at submicron level at the required magnification[12].

To our knowledge, few studies have assessed the adaptation of the sealers used in this study. Therefore, the current study was adopted to compare the adaptation of three sealers; AH Plus, Bioroot RCS and Endoseal MTA. The null hypothesis was that there is no significant difference between the three sealers in their adaptation to root canal walls.

MATERIALS AND METHODS

Specimen Preparation

Twenty-six freshly extracted human single-rooted mandibular premolar teeth without caries, apical or surface resorption and cracks were selected. Teeth with curved roots, abnormal canal morphology and having pulpal calcifications were excluded from the study. To preserve the humidity of dentinal tubules, teeth were stored in saline solution till the time of use.

Teeth were decoronated to standardize the length of the root canal at 14 mm. The working length was determined by inserting a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal until the file was just visible at the apical foramen and then subtracting 1mm. The root canals were instrumented using ProTaper rotary files (Dentsply Maillefer) up to size F3. Canals were irrigated with 3mL of 5.25% sodium hypochlorite (NaOCl) between instruments. After complete preparation, smear layer was removed using 5mL of 17% ethylenediaminetetraacetic acid (EDTA) solution, 5mL of 5.25% NaOCl and finally flushed with 10mL distilled water. The specimens were dried using sterile absorbent paper points (Dentsply, Maillefer).

Two random specimens were selected to serve as blank control group to assess the smear layer removal from the dentinal walls. The remaining 24 samples were randomly and equally divided into 3 groups (n = 8) according to the type of sealer used: group I: AH Plus, group II: Bioroot RCS and group III: Endoseal MTA.

In AH Plus and BioRoot RCS groups, the sealers were prepared according to manufacturer's instructions and introduced into the canal with size #25 Lentulospiral (Dentsply, Maillefer, Ballaigues, Switzerland) at 300 rotations/min to the working length until complete filling of the canal. In Endoseal MTA group, the sealer was injected into the root canal using intracanal tip supplied by the manufacturer, to fill the apical part then slowly withdrawn while sealer was injected until complete filling of the canal.

For the three experimental groups, lateral condensation technique was employed for root canal obturation with master cone size 30/0.06 and completed with size 25/0.02 auxiliaries. The coronal access of all groups was sealed with temporary filling material and the samples were stored in 100% humidity at 37˚C for 10 days to allow the sealers to set.

Assessment of adaptation

After the storage period, the roots were embedded into acrylic resin vertically and sectioned horizontally with Isomet precision cutting machine (Buehler, Germany) at 3, and 7 mm from the apex representing the apical and middle thirds, respectively. Specimens of the control group were further cut vertically for assessment of smear layer removal. Sections were then washed with distilled water for 5 min and dehydrated for observation by scanning electron microscope (SEM). The samples were mounted on an aluminum stub and viewed under SEM (Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses) (FEI company, Netherlands), with accelerating voltage 30 KV. Interfacial gaps between the sealer and root dentin interface were evaluated under ×2000 magnification at middle and apical halves of the root canal by taking photomicrographs. For each section, the maximum gap in microns (µm) was recorded.

Statistical analysis

The mean and standard deviation values were calculated for each group. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests, data showed parametric (normal) distribution. Paired wise sample t-test was used to compare between two groups in related samples. One-way ANOVA followed by Tukey post hoc test was used to compare between more than two groups in non-related samples. Two-way ANOVA was used to test the interaction between different variables. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

The control group showed open dentinal tubules, despite some remaining debris, which did not occlude the tubules Figure (1).

The results were summarized in Table (1) and Figure (2). AH Plus showed the least gap width at middle and apical levels figure (3A), followed by Bioroot RCS figure (3B), the Endoseal MTA figure (3C), which showed the highest mean gap width. In all groups the mean gap width was higher at middle than at the apical third.

At the apical level, a statistically significant difference was found between Bioroot RCS, AH Plus and Endoseal MTA groups where (p<0.001). A statistically significant difference was also found between Endoseal MTA group and each of Bioroot RCS and AH Plus groups where (p<0.001). However, no statistically significant difference was found between Bioroot RCS and AH Plus groups where (p=0.502).

At the middle level, a statistically significant difference was found between Bioroot RCS, AH Plus and Endoseal MTA groups where (p<0.001). A statistically significant difference was also found between Endoseal MTA group and each of Bioroot RCS and AH Plus groups where (p<0.001). On the other hand, no statistically significant difference was found between Bioroot RCS and AH Plus where (p=0.986).

In AH Plus group, a statistically significant difference was found between (Apical/3mm) and (Middle/7mm) where (p=0.002). The highest mean value was found in (Middle/7mm) while the lowest mean value was found in (Apical/3mm).

While in Bioroot RCS group, no statistically significant difference was found between (Apical/3mm) and (Middle/7mm) where (p=0.056), although the highest mean value was found in (Middle/7mm) and the lowest mean value was found in (Apical/3mm).

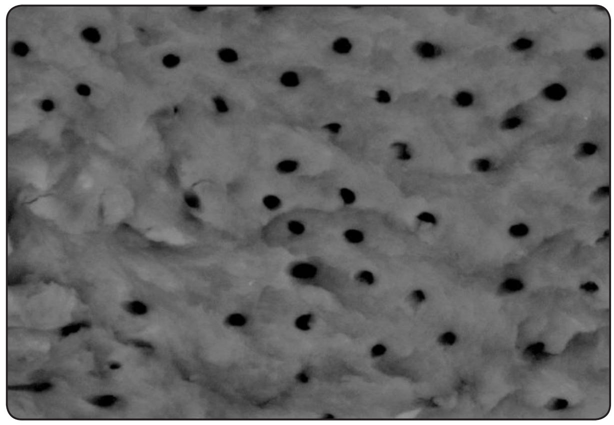

Fig. (1): SEM showing opened dentinal tubules of the control group

As for the Endoseal MTA group, a statistically significant difference was found between (Apical/3mm) and (Middle/7mm) where (p=0.001), the highest mean value was found in (Middle/7mm) and the lowest mean value was found in (Apical/3mm).

Data in Table (2) shows the results of Two-way ANOVA analysis for the interaction of different variables. The results showed that different sealers had a statistically significant effect at p-value <0.001. Also, root level had a statistically significant effect at p-value <0.001. The interaction between the two variables had a statistically significant effect at p-value =0.005.

TABLE (1): The mean and standard deviation (SD) of gap width in different thirds of different groups.

Variables	Gap width						
	Bioroot RCS		AH Plus		Endoseal MTA		p-value
	Mean	SD	Mean	SD	Mean	SD	
Apical/ 3mm	3.25 ^{aB}	2.05	^{bB} 2.47	0.80	7.55 ^{bA}	0.84	**<0.001***
Middle/ 7mm	5.02 ^{aB}	3.04	^{aB} 4.80	1.58	14.17 ^{aA}	3.31	**<0.001***
p-value	**0.056ns**		**0.002***		**0.001***		

Means with different small letters in the same column indicate statistically significant difference; means with different capital letters in the same row indicate statistically significant difference. *; significant (p<0.05) ns; non-significant (p>0.05)

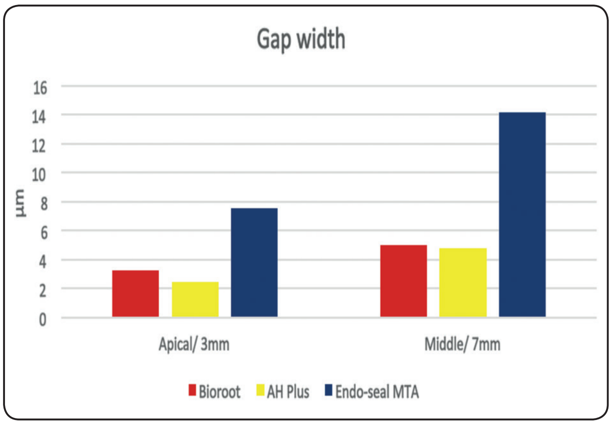

Fig. (2): Bar chart representing gap width of different groups

TABLE (2): Results of Two-way ANOVA for the effect of different variables on gap distance evaluation.

	Type III	df	Mean Square	*F* - value	*P* - value
Sealers	520.796	2	260.398	55.343	<.001*
Root level	153.117	1	153.117	32.542	<.001*
Sealers x root level interaction	56.073	2	28.036	5.959	.005*

df: degrees of freedom = (n-1), * Significant at P ≤ 0.05

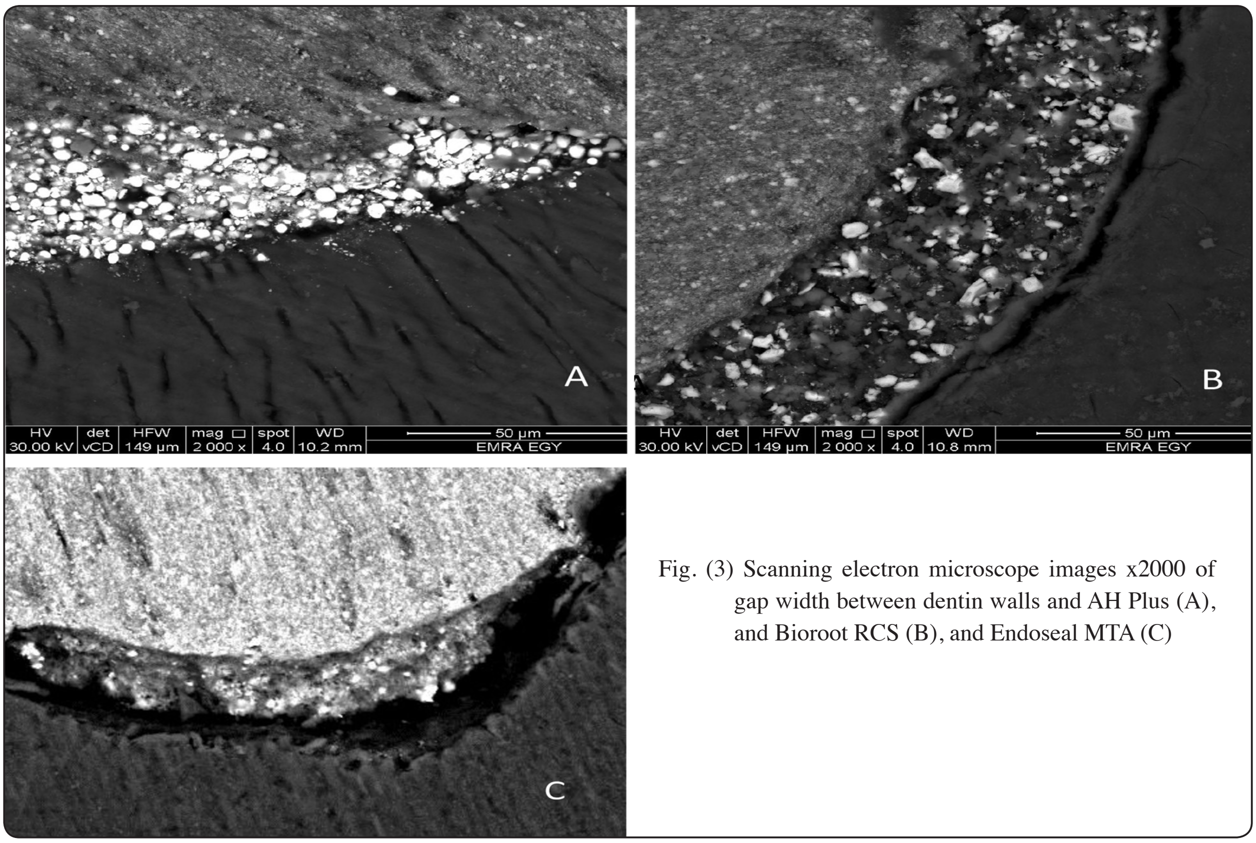

Fig. (3) Scanning electron microscope images x2000 of gap width between dentin walls and AH Plus (A), and Bioroot RCS (B), and Endoseal MTA (C)

DISCUSSION

At the apical and middle root levels, a statistically significant difference was found between AH Plus, BioRoot RCS and Endoseal MTA groups (p<0.001). Based on these results, the null hypothesis was rejected in this study.

The main function of a root canal sealer is to fill the imperfections and increase the adaptation of the filling material to the root canal walls. Ideal root canal sealer must be biocompatible, and should have low surface tension to allow penetration into irregularities and good wettability to provide fluid tight seal.[13]

In this study, scanning electron microscope was used for the assessment of marginal gap along with horizontal cross-sectioning of the specimens. This allowed observing the adaptation and measuring the defects around the entire canal lumen at the selected levels for a more precise and complete evaluation, unlike vertical sectioning of specimens which permits evaluation in only two selected planes.

The lateral condensation of gutta-percha with the root canal sealers was chosen to be the obturation method in this study. Since the sealer may display a variable level of solubility, depending on its physical and chemical nature, it is crucial to utilize a sealer that has a minimum film thickness adjacent to the dentinal wall[14,15]. Moreover, by revealing a thin film thickness, the sealer may also infiltrate deeper into the canal irregularities. Hence, the supreme outcome of root canal obturation is to achieve a great volume of gutta-percha and a least volume of sealer within the root canal space [12,16] with improved permeation into the canal irregularities and dentinal tubules.

Degree of adhesion and penetration of sealers into dentinal tubules is influenced by several factors such as physical and chemical properties of the sealers, dentin permeability, filling technique, and smear layer removal[17]. The significance of smear layer removal was assessed by Oksan et al. 1993[18], Kouvas et al.1998[19], Kokkas et al. 2004[20], and Sonu et al. 2016[21], who concluded that the removal of smear layer had lead to the deeper penetration of sealers into dentinal tubules, thus reducing microleakage and increasing the root canal treatment success rate.

The use of a chelating agent such as 17% EDTA solution to remove the inorganic component, followed by 5.25% NaOCl solution to dissolve any remaining organic component is the most commonly used regimen to remove the smear layer[22,23,24]. EDTA easily enters dentinal tubules due to its low surface tension and eliminates smear layer up to the depth of 2.5-4 µm [25]. Thus bonding and adaptation of sealers to root canal walls are expected to increase[26]. Finally, distilled water was used to counteract the lasting effect of irrigants used.

In contrast with the results of previous studies[27,28], in this study less gap width was observed at apical level for all the sealers than at middle level. This difference could be accounted to the round cross section at the apical region conforming more to the round cross section of the filling core material. Greater mean gaps at the middle root area could be ascribed to the difficulty posed by the premolar root canals' oval shape. Published literature had indicated that this area could prove challenging during preparation and subsequent filling, especially with cold lateral compaction[29] hence might affect the sealer diffusion into the root dentin[30].

AH Plus sealer exhibited the least gap width values with the root dentin, a result which came in agreement with many previous studies[31,32,33]. AH Plus enhanced interfacial bonding and adaptation could be attributed to many factors. Its chemical bonding to root dentin by forming covalent bonds between the epoxy resin and any exposed amino groups in collagen might be one of the causes of the excellent adaptation. Besides, being chemically cured, AH Plus compensates for polymerization shrinkage and exhibits zero polymerization stresses[34,35]. AH Plus also shows pseudoplastic behavior, a term describing liquids exhibiting a thixotropic behavior by a decrease in viscosity when there is an increase in shear rate during compaction[36]. Furthermore, the slight acidity of AH Plus might result in self-etching when comes in contact with dentin, thereby enhancing bonding and adaptation[17].

In our study, BioRoot RCS showed similar adaptation to dentinal walls as AH Plus. It has been shown that infiltration of BioRoot RCS mineral content into the intertubular dentin, results in the formation of a mineral infiltration zone after denaturation of the collagen fibers by the strong alkalinity of the sealer[37,38].

Moreover, previous studies demonstrated that BioRoot RCS had higher calcium ion release over a prolonged duration than other sealers. This prolonged mineralizing ion release triggers the nucleation of calcium phosphate resulting in the formation of hydroxyapatite along the mineral infilteration zone which may improve the adaptation and sealing of the sealer[39,40,41].

Although Endoseal MTA has also demonstrated an alkaline nature and calcium release, yet in this study it showed more interfacial gaps and the least adaptation to dentinal walls. Previous studies suggested that the reason of the low bonding efficiency of MTA-based sealers to dentinal tubules was due to the formation of apatite by MTA over its own surface, hence creating poor microtags on setting[42,43].

Another possible explanation could be as described by Türker et al[44], who evaluated the effect of smear layer removal on the adhesion of MTA-based sealer with root canal dentin, and concluded that the smear layer had an essential role in the formation of the interfacial layer between the MTA-based sealers and root dentin. Yildirim et al[45] reported that due to the humidity of the root canal wall, the smear layer, which acts a coupling agent, might have a helpful effect on the adhesion of MTA-based sealers to the root canal dentin.

Since not used in bulk, the MTA-based sealers in the middle part of root might remain unset due to lack of water for hydration reaction unlike the apical part of the root, which might be the cause of increased interfacial gaps in the middle root sections[46].

CONCLUSIONS

Within the limitations of this study it could be concluded that AH Plus and BioRoot RCS were better than Endoseal MTA regarding adaptation to the root canal walls. Although MTA has confirmed to be successful in numerous other clinical applications, further researches should be conducted to decide whether MTA-based sealers themselves or the method of their placement could be adjusted to enhance their performance as a root canal sealers.

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