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INTERFACIAL ADAPTATION AND PENETRATION DEPTH OF RECENT BIOCERAMIC ROOT CANAL SEALERS TO DENTIN: AN IN VITRO STUDY

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ABSTRACT

Introduction: This study aimed to investigate the interfacial adaptation of CeraSeal and Bio-C Sealer in comparison to AH Plus using scanning electron microscope (SEM). Additionally, to compare penetration depth of CeraSeal and Bio-C Sealer with AH Plus using confocal laser scanning microscopy (CLSM).

Materials and Methods: Sixty-six extracted single rooted human premolars were collected and randomly divided into three groups according to sealer type AH Plus, Bio-C Sealer and CeraSeal using single cone obturation technique. Then each group was divided into 2 subgroups (n=11), one of the subdivided groups was obturated, then samples were cut longitudinally to be evaluated for interfacial adaptation using SEM at magnification 500x. For the other 33 samples, the sealer was mixed with Rhodamine B dye obturated, then the samples were cut horizontally at (3, 6 and 9 mm) to be evaluated for depth of penetration using CLSM.

Results: For interfacial adaptation, AH Plus showed better adaptation without statistically significant difference between them. The maximum depth of penetration, Bio-C Sealer showed the maximum penetration, without statistically significant difference between them. While for penetration %, there was a statistically significant difference between the three sealers, with the Bio-C showed the best penetration percentage.

Conclusions: Within the limitations of this study, it was concluded that AH Plus showed the best insignificant sealer adaptation to the root canal wall, while Bio-C sealer showed the most optimal dentinal tubular penetration of the tested sealers.

KEY-WORDS: Adaptation, Bioceramic, Interfacial, Penetration, sealers

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INTRODUCTION

Endodontic treatment aims to afford 3D root canal filling with a hermetic seal to eliminate or prevent apical/coronal leakage that lead to apical periodontitis [1]. Endodontic sealers are used in root canal obturation in conjunction with gutta-percha to seal the main, lateral and accessory canals, fill voids & irregularities, creating a bond between gutta-percha and root canal wall, furthermore they act as a lubricant to facilitate placement of core material [2]. Thus, sealer penetration and interfacial adaptation to root canal walls are necessary properties [3] as they increase the surface contact between root canal wall and core material [4]. In the same way, interfacial adaptation between root canal filling material and dentinal walls is a crucial issue to be concerned, as most failures occur at core-sealer interface and dentin boundary lead to failure of endodontic treatment. Resin based sealers are the most widely used sealers due to their low solubility and better sealing ability, but their chief disadvantages are cytotoxicity, hydrophobicity and mutagenicity [5], to subside these problems, calcium silicate-based sealers have been advocated.

Bioceramic sealers have been used in endodontics 30 years ago, they are ceramic materials composed of alumina, zirconia, calcium phosphates, hydroxyapatite, glass ceramics and calcium phosphates with superior biological and physical properties. Their major advantages are great biocompatibility to surrounding tissues that permit chemical reaction with hydroxy apatite of tooth structure improving the bond of sealer-to-root dentin. CeraSeal (Meta-BioMed, Co., Cheongju, Korea), and Bio-C Sealer (Angelus, Londerina, PR, Brazil) are recent calcium silicate-based sealers that have similar properties. This study investigated them in a comparison with AH Plus which is considered a bench for comparison, using scanning electron microscope (SEM) to determine interfacial adaptation with dentinal walls, while using confocal laser scanning microscope (CLSM) to assess depth of sealer penetration into dentinal tubules.

Null hypothesis

There was no significant difference between all tested sealers regarding their interfacial adaptation as well as the depth of penetration.

MATERIALS AND METHODS

The experiment samples size was analysed according to G*Power, using one-way ANOVA test clarified that total sample size of 66 samples was satisfactory to detect the effect size of 0.4. Freshly extracted sound unidentified human single rooted premolars were disinfected in sodium hypochlorite (5.25%), then kept in 10% natural buffered formalin at room temperature to preserve their humidity. Samples were equally and randomly distributed into three main groups and six sub groups (n=11) based on type of sealer used and the assessment method.

All teeth were decoronated at 1mm coronal to the cement enamel junction, in order to standardize root sections of 12mm length, using diamond disk (Dica, Dendia, USA) under copious amount of water irrigation. Canal patency was checked using #10K file, then the working length for each tooth was determined. Preparation of canals was completed using TruNatomy rotary file system (Dentsply Sirona, Ballaigues, Switzerland) at 500 rpm and 1.5 torque till final apical diameter reach #35 taper 0.04. Irrigation was done using 3ml of 2.5% sodium hypochlorite between each file with total volume 15 ml per each sample using (#30 gauge) side vented endodontic needles.

To remove smear layer, 2 ml of 17% EDTA was used as final irrigation for 1 minute. A final rinse was then obtained with 5ml of distilled water, followed by dryness of canals using multiple sterile absorbent paper points.

A- Adaptation of sealers on dentinal wall

I- Obturation of root canal specimens

After instrumentation, the #35 taper 0.04 gutta-percha master cone was verified for retention and resistance, then the root canals were obturated each group with its type of sealer, group 1 (n=11) was obturated using AH Plus (Dentsply DeTray GmbH, Konstanz, Germany) 1:1 according to manufacturer instructions, group 2 (n=11) was obturated using Bio-C Sealer (Angelus, Londerina, PR, Brazil) and group 3 (n=11) was obturated with CeraSeal (Meta BioMed, , Chungcheongbuk-do, Korea) which are pre mixed sealers and for standardization 3mm of each sealer was measured on a glass slab using millimeter rule. After that the sealer was introduced into the prepared canal using the single cone technique. The master point was then cut off at the orifice level using a heated endodontic plugger. The orifice was then sealed using Medifil glass ionomer filling (Domagkstrasse , Neumuenster. Germany).

II- Preparation of specimens for SEM

The roots sectioned vertically with IsoMet 4000 microsaw (Buheler, Dusseldorf, Germany) at 2500 rpm for 10 mm/min under water cooling. The roots were then fixed on an aluminum end, positioned in a vaccum and then targeted sputter coated with gold. Then specimens were viewed using SEM (Ametek edax, New Gersey, USA). Gaps were assessed under 500x magnification at coronal, middle, and apical cuts by taking photomicrographs. (Figure 1)

B- Penetration depth of sealers

I- Obturation of root canal specimens:

Before obturation, tested sealers were mixed with the Rhodamine B dye 0.1% (Sigma-Aldrich, ST, Louis, MO, USA). For test standardization, 10 parts of the tested sealer were mixed with 1part of dye solution. Then each sealer was introduced in root canals using #35 taper 0.04 gutta-percha master cone in a single cone technique.

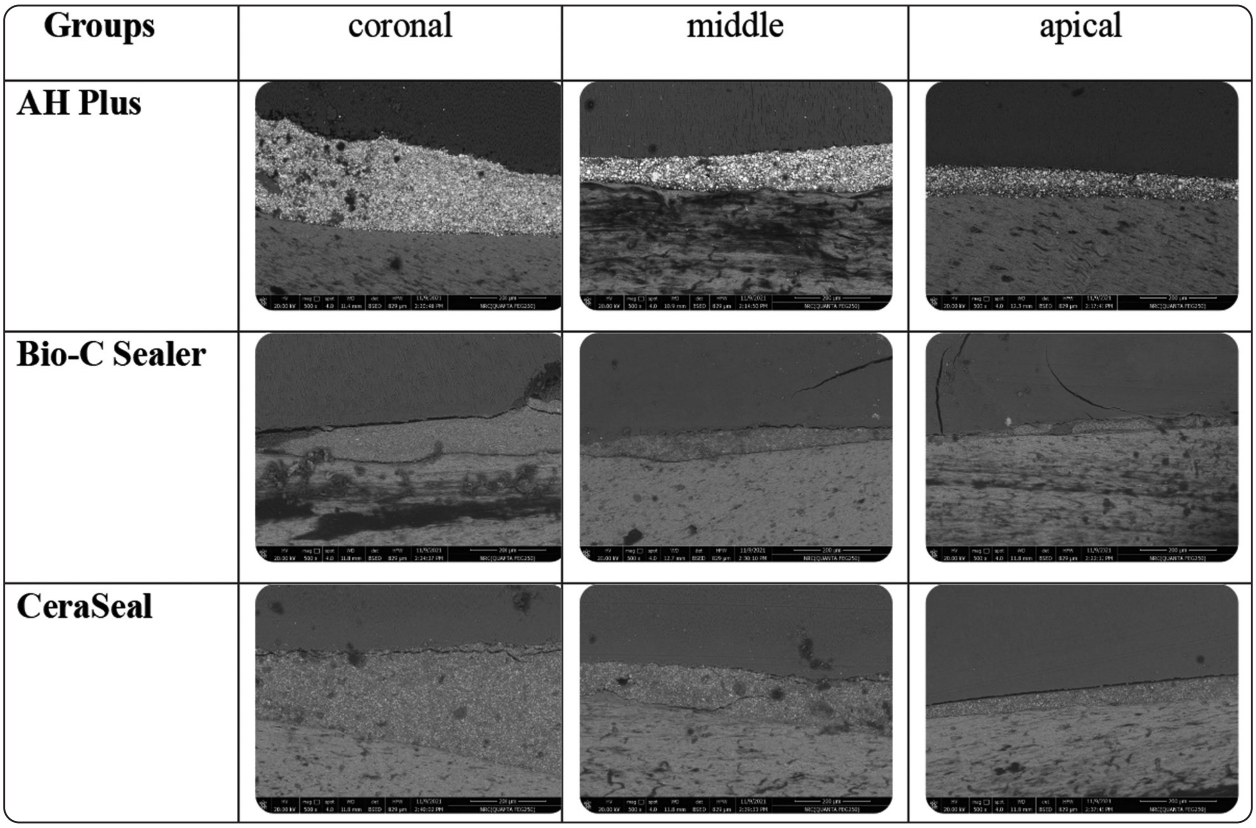

Fig. (1) Scanning electron microscope images showing interfacial adaptation of the three groups

II-Preparation of specimens for CLSM

The 33 roots were sectioned horizontally at 3mm, 6mm and 9mm from apex using IsoMet 4000 microsaw under copious amount of water to be examined under CLSM (Leica DMi8, Germany).

Data were collected and analyzed using image J software, for interfacial adaptation, data was then statistically analyzed using Kruskal-wallis and Mann witney test and $p \leq 0.05$ was considered statistically significant. While for depth of penetration, statistical analysis was performed using ANOVA test (one and two way) followed by post hoc considering $p \leq 0.05$ was statistically significant.

Pearson correlation was conducted to determine the correlation between both interfacial adaptation and penetration %, and maximum penetration and penetration %. (Figure 2)

RESULTS

For interfacial adaptation, no statistical difference among the three sealers was found, taking into consideration that AH Plus showed better adaptation than Bio-C and CeraSeal. For AH Plus and CeraSeal gaps increased in a corono-apical direction, while for Bio-C the least gaps were at the middle followed by apical and then the coronal thirds .

For maximum depth of penetration, no significant difference between the three sealers, but Bio-C Sealer showed the maximum penetration followed by CeraSeal and then AH Plus. For each sealer the depth of sealer penetration increased in an apico-coronal direction.

For penetration %, a statistical change was recorded between the three sealers, with the Bio-C showed the best penetration %, followed by CeraSeal and then AH Plus showed the least depth of sealer penetration. There was a strong positive correlation between maximum penetration depth and the penetration percentage results for the three groups of sealers .

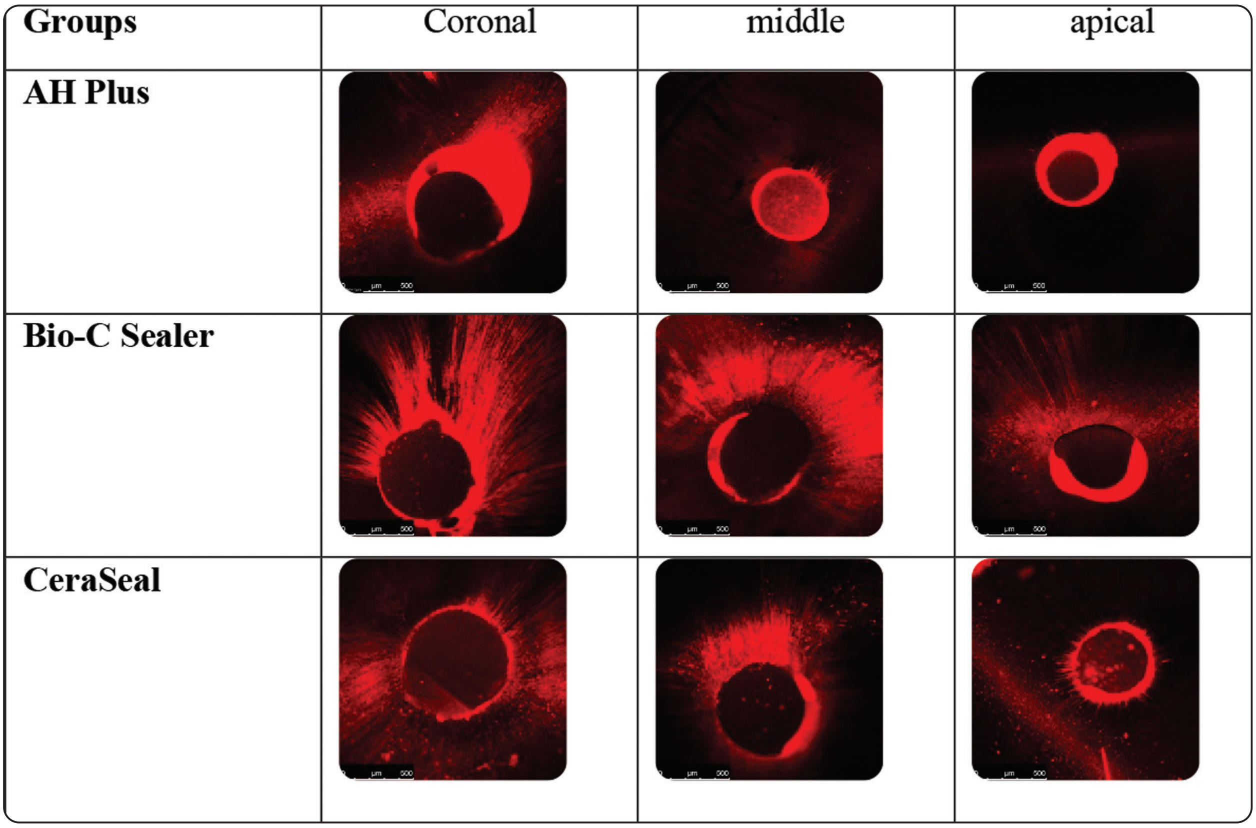

Fig. (2) Confocal laser scanning microscope images showing sealer penetration of the three groups

TABLE (1) Correlation between the Maximum penetration depth results and the penetration percentage results for the three groups.

	r**	P-value	Correlation type
AH Plus	**0.917**	**0.001[HS]**	Strong positive
Bio-C	**0.786**	**0.012[S]**	Strong positive
CeraSeal	**0.853**	**0.003[S]**	Strong positive

There was a moderate positive correlation between adaptation and penetration percentage results for the three groups of sealers

TABLE (2) Correlation between the adaptation results and the penetration percentage results for the three groups.

	r**	P-value	Correlation type
AH Plus	**0.665**	**0.048[S]**	Moderate positive
Bio C	**0.493**	**0.177[NS]**	Moderate positive
CeraSeal	**0.471**	**0.200[NS]**	Moderate positive

DISCUSSION

Ideally sealer should be biocompatible with good flowability to allow penetration into canal irregularities and good wettability to provide fluid tight seal(6). This outcome was gained despite of a high % of gutta percha and minimal sealer (7). Adhesion and penetration of sealers into dentin is influenced by some factors as chemical and physical properties of the used sealer, dentin permeability, root canal filling method, and removal of smear layer (8,9).

The test null hypothesis of the current study was partly rejected, for interfacial adaptation our results showed no significant difference between the tested sealers, taking into consideration that AH Plus showed the least gap, followed by Ceraseal and the highest gap value was recorded in Bio-C sealer. This result was in agreement with (10–12), who showed that AH Plus offered better adaptation than other sealers. While it was disagreed with (13–15). Good adaptation of AH Plus might be because of chemical bond formation with root dentin (16). Therefore, AH Plus lead to formation of regular, and streamline chemical adhesion with root canal walls. Moreover, the minor acidity of the sealer might cause self-etching to root dentin, so enhance adaptation and bonding (17).

In the current study, AH Plus and Ceraseal recorded the least gap values in the coronal third followed by the middle third then the apical third. This finding was consistent with those of previous studies (8,18,19). High mean gaps values at the middle and apical root region might be attributed to the oval shape of premolar root canals used in this study. That might explain the presence of high interfacial gaps in these areas (20). Another possible clarification could be due to the cementum like structure and atubular dentin in these regions, moreover the reduced effectiveness of smear layer removal techniques closer to the apex (21).

While for Bio-C sealer the highest gap value was in the coronal third followed by the apical third and then the middle third. This result may be due to variations in root canal anatomy (22).

Confocal laser scanning electron microscope (CLSM) is a widely used method for evaluation of the penetration ability of sealers (23,24). As it could provide a detailed view of the spread of sealers inside dentinal tubules along the canal circumference of each sample using fluorescent dye, it has the capability to gather a lot of sections, even from thick sample (25). Also, the samples under CLSM can be visualized in various depths (26,27,28).

Some studies advocated maximum penetration(4,29). Others used penetration percentage(30). We endorsed both methods in this study to reproduce more reliable results (31).

For the three sealers maximum penetration, no statistical difference between coronal, middle and apical thirds in penetration was recorded. Although the maximum penetration was the highest coronally then decreased in the middle third and showed the least values in the apical one. This came in harmony with the fact of presence of higher number of dentinal tubules in the coronal third as well as their diameter is larger allowing for more sealer penetration, as well as the formation of sclerotic dentin and cementum like structure apically might reduce sealer penetration [32]. This result was in agreement with previous studies recorded fewer tubule penetration in the three thirds [10,32,33,34,35]. Worth meanings, no significant difference was recorded among the three sealers in their maximum penetration. Taking into consideration that maximum penetration values was displayed by Bio-C followed by Ceraseal, while AH Plus showed the least value of maximum penetration. This comes in agreement with [13] who reported that Bio-C Sealer had better penetration than AH Plus. But it was against [6,20,36].

For the three sealers penetration percentage, there was a statistically significant difference between coronal, middle and apical thirds, with the higher percentage of penetration recorded at the coronal third then gradual decrease at the middle and then the least penetration values were apically because of histological characteristics of the apical root dentin, described as sclerotic and poorly permeable dentin that has fewer dentinal tubules compared to middle and coronal thirds dentin [10] .These results were consistent with previous studies [30,31,37]. A statistically significant difference between the tested sealers was recorded with the maximum penetration percentage in Bio-C followed by CeraSeal then AH Plus in all the three thirds. These findings were in agreement with [13,29,30] who showed that bioceramic allow greater penetration than AH Plus.

While these findings were against other studies[10,23,38]. High penetration of Bio-C Sealer might be related to its high flowability, its consistency and nanometric particles as it is less than 2µm according to its manufacturer, which subsequently affects sealer penetration into irregularities of root canal systems[39]. As in earlier studies that revealed that Bio- C Sealer showed higher flowability than Ceraseal [40,41]. Also, according to recent study, CeraSeal did not properly optimize the ANSI/ADA standardization of sealers flowability [42]. In contrast, AH Plus having larger size, so polymerization shrinkage or problems during mixing might diminish its permeability into the dentinal tubules, the mixing process of sealers might have a factor in the sealer penetration into dentinal tubules [13].

Finally, by correlating interfacial gap results to the penetration percentage, there was a positive correlation. The previous result was inconsistent with the published study [43], where they found that there is no correlation between interfacial gap and penetration percentage. Different assessment methods might be the reason, as they used SEM to detect both parameters. In the current study, independent models were conducted.

CONCLUSION

Within the limits of the present study, it was concluded that AH Plus showed the highest adaptation to the root canal wall, while Bio-C sealer displayed the most optimal tubular penetration of the tested sealers.

Significance

Sealer penetration and interfacial adaptation are desirable properties as they decrease microleakage, by increasing the surface contact between root canal wall and obturating material, thus improving quality of root canal treatment

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