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ADAPTATION AND PENETRATION OF THREE DIFFERENT ROOT CANAL SEALERS WITH SINGLE CONE OBTURATION TECHNIQUE: A SCANNING ELECTRON MICROSCOPE STUDY (AN IN VITRO STUDY)

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ABSTRACT

INTRODUCTION: Obturation materialswith superior adaptation and sealing capacity are crucial for root canal treatment success.
OBJECTIVE: To compare adaptation and tubular penetration depth of three different sealers (Ceraseal, AH Plus and Endofill)with single cone obturation technique using scanning electron microscope (SEM).
MATERIALS AND METHODS: Thirty-six extracted single canaled mandibular premolar teeth were decoronated and prepared using ProTaper Universal rotary files till F3. Teeth were randomly divided into three groups according to sealer type used: Group I: Ceraseal (Bioceramic), Group II: AH Plus (Resin), Group III: Endofill (Zinc Oxide Eugenol). All roots were obturated with a single F3 cone with one of the assigned sealers. Teeth were sectioned into threethirds horizontally and analyzed for sealer adaptation and penetration using SEM.
RESULTS: Regarding sealer adaptation,roots filled with AH Plus showed better adaptation (less mean gap sizes) than other sealers in the three canal thirdsand the least mean gap sizes were recorded in the apical third in all groups. Along the complete canal length,a significant difference was found between AH Plus group and other groups ($P <0.0001$).Regarding tubular penetration depth, Ceraseal showed greater mean penetration depth in the middle and apical thirds than other sealers. However, along complete canal length, no significant difference was found between Ceraseal and AH Plus groupswith a significant difference noted between both groups and Endofillgroup ($P <0.0001$).
CONCLUSION: Single cone obturation with AH Plus and Ceraseal could result in significant better adaptation and tubular penetration than Endofill sealer.
KEYWORDS: AH Plus, Ceraseal,Endofill, Single cone obturation, Sealer adaptation and penetration.
RUNNING TITLE: Adaptation and penetration of three sealers with SC.

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INTRODUCTION

Following biomechanical preparation of root canals, residual bacteria or their byproducts may still be present in the complex canal anatomy and dentinal tubules (DT). Accordingly, a tight seal of the root canal system is mandatory to prevent micro-leakage and re-infection (1,2).

There is a tendency for the remnant bacteria and their byproducts to enter the DT due to its permeability.As a result, obturating complex root canal systems using filling materials that has high adaptability to the canal walls and superior sealing capacity isdesirable to entomb the residual bacteria(2,3).

The contact between the obturation material and dentin is increased by deep tubular sealer penetration depth, which improves the root canal system's sealing capacity. Moreover, when in direct contact with microorganisms, its antibacterial effect increases and may inhibit bacteria from escaping through the DT.It has also been speculated that greater tubular penetration depth might strengthenendodontically treated teeth(4).

Numerous techniques have been described to establish a root canal space free of voids and to ensure that the filling materials adapt well to root canal dentin (4).Severalstudies showed acceptable results when using single cone(SC) obturation techniquecorresponding to the taper and apical diameter of canals prepared using nickel titanium engine driven files (4-5).

The SC technique is a simple obturation technique that doesn't require much time or a lot of skill from the operator to be executed (4-5). However, The main disadvantage of the SC obturation technique is the use of large volume of sealer that can result in gaps and voids in the final obturation mass due to the contraction of sealer upon setting leading to treatment failure. However, it has been claimed that sealers that are dimensionally stable, such as bioceramic sealers can overcome this problem (6).

Bioceramicsealers introduced since 2007(7). They have a number of benefits, such as being biocompatible, safe, antibacterial, and stable in biological environments (7).Bioceramics are not affected by moisture or blood contamination, so they are usually easier to work with than other materials.They slightly expand as they set, but once they are set, they are dimensionally stable and might provide a good seal in the root canal system(7).

Ceraseal is a new pre-mixed endodontic sealer made by Meta Biomed Co. in Cheongju, Korea. It contains calcium silicates, zirconium oxide, and a thickening agent(8).However, studies regarding its adaptation and penetration into the DT using SC obturation technique are still lacking.

AH Plus is an Epoxy resin based sealer and is considered the gold standard sealer due to its superior physical and chemical properties having low volumetric changes after setting and good flowability which makes it optimal for testing and comparison of adaptation and penetration into the dentinal tubules (9).

Zinc oxide eugenol based sealers were introduced to the market by Grossman in 1936 to be used with gutta-percha and silver cones, it has good mechanical and physical properties and it may slightly expand after setting due to water sorption by its components after polymerization, making it ideal for testing for adaptation into the dentinal tubules(10).

Accordingly, the research question was would the bioceramic sealer (Ceraseal) show better adaptation and tubular penetration abilities compared to the gold standard epoxy resin sealer (AH Plus) (9) and zinc oxide-eugenol-based sealer (Endofill).

The null hypothesis of this research was that there would be no difference between the tested sealers regarding their adaptation and tubular penetration abilities.

MATERIALS AND METHODS

ThisstudywasacceptedbytheethicalcommitteeattheFacultyofDentistry,AlexandriaUniversity (serial #0257-06/2021).

Sample size estimation

The sample size was calculated using GPower version 3.1.9.2(11). Based on the results of a previous study (12), by adopting a power of 80% to detect a standardized effect size in the tubular penetration (d=0.550) (large-sized standardized effect), the minimum required sample to achieve a power of 80%(βerror) and an (α error) of 5 % (p-0.05) to detect a significant difference was calculated to be 36 teeth (n=12 per group). Any sample loss during the study was replaced to maintain the sample size (13).

Canal preparation

This study was conducted on 36 human single-rooted mandibular premolar teeth extracted for orthodontic purposes.Teeth selected were free from caries, cracks, and fractures. Any debris and calculus were removed from the teeth, then buccal and proximal view radiographs were done to include teeth with single canal.

Teeth were decoronated to standardize a length of 14mm and the working length (WL) was measured by introducing a size 10 K-file (Mani, Tochigi, Japan)in the canal till it was just visible at the apical foramen then subtracting 1mm from this length (12).Size #15 hand K file (Mani, Tochigi, Japan) was used to establish the glide path,then canals were mechanically instrumented using ProTaperUniversal rotary file system (Dentsply Maillefer, Ballaigues, Switzerland) mounted on XSmart plus endodontic motor (Dentsply Maillefer, Ballaigues, Switzerland)till size F3 (12).EDTA chelating gel (MD.ChelCream, MetaBiomed, Korea) was placed on each rotary file during preparation and canals were irrigated with 4mL of 2.5% NaOCl (Clorox for Chemical Industries, A.R.E)after each file change (12). Size #10 K filewas used to maintain apical patency.

For smear layer removal, canals were irrigated with 10 mL of 17% EDTA (Calix EDTA, DHARMA Research, USA) and 10 mL of 2.5 %NaOCl followed by a final rinse of distilled water for 5 minutes (14).

All irrigating solutions were delivered using 30G side vented needle (PPH CERKAMED, StalowaWola, Poland) 1mm shorter than the WL. After which, canals were dried using F3 paper points (Dentsply Maillefer, Ballaigues, Switzerland) (14).

Two specimens (not included in the sample size) were randomly selected and examined under SEM to verify the absence of the smear layer (Figure 4).

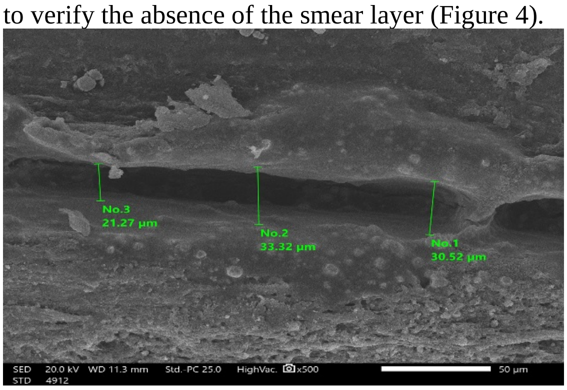

Figure (3): Representative SEM micrograph (×500) of sealer-dentine interface showing Endofill maximum penetration at Coronal third and maximum gaps recorded at the same third.

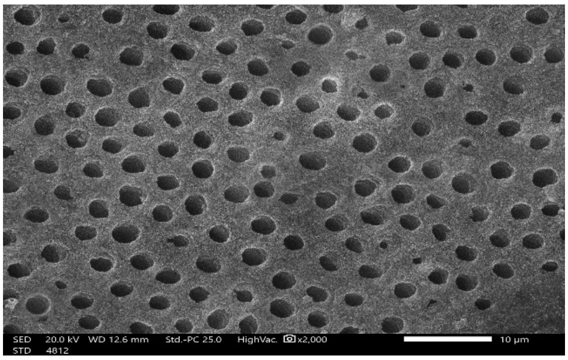

Figure (4): Representative SEM micrograph (×2000) showing smear layer removal in control group.

Root canal filling

The specimens were numbered and randomly assignedinto 3 equal groups (n=12). Using permuted block technique,where samples were assigned in blocks of 4 (15).

Study Group I: Ceraseal (Meta Biomed Co., Cheongju, Korea)(Bioceramic sealer group).

Study Group II: AH Plus (Dentsply Sirona, York, PA, USA) (Resin sealer group).

Study Group III: Endofill(Dentsply, Petropolis, Brazil) (Zinc oxide eugenol group).

In group I, the sealer was introducedby using a manufacturer-provided application tip. The tip of the applicator was placed 3mm shorter than the WL. without binding in the canal and slowly injected while moving in a coronal direction till the sealer was visible at the orifice level. The F3 cone was then coated with the sealer, placed in the canal and seared at the orifice level using a hot plugger.

In groups II and III, sealers were mixed according to manufacturer instructions and placed in the root canals using a lentulo spiral size #25(Dentsply Maillefer, Ballaigues, Switzerland). The F3 cone was then coated with the assigned sealer, placed in the canal and seared at the orifice level using a hot plugger. The obturation quality was checked with two periapical radiographs (buccal and proximal views). ColtosolF temporary filling (Coltene/Whaledent AG, Switzerland) was then used to seal the root canal orifices.

Specimens were then incubated at a temperature of 37ºC and 100% relative humidity for 7 days allowing complete setting of the sealers. All specimens were prepared and filled by the same operator.

Scanning electron microscope analysis

After incubation, root slices were cut at 2, 5 and 8 mm fromthe apex using a water-cooled diamond saw. The samples were dehydrated, sputter coated with gold, mounted on a copper stub, and observed under SEM (JEOL Ltd,Tokyo, Japan) at the three levelsrepresenting apical, middle and coronal thirds. Photographs were taken for each thirdwherethe maximum gap size in micrometers (adaptation) and the point of maximum visible tubular penetration was measured for each specimen.

Two experienced observers examined the images two times at different time intervals. Blinding of the specimens was provided.

Statistical analysis

Normality of penetration and adaptation in micrometer were checked using Shapiro Wilk Test, descriptive and box plot. Data was found to be normally distributed. All variables were mainly presented by mean and standard deviation (SD) in addition to median, minimum, maximum and inter quartile range. Comparison of adaptation and penetration in micrometer between groups were done using One Way ANOVA followed by pairwise comparisons with Bonferroni adjustments.Repeated measures ANOVA was used to assess differences between coronal, middle and apical thirds.Significance level was set at P value of 0.05. Data were analyzed using SPSS for windows version 23. Intra-examiner and inter-examiner reliability were assessed and showed moderate to excellent agreement (kappa ranged from 0.77-0.99).

RESULTS

Sealer adaptation

In the coronal third, the least mean gap size was found in group II with mean gap size of 5.56 ± 1.31 µm. In addition, a statistically significant difference was found between the three study groups (P<0.0001). In the middle & apical thirds the least mean gap size was also found in group II with mean gap size of 4.41 ± 1.45 µm. However, no statistically significant difference was found between groups I & II, while a significant difference was noted between both groups and group III (P<0.0001). Along complete canal length, group II showed the least mean gap size measurement of 4.03± 0.6 µm, with a statistical significant difference with other study groups (P<0.0001) (Table 1& Figures 1,2).

Table (1): Comparison of mean marginal gap sizes (adaptation) in µm in the three canal thirds and along complete canal length in all study groups.

		Group I (n=12)	Group II (n=12)	Group III (n=12)	Test (p value)
Coronal	Mean (SD)	7.30 (1.55)^{a}	5.56 (1.31)^{b}	15.90 (2.20)^{c}	123.666 **(<0.0001*)**
	Median (IQR)	7.30 (1.65)	5.42 (1.97)	16.35 (4.51)	
	Min - Max	4.45 – 10.55	3.48 – 7.65	12.81 – 18.76	
Middle	Mean (SD)	5.23 (0.96)^{a}	4.41 (1.45)^{a}	14.18 (3.07)^{b}	84.677 **(<0.0001*)**
	Median (IQR)	5.17 (1.280	3.81 (2.34)	14.79 (4.07)	
	Min - Max	3.46 – 6.96	2.74 – 6.92	8.31 – 18.71	
Apical	Mean (SD)	3.78 (1.41)^{a}	2.13 (1.69)^{a}	13.47 (2.96)^{b}	99.298 **(<0.0001*)**
	Median (IQR)	3.44 (2.74)	1.90 (3.15)	13.96 (5.52)	
	Min - Max	2.11 – 6.31	0.0 – 5.16	8.15 – 16.58	
Test (p value)		23.520 **(<0.0001*)**	2.498 (0.105)	13.051 **(<0.0001*)**	
Pairwise comparisons		**P_1=0.007*** **P_2≤0.0001*** P_3=0.052	-	P_1=0.242 **P_2=0.001*** **P_3=0.040***	
Overall	Mean (SD)	5.44 (0.84)^{a}	4.03 (0.60)^{b}	14.52 (1.64)^{c}	310.746 **(<0.0001*)**
	Median (IQR)	5.21 (0.94)	4.06 (1.14)	14.25 (2.99)	
	Min -	4.30 – 7.06	3.27 –	12.65 –	

	Max		5.06	17.63	

* Statistically significant difference at p value≤0.05
abc Different superscript letters denote statistically significant difference between groups within the same row
P1: Comparison between coronal and middle thirds,
P2: Comparison between coronal and apical, P3: Comparison between middle and apical

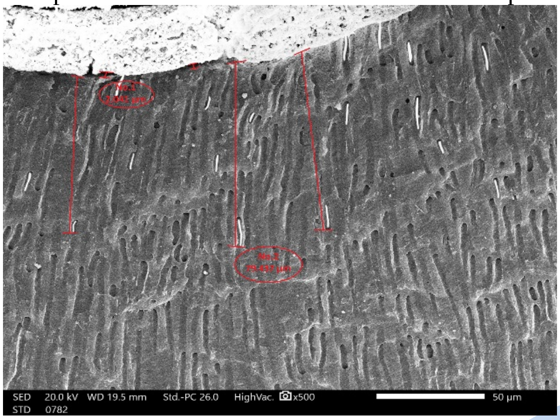

Figure (1): Representative SEM micrograph (×500) of sealer-dentine interface showing Ceraseal maximum penetration at Apical third andmaximum gap recorded at the same third.

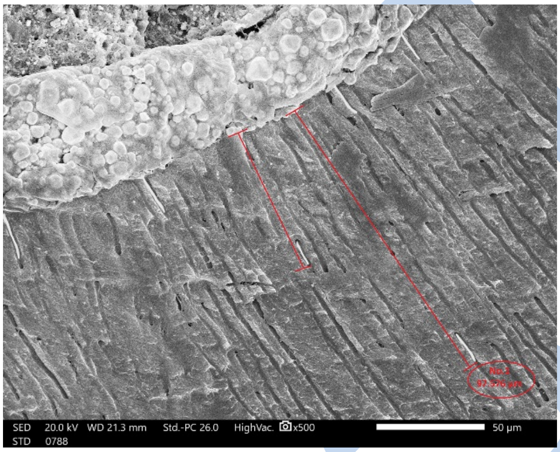

Figure (2): Representative SEM micrograph (×500) of sealer-dentine interface showing AH Plus maximum penetration at Middle third and maximum gap recorded at the same third.

Sealer tubular penetration

In the coronal third, the maximum mean penetration depth was recorded in group II(54.68 ± 13.00), while the maximum penetration depth in the middle and apical thirds was recorded in group I (60.12 ± 13.17and 65.79 ± 12.42, respectively). No sealer penetration was evident in group III in the three canal thirds. Along the complete canal length and in the three canal thirds,no statistical significant difference was found between groups I and II while a significant difference was noted between both groups and group III (Table 2& Figures 1-3).

Table (2): Comparison of mean penetration in µm in the three canal thirds and along complete canal length in all study groups.

		Group I (Ceraseal) (n=12)	Group II (AH plus) (n=12)	Group III (Endofill) (n=12)	Test (p value)
Coronal	Mean (SD)	52.57 (13.10)[a]	54.68 (13.00)[a]	0 (0)[b]	101.368 (<0.0001*)
	Median (IQR)	51.19 (24.58)	55.67 (23.50)	0 (0)	
	Min - Max	35.27 – 76.16	31.82 – 72.70	0 - 0	
Middle	Mean (SD)	60.12 (13.17)[a]	57.06 (11.31)[a]	0 (0)[b]	137.024 (<0.0001*)
	Median (IQR)	57.20 (26.33)	56.33 (18.04)	0 (0)	
	Min - Max	41.24 – 80.16	42.78 – 76.89	0 - 0	
Apical	Mean (SD)	65.79 (12.42)[a]	60.40 (10.97)[a]	0 (0)[b]	175.033 (<0.0001*)
	Median (IQR)	64.26 (19.46)	61.17 (16.76)	0 (0)	
	Min - Max	45.89 – 84.45	45.76 – 78.78	0 - 0	
Test (p value)		3.636 (0.053)	0.992 (0.287)	-	
Overall	Mean (SD)	59.49 (8.35)[a]	57.38 (8.51)[a]	0 (0)[b]	288.608 (<0.0001*)
	Median (IQR)	57.22 (13.02)	56.86 (10.65)	0 (0)	
	Min - Max	47.31 – 76.71	40.17 – 74.63	0 - 0	

* Statistically significant difference at p value≤0.05
ab Different superscript letters denote statistically significant difference between groups within the same row

DISCUSSION

To achieve a successful root canal treatment, a dense root canal filling is desirable following the cleaning and shaping step to prevent microleakage and reinfection(3). Therefore, it is critical to choose obturation materials that have good adaptation and sealing properties for establishing a void-free environment.

This study investigated the adaptation and penetration abilities of three different root canal sealers using SC obturation technique in extracted mandibular first premolar teeth using SEM for analysis.

This study included mandibular first premolars extracted for orthodontic purposes. as these teeth tend to be within the same age group in an attempt to have a nearly similar DT width and number in all teeth as this parameter affects sealer adaptation and penetration greatly as revealed by Yang et al (16).

Mandibular first premolars with single oval shaped canals were selected as it was shown previously in the year 2021 by Mancino et al (17) proving difficultyto accomplish void free root canal filling using SC technique in such canals. Accordingly, using the SC obturation technique in such canals would provide more volume for the sealer inside the canal to be in contact with the dentinal wall to evaluate the sealers adaptability and penetration. On the other hand, canals with round cross section would not allow this feature.

In this study, mechanical instrumentation was done up to file F3 ProTaper Universal system (# 30 / 9% v taper) to preserve canal anatomy for better evaluation of the tested sealers as described in 2017 by Chen et al (12).

During biomechanical preparation, the concentration of NaOCl used for irrigation was 2.5% to avoid the harmful impact of high concentrations on dentin mechanical characteristics as described in the year 2001 by Grigoratos et al (18). This concentration gives a chance to prevent dentinal erosion affecting crystalline structure of dentin as mentioned by Kaya et al (19). On the other side, prior research conducted by Saraf-Dadpe et al (20) and Sonu et al (21) utilized various concentrations of NaOCl, 1% and 5.25 %, respectively.

In the bioceramic sealer (Ceraseal) and resin (AH Plus) groups, teeth were dried using three paper points after final rinse per canal using ProTaper paper points F3. This was done in order to remove the irrigant without excessively drying the canals since moisture is essential for the bioceramic sealer to fully set as previously mentioned by Pedulla et al in the year 2020 (22). In addition, as for the resin sealer it was shown in 2012 by Nagas et al (23) that It may be preferable to keep the canals moderately moist prior to filling, as this may lead to greater bonding of the sealer to dentinal walls due to swelling of the epoxy resin component of AH Plus improving its resistance to dislodgment.

In the present study, the obturation method of choice was SC obturation technique because it represents technical simplification that takes minimal time and no exceptional skill for the operator to accomplish. Moreover, this allows the sealer to be the primary component of the root filling, allowing better test conditions for the sealers and their properties as described by Angerame et al (24). On the contrary, Iglecias et al (25) showed that this technique is questioned for using a high volume of sealerthat can result in treatment failure due to the contraction and dissolution of sealers.

Ceraseal is a relatively newly introduced biocreamic sealer in the market and it is claimed by the manufacturer that it has excellent sealing ability and can be used with SC obturation technique (8). There is a knowledge gap regarding Ceraseal in comparison to other sealers that are commonly used in the market regarding its adaptation and penetration abilities. Another sealer used in this study was AH Plus (resin sealer) which is the gold standard sealer that has been studied for all aspects along the years (9, 26). Endofillsealer (zinc-oxide eugenol) was also tested as it has been used for a long time in root canal treatment and showed good results (10).

AH Plus and Endofill sealers were mixed upon manufacturer’s instructions and introduced in the canal by lentulo spiral as it was shown previously that this method results in better penetration of sealer into the DT than other methods eg: bidirectional file and ultrasonics (27). For the Ceraseal sealer, it was injected into the canal using a disposable propylene tip placed 3 mm short of the apex to allow the sealer to be the main filling material in the canal.

The method selected in the current study for examination of sealer adaptation and penetration was SEM which is in accordance with numerous studies (28, 29). At high magnification, SEM micrographs enable precise viewing of sealer penetration depth into DT as it enables the detection of sealer at remote areas from the root canal wall where tubule density is reduced. However, the primary downside of this approach is the difficulty to gain a detailed overall image at low magnification. Furthermore, artifacts may be produced during the preparation of the specimens for SEM as revealed by Mamootil et al (30).

Confocal laser scanning microscopy is another method used to assess sealer penetration in DT. In this technique, a fluorescent dye such as Rhodamine B is used to stain the sealers. However, it was shown that this may be unreliable for determining the sealer’s penetration depth into DT as Rhodamine B diffuses passively into DT (31). Accordingly, when this approach is used, the penetration depth of the sealer into DT is significantly overestimated (31).

In the current study, it was found that the least mean gap size (best adaptation) along the complete canal length was present in AH Plus group followed by Ceraseal group, while the widest mean gap size (worst adaptation) was found in Endofill group with a significant difference between the three study groups. Moreover, a significant difference was found between AH Plus and Ceraseal in the coronal third only while no difference was found in the middle and apical thirds. These results are in accordance with multiple previous studies (32, 33) demonstrating that AH Plus presents the best adaptationalong the canal wall. Therefore, the null hypothesis regarding the adaptation of the three tested sealers was rejected

Epoxy resin sealers like AH Plus are associated with increased adhesion to dentin and gutta-percha leading to superior adaptation abilities (4). This may be due to the excellent flow characteristics of this sealer and it sets via addition polymerization which improves long term dimensional stability (34).In addition, the epoxy resin present in AH Plus might react with the amine group of the collagen network found in dentin creating a covalent bond between sealer and dentin (35)

. This characteristic will have two beneficial effects: first, on sealing, because the sealer and dentin have enhanced surface contact. and second, on antibacterial activity, due to the remaining microorganisms being entombed in the DT as previously stated in the year 2000 by Siqueira et al (36).

In addition, the acceptable adaptation results of Ceraseal (Bioceramic sealer) might be due to that it chemically bonds to dentine during setting through the formation of hydroxyapatite through the "mineral infiltration zone," as implied by its micromechanical interaction characteristic. Bioceramics uses the moisture found in DT to complete its setting reaction without shrinking, leading to a gap-free contact between the obturation materials and dentin as reported by Gade et al (37). On the contrary, Endofill and other zinc oxide-based root canal sealers have poor particle cohesion and a weak binding to dentine, which may allow for the formation of gaps particularly when used with manual filling techniques as mentioned by Tedesco et al (38).

Regarding the penetration abilities of sealers tested, no significant difference was found between Ceraseal and AH Plus sealers while a significant difference was noted between both sealers and Endofill sealer in all canal thirds and along complete canal length. This might be due to the good flowability of Ceraseal and AH Plus as the flow of both sealers is more than 17 mm which fulfills the requirements of ISO 6867/2012 criteria for flow of endodontic sealers as previously shown by Park et al (39).

It is noteworthy to mention that the Endofill sealer (ZOE-based sealer) showed no evident sealer penetration in any tested specimen. The reason for this might be the SC obturation technique used as the obturation technique has a significant impact on the penetration of ZOE-based sealers.A previous study (40) demonstrated that when utilized in conjunction with a thermoplasticized technique, ZOE-based sealers displayed better tubular penetration than when used in conjunction with a cold lateral condensation technique. Accordingly, the null hypothesis regarding the penetration of the tested sealers was rejected.

Based on the promising results of Ceraseal regarding its adaptation and tubular penetration abilities shown in the present study, the authors recommend that further studies should be conducted to evaluate its solubility and retreatability before recommending its routine clinical use in endodontic practice.

The results of the present study cannot be generalized as only mandibular first premolar teeth with single straight canals were used. Canals with different cross sections and curvature such as mesial canals of mandibular and maxillary molars, distal canals of mandibular molars and palatal canals of maxillary molars, might have shown different results. This could be considered as a limitation of the present study.

CONCLUSIONS

Within the limitations of this study, it may be concluded that SC obturation with AH Plus and Ceraseal in mandibular first premolar teeth could result in significant better adaptation and tubular penetration than Endofill sealer. In addition, AH Plus showed significant better adaptation than Ceraseal, however, no significant difference was noted between both sealers regarding their tubular penetration abilities.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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