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Original Article

Evaluation of dentinal tubules penetration of bio-ceramic and resin root canal sealers using different obturation techniques: An in-vitro study

Mohamed Omaia [1], *Hadil A. Sabry* [2], *Mostafa Shaker* [1]

[1]Lecturer of Endodontics, Department of Endodontics, Faculty of Dentistry, October 6 University.

[2] Professor of Dental Materials, Department of Biomaterials, Faculty of Dentistry, October 6 University.

Email: Mohamed.omaia@dentistry.cu.edu.eg

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Abstract

Aim: This study was conducted to evaluate contact angle and dentinal tubules penetration depth of Epoxy resin-based sealer Adseal and calcium silicate-based sealer CeraSeal with different obturation techniques using Scanning Electron Microscope (SEM).

Subjects and methods: 56 freshly extracted upper central incisors were prepared in the present study. The specimens were divided into four experimental groups (each n= 14) which were filled with different sealers (Ceraseal and Adseal) by different obturation techniques {Cold lateral compaction (CLC) and Warm vertical compaction (WVC)} and respective core filling materials. Group 1 (Ceraseal/ WVC), Group 2 (Ceraseal/CLC), Group 3 (Adseal/ WVC) and Group 4 (Adseal/ CLC). Root slices were cut with a thickness of 1 mm at 2 to 3, 5 to 6 and 8 to 9 mm from the root tip using diamond saw with coolant then scanning electron microscope at 1600x magnification was employed to assess the tubular penetration of different sealers. One Way ANOVA test was used to compare different groups, followed by Tukey`s Post Hoc test for multiple sections comparison. The significance level (P-value) was set as P-value < 0.05.

Results: CeraSeal /CLC group and Adseal / WVC group showed the highest dentinal tubules penetration (11.75±3.72 µm and 10.45±4.73 µm, respectively) with statistically insignificant difference between them (P>0.05). Adseal/CLC group showed the lowest penetration (6.63±2.73 µm), while CeraSeal/ WVC group (9.41±2.71 µm) revealed insignificant difference with the other groups (P>0.05).

Conclusion: Obturation technique should be selected according to sealer type for a successful endodontic treatment. Bioceramic sealer had higher ability of dentinal tubules penetration than epoxy resin sealer.

Keywords: Bioceramic sealers, cold lateral compaction, contact angle, dentinal tubules penetration, Epoxy resin-based sealer, warm vertical compaction

Introduction:

The aim of root canal treatment is the eradication of microorganisms and its byproducts from the root canal system and to fill the cleaned space with restorative material that prevents leakage and reinfection of the root canal space [1,2].

Application of appropriate irrigation protocol is mandatory to eradicate the microorganisms from the complex structure of root canal system (recesses and irregularities, lateral canals, ramifications and apical deltas) [2,3]. The removal of smear layer exposes the dentinal tubules enabling the irrigants to penetrate them. Introducing the sealer into them during obturation gives better seal of the canal [4].

Obturation should provide hermetic seal for the prepared root canal system. This step is the last step of endodontic treatment that traps the inaccessible bacteria and their byproducts inside the canal, in addition avoids the movement of fluids and nutrients into it. [5]

Sealers play major role in sealing the irregularities inside the canal system and gaps between gutta-percha. Furthermore, dentinal tubules penetration entomb the bacteria potentially surviving inside them. This significantly has high impact on endodontic treatment success [6]. That is why the percentage and depth of sealer penetration, combined with antimicrobial properties, have implications for success of root canal treatment. Also, the sealer penetration improves the retention of the obturating material with the canal walls as it acts as mechanical lock inside the dentinal tubules. [7, 8]

The lateral compaction obturation technique is very effective in confining the obturating material inside the root canal system, but several reports suggested that this technique causes voids and spaces due to inhomogeneous distribution of sealer within the obturation [9]. On the other side, warm vertical compaction technique is beneficial as the plasticized obturation material is more adapted to the irregularities resulting in homogenous and denser filling with lesser chance of porosity than the lateral compaction technique [10].

Calcium silicate-based sealer was first released by **Brasseler** [11] in 2009. Nowadays, many different formulas were introduced for calcium silicate-based sealers giving a promising result.

Their excellent physicochemical properties made them widespread used due to their small particle size, biocompatibility, and antibacterial effect [12]. These sealers have high sealing ability caused by the setting expansion [13] and bonding to dentin chemically [14], due to hydroxyapatite formation at the interface between the dentin and sealer [15].

The physicochemical properties and easy manipulation of Epoxy-resin based sealers leads to their usage as control group in recent studies [16]. The epoxy ring of resin sealers produces a high covalent bond to the amino group of dentine with lowest polymerization shrinkage [17], resulting an interlock with canal walls micro-mechanically [18]. Epoxy resin-based sealers were recommended to be introduced with warm vertical compaction technique due to the reduction of gutta-percha sealer interface and compensation of shrinkage that cause gap formation between the sealer and gutta-percha [19].

There are different techniques used for evaluation of sealer penetration through the dentinal tubules including microcomputed tomography, scanning electron microscopy, stereomicroscopy, spiral computed tomography and confocal laser scanning microscopy [20].

Contact angle expresses the spread of a liquid on a solid surface, their measurements give a better knowledge on solids and liquids interactions. Contact angle is inversely proportional with wettability [21, 22].

Wetting ability of sealer is affected by its particle size, hydrophilicity, and contact angle. These properties enable the sealer to easily distribute into the dentinal tubules and create a gapless bond between the sealer and dentinal walls [23].

The aim of the present study is the evaluation of calcium silicate sealer CeraSeal contact angle and dentinal tubules penetration with different obturation techniques (cold lateral compaction and warm vertical compaction) in comparison to Epoxy resin sealer Adseal using vertical profile projector and Scanning Electron Microscope (SEM).

Subjects and Methods:

Table 1: Materials tested and their data compositions according to manufacturer's data sheets

Materials	Manufacturer	Composition	Batch
CeraSeal	*Meta Biomed, Chungju, Korea*	Calcium silicates, zirconium oxide and thickening agent	CS18020501
Adseal	*Meta Biomed, Chungju, Korea*	Base: 25%–50% bisphenol A 10%–25% zirconium dioxide NS calcium tungstate NS iron oxide Catalyst: 2.5%–10% N, n-dibenzyl-5-oxanonandiamin-1,9 2.5%–10% amantadine	ADS1608271

I. Contact Angle (θº):

Sample size calculation:

Sample size of contact angle was calculated depending on a previous study [24]. According to statistical calculations, the accepted sample size was 10 samples per group minimally, when each subject group response was distributed normally with standard deviation 0.91, the mean difference estimation was 1.2, when the power was 80 % and type I error probability was 0.05.

Specimen preparation:

Single-rooted teeth were examined to ensure the absence of cracks, fractures, root caries and signs of external/internal resorption or calcification, with mature apices to be included in the study. The teeth were collected from oral surgery department, which were extracted due to periodontal diseases. After de-coronation, the roots of the teeth were split longitudinally producing 20 circular discs, with water cooled diamond disc running in low-speed (Buehler, Lake Buff, IL, USA).

Twenty dentin slices were ground smoothly by 400–600 grit polishing papers under distilled water for surface scratches removal. A diamond disk was used to standardize a 10-mm segments [25].

The sealers were prepared according to the manufacturers' instructions. A standard volume (0.1 mL) of each examined sealer were injected from an insulin syringe on dentin specimens till complete setting. Measuring of contact angle by shadowing and semi reflection techniques was performed using vertical profile projector Mitutoyo PJ-A3000 [21].

Profile projector or optical comparator is a device that applies the principles of optics to the inspection of samples. The magnified silhouette is projected upon an angle-scale screen.

The specimens were mounted on the projector table to obtain shadow projection clearly visible when viewed from above. The contact angles were measured with 10x magnification. When the specimen was placed over the microscopic slide, X and Y coordinates were adjusted to initialize the measuring procedure. The profile projector is adjusted accordingly to measure the angle formed between the droplet and the prepared dentine surface (Figure 1).

Figure 1: Profile projector device

II. Dentinal Tubules penetration:

Sample size calculation:

Sample size of dentinal tubules penetration was calculated depending on a previous study [26]. According to statistical calculations, the accepted sample size was 14 samples per group minimally, when each subject group response was distributed normally with standard deviation 25.28, the mean difference estimation was 28, when the power was 80 % and type I error probability was 0.05.

Specimen preparation:

The study was approved by research Ethics committee at Faculty of Dentistry, October 6 University with approval No. (RECO6U/12-2020) and conducted following the principles of declaration of Helsinki. Fifty-six freshly extracted upper central incisors were used in the present study. The teeth were collected from oral surgery department, which were extracted due to periodontal diseases. The extracted teeth were investigated for cracks and resorption under magnification of 2.5X using surgical microscope (Karl Kaps, SOM 62, Germany). The cracked and resorbed teeth were excluded and replaced by other incisors free of cracks and resorption. Radiographic examination was performed from proximal view to ensure the integrity of apical foramen and the presence of single canal. All teeth were decapitated to a full length of 15 mm. Patency of the canals were checked using K-file #15 and working length (WL) of 14 mm was established.

Root canal preparation was performed using ProTaper Next system (Dentsply Sirona, York, PA, USA) till size X4 (#40/0.06). Irrigation was done by using 2.5 ml of NaOCl 2.6% before the insertion of each file used for canal preparation. Final flush of all root canals was done after instrumentation using 5 ml of NaOCl 2.6% followed by ultrasonic activation (IRR20-21, Satelec, Acteon, France) for 30 sec. 5 ml EDTA 17% (META BIOMED Co., Chungbuk, Korea) then a final flush with 5 mL NaCl 0.9%. All specimens were kept at 37ºC and 100% humidity for 24 hours before root canal obturation in an incubator to simulate body temperature [27].

A random distribution of specimens was performed into four experimental groups according to type of sealer and obturation technique applied (each group, n = 14) as follows:

Group 1: CeraSeal and Warm vertical compaction technique (CeraSeal/ WVC)

Group 2: CeraSeal and the Cold lateral compaction technique (CeraSeal /CLC)

Group 3: Adseal and Warm vertical compaction technique (Adseal / WVC)

Group 4: Adseal and the Cold lateral compaction technique (Adseal/CLC)

The roots of teeth obturated with warm vertical compaction technique (Group 1 and 3) were placed into plastic vials then mixed alginate (Hydrogum 5, Zhermack, Italy) was poured around the roots for simulation of heat dissipation through the tissues on using warm vertical obturation technique [27], while the other two groups (Group 2 and 4) were left in the incubator without coating as cold lateral compaction technique would be performed for them.

All teeth were dried using sterile paper points. Preparation of sealers was performed following the manufacturer's instructions. For groups assumed to be obturated with CLC technique (Group 2 and 4) a master cone (size 40/0.04 taper) (META BIOMED Co., Chungbuk, Korea) was inserted then WL and Tug-back were checked. The master cone was inserted inside the canal after coating it with sealer till reaching the full working length then lateral compaction technique was done using appropriate size of finger spreader and auxiliary cones of the same size of the spreader.

For groups obturated with WVC technique (Group 1 and 3), a master cone (size 40/0.04 taper) was inserted then WL and Tug-back were checked. The master cone was inserted inside the canal after coating it with sealer till reaching the full working length. Appropriate tip of heated plugger was selected and heated at 230°C then applied inside the root canal keeping 5 mm of gutta-percha apically by the obturation device (EQV, META BIOMED Co., Chungbuk, Korea) then thermosplasticized injectable gutta-percha Obtura III (EQV, META BIOMED Co., Chungbuk, Korea) was used for backfilling the rest of the canal.

Confirmation for the obturation quality was performed using two radiographs (1 labial and 1 proximal). Orifices of all specimens were filled using Scotchbond universal adhesive (3M ESPE Deutschland Neuss, Germany) and resin composite (3M Filtek Z250 XT, Universal, St Paul, USA) All groups were kept in an incubator (100% humidity and 37 °C) for one week to allow complete setting of the sealers [28].

After incubation period, the specimens were removed from the vials and submerged into resin (Technovit 4071, Hereaus Kulzer, Hanau, Germany) horizontally. The mesial and buccal aspects of the tooth were marked, then the root was cut into 1 mm thickness slices using a diamond saw under coolant (Leitz, Wetzlar, Germany) at 2 to 3, 5 to 6 and 8 to 9 mm from the root tip. EDTA 17% was used for rinsing the specimens for 15 seconds followed by distilled water rinsing for the same time to remove any smear layer, then stored in a closed container.

Evaluation of sealer penetration was performed using SEM (Quanta FEG 250, Netherland). Each root section was divided into four quadrants (mesial, distal, buccal, palatal), and images were captured for each quadrant at 1600x magnification. The sealers penetrating the dentinal tubules were identified by EDX analysis as the sealers used in the study contain a radiopacifier (zirconium oxide). Each quadrant was evaluated for the highest penetration depth of sealer, then statistical analysis was done.

Statistical analysis:

All data were presented as mean and standard deviation. Statistical analysis was done using SPSS 16® (Statistical Package for Scientific Studies, IBM Co., New York, USA), Graph pad prism & windows excel.

Shapiro-Wilk test and Kolmogorov-Smirnov test for normality were applied on the given data which revealed that the significance level (P-value) was insignificant as P-value > 0.05 which reject the alternative hypothesis, and the concluded data arises from normal distribution (parametric data) resembling normal Bell curve.

Independent t-test was used to compare between 2 different groups, while One Way ANOVA test was used to compare between 4 groups, Repeated measures ANOVA test were used for comparing the three sections of the same root followed by Tukey`s Post Hoc test for multiple comparisons.

Results:

Contact angle: Table (2) & Figure (2).

Independent t-test was used for comparing contact angle results of both sealers which

showed that Adseal (51.93 ± 0.18) was significantly higher than Ceraseal (47.83 ± 0.51) as P < 0.05.

Table 2: Maximum, minimum, mean and standard deviation of contact angle (θ °) in CeraSeal and Adseal groups

N=10	Minimum	Maximum	M.	SD>	P value
CeraSeal	47.18	48.44	47.83	0.51	<0.0001*
Adseal	51.68	52.08	51.93	0.18	

*M: mean SD: standard deviation *: significant difference as P <0.05*

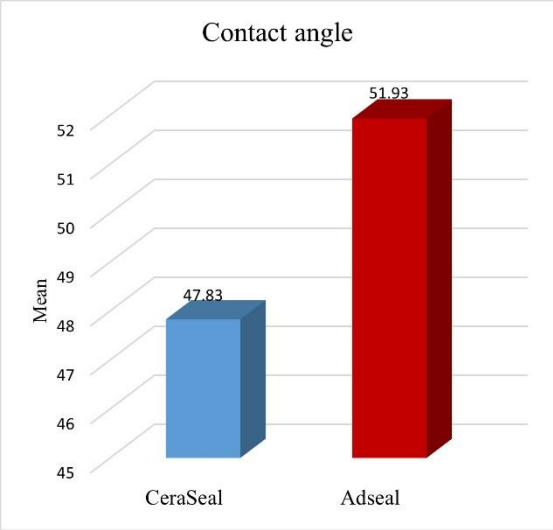

Figure 2: bar chart showing contact angle in CeraSeal and Adseal groups

Dentinal tubules penetration: Table (3) & Figure (3).

Comparison between different groups:

One Way ANOVA test was used to compare different groups, which showed significant difference between them in all sections as P <0.05, followed by Tukey`s Post Hoc test for multiple comparisons which showed significant difference in means with different capital superscript letters as P < 0.05, while showed insignificant difference in means with the same capital superscript letters as P > 0.05.

In coronal section: Adseal / WVC group was significantly the highest, followed by CeraSeal/ WVC group and CeraSeal /CLC group with insignificant difference between them, while Adseal/CLC group was significantly the lowest.

In middle and apical sections: CeraSeal /CLC group was significantly the highest, followed by CeraSeal/ WVC group and Adseal / WVC group with insignificant difference between them, while Adseal/CLC group was significantly the lowest.

In overall: CeraSeal /CLC group (figure 4) and Adseal / WVC group (figure 5) showed the highest dentinal tubules penetration with statistically insignificant difference between them. Adseal/CLC group (figure 6) showed the lowest penetration, while CeraSeal/ WVC group (figure 7) revealed insignificant difference with the other groups.

Comparison between different sections:

In CeraSeal/ WVC group and in Adseal / WVC group: there was a significant difference between all sections (coronal, middle and apical) as P < 0.05, where the coronal section had the highest dentinal tubule penetration, while the apical one had the lowest.

In CeraSeal /CLC group and Adseal/CLC group: the results showed that coronal and middle sections had the highest dentinal tubule penetration, while the apical section had the lowest.

Table 3: Mean and standard deviation of dentinal tubules penetration in coronal, middle and apical sections in (CeraSeal/ WVC) (µm), (CeraSeal /CLC) (µm), (Adseal / WVC) (µm) and (Adseal/CLC) (µm) and comparison between different sections

N=14	(CeraSeal/ WVC) (µm)		(CeraSeal /CLC) (µm)		(Adseal / WVC) (µm)		(Adseal/CLC) (µm)		P value
Groups	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	
Coronal	11.83 $^{A}_{a}$	1.03	12.90 $^{A}_{a}$	5.13	16.13 $^{B}_{a}$	2.12	8.56 $^{C}_{a}$	2.96	<0.0001*
Middle	9.99 $^{A}_{b}$	2.18	12.66 $^{B}_{a}$	1.38	9.70 $^{A}_{b}$	1.46	7.97 $^{C}_{a}$	0.57	<0.0001*
Apical	6.33 $^{A}_{c}$	0.85	9.65 $^{B}_{b}$	2.44	5.54 $^{A}_{c}$	1.06	3.83 $^{C}_{b}$	0.31	<0.0001*
Overall	9.41 ^{AB}	2.71	11.75 ^{A}	3.72	10.45 ^{A}	4.73	6.63 ^{B}	2.73	0.003*
P value	<0.0001*		0.02*		<0.0001*		<0.0001*		

Means with different superscript capital letters per row/subscript small letters per column were significantly different as P <0.05
Means with the same superscript capital letters per row/subscript small letters per column were insignificantly different as P >0.05
WVC: Warm vertical compaction
CLC: Cold lateral compaction

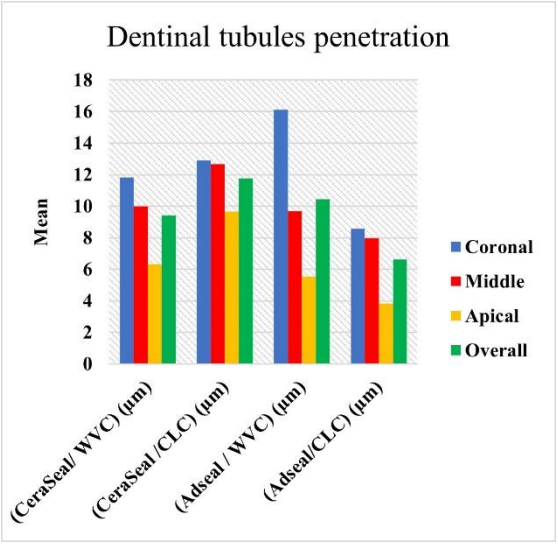

Figure 3: bar chart showing dentinal tubules penetration in (CeraSeal/ WVC) (µm), (CeraSeal /CLC) (µm), (Adseal / WVC) (µm) and (Adseal/CLC) (µm)

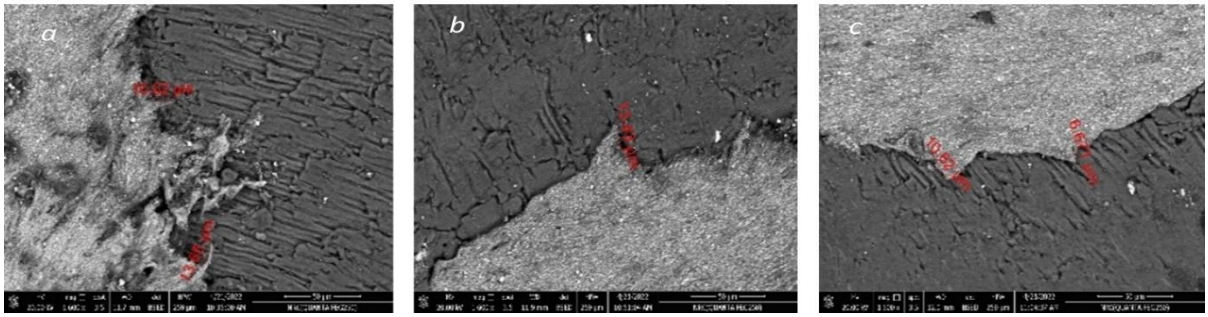

Figure 4: SEM 1600x magnification, showing the dentinal tubules penetration of CeraSeal/ CLC (µm) a) Apical section. b) Middle section. c) Coronal section

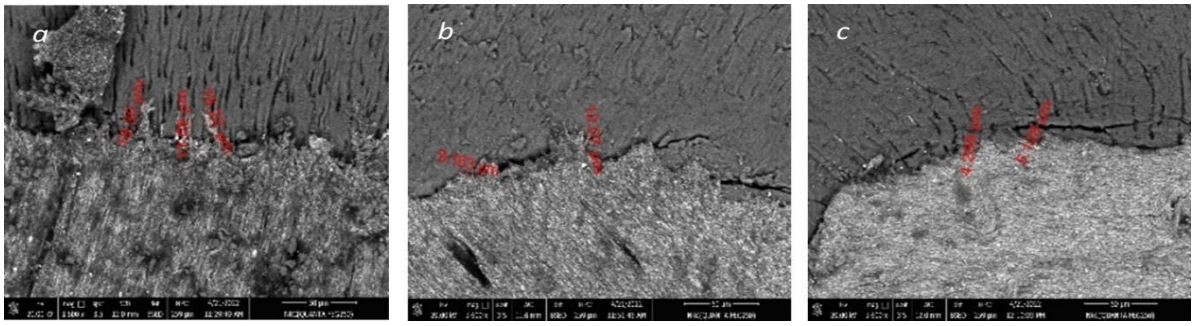

Figure 5: SEM 1600x magnification, showing the dentinal tubules penetration of Adseal/ WVC (µm) a) Apical section. b) Middle section. c) Coronal section

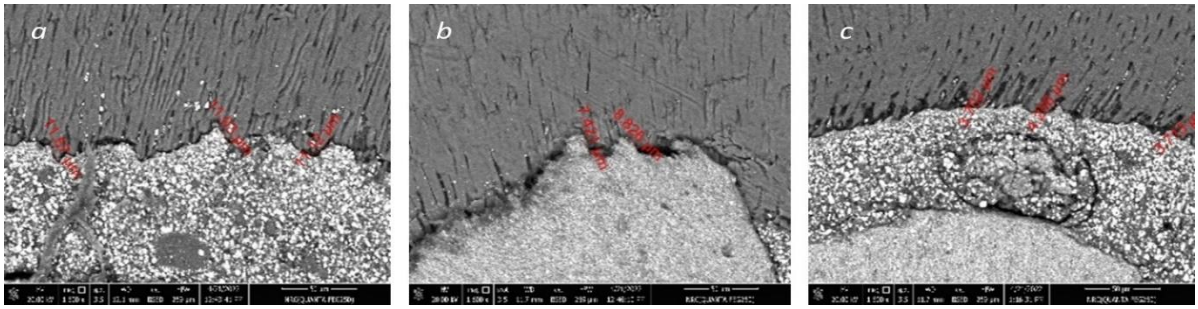

Figure 6: SEM 1600x magnification, showing the dentinal tubules penetration of Adseal/ CLC (µm) a) Apical section b) Middle section. c) Coronal section

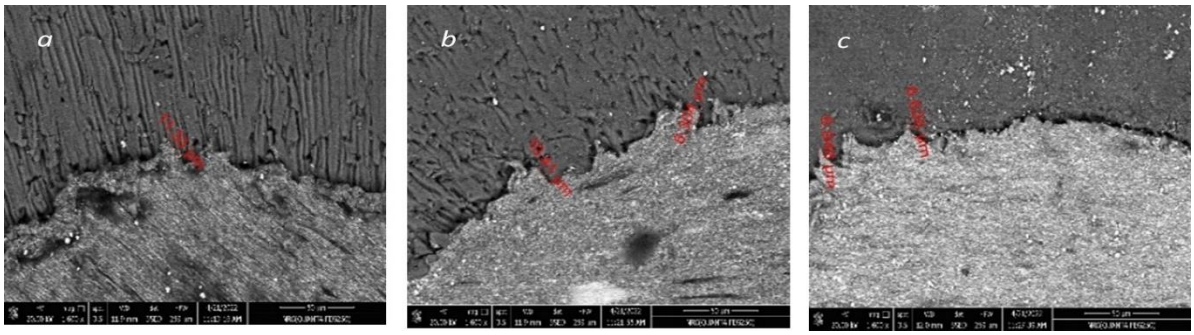

Figure 7: SEM 1600x magnification, showing the dentinal tubules penetration of CeraSeal/ WVC(µm) a) Apical section b) Middle section. c) Coronal section

Discussion:

The dentinal tubules penetration by root canal sealer has high clinical importance. Sealer penetration into dentinal tubules has been reported by many studies [13]. Scanning electron microscopy (SEM) [8], light microscopy [29], and confocal laser scanning microscopy (CLSM) [30] have been used to analyze sealer penetration.

Among the properties of Epoxy resin sealers that resulted in their wide spread use is their low cytotoxicity and solubility, good handling properties and compatibility with core filling material.

Calcium-silicate based Bioceramic sealers have been recently used for their dimensional stability, biocompatibility and bioactivity [5].

Bioceramic sealers reduce hydraulic conductance through occluding the dentinal tubules [31]. Therefore, evaluation of tubular penetration of sealers with different obturation techniques could reveal their sealing potential in obturated teeth. In the present study, we compared Bioceramic sealer (Ceraseal) with Epoxy resin sealer (Adseal) with warm vertical compaction and cold lateral compaction techniques.

Penetration of sealer is affected by several variables as the number and diameter of dentinal tubules, the flow of the sealer and the obturation method [32,33].

The results of the present study showed better sealer penetration in the coronal thirds in all experimental groups than in the apical thirds. These results are in agreement with those of other studies [29, 34-37]. This may be referred to the higher efficiency of smear layer removal in coronal thirds than in apical thirds of root canals.

The most common combination used for smear layer removal from the root canal system is EDTA and NaOCl where the EDTA can eliminate smear layer from the coronal and middle thirds efficiently rather than the apical third. This may be caused by the higher surface tension of 17% EDTA [38].

Among the factors affecting the sealer flow are the temperature, particle size and setting time [39].

In this study, the contact angle test showed that Adseal was significantly higher than Ceraseal as $P < 0.05$. The lower contact angle of Ceraseal (CLC) reveals higher flow of the material that was found in the middle and apical sections while in the coronal section Adseal (WVC) showed higher values and this could be due to the effect of high temperature used in WVC technique which increased the flow of polymeric materials (resin sealers) while it decreases the flow of the Bioceramic sealers due to the evaporation of humidity inside the canal and sealers, which may also affect the setting of the Bioceramic sealers [28].

The obturation technique influences the ability of sealer penetration into the dentinal tubules as proved by **De Deus et al** where the warm vertical condensation technique reported a higher penetration than cold lateral compaction technique. The results of the present study were in agreement with **De Deus and Ordinola-Zapata et al** results. [30, 34]

According to this explanation the absence of significance was found between the group obturated with Ceraseal using CLC technique, and the group obturated with Adseal using WVC technique, as the effect of heat generated during WVC affect the obturating sealer and increases the flow of the Adseal compensating the difference in its flow in the cold form, in addition to the force of vertical condensation that increases the penetration of the sealer mainly in the coronal third, due to the increased number and diameter of dentinal tubules.

The application of heat reduced the sealer porosity in resin-based sealers, and this is of benefit as porosities could be detrimental to the seal of the root fillings [40].

Better results of dentinal tubules penetration were observed in the group of Ceraseal by the using of CLC than the group which used WVC. This might be due to the adverse effect of the raise of the temperature in the canal atmosphere during WVC which decreases the humidity inside the canal, needed by the Bioceramic sealer to flow and to complete the setting reaction, as it is considered a hydrophilic material. A previous study reported that extra pressure created by warm vertical compaction has no effect on the depth of tubular penetration using calcium silicate sealers at the 4-mm level from the apex [19, 41] this was in agreement with the results of the present study related to the apical third section.

Premixed calcium silicate sealer consists of an inorganic matrix of calcium silicate hydrate surrounding the unreacted silicate granules with micro spaces in between containing water. Porous calcium silicate hydrate sets into a solid network. Several days are required to complete the complete setting of the material [42]. Heat application would result an increase in Water desorption. The end result of this is the loss of water adsorbing inside the pores, the relatively high water-cement ratio of the sealer should be considered [5].

Limitations:

Presence of dentinal fluid in clinical condition may decrease the sealer penetration into dentinal tubules.

Results were entirely based on the observation under SEM. Micro-computed tomography was proved to have higher effectiveness than SEM in evaluation of voids and gaps formation. So combining SEM and Micro-CT may reveal a more accurate results for assessment of root canal sealers penetration into dentinal tubules as well as the preparation of samples for confocal laser scanning microscopy reduce the formation of artifacts in sample preparation than SEM.

Conclusion:

1. Obturation technique should be selected according to sealer type for a successful endodontic treatment.

2. Bioceramic sealer has higher ability of dentinal tubules penetration than epoxy resin sealer according to the restriction of the present study. Also, the warm vertical compaction increases the penetration of epoxy resin sealers, contrarily, it adversely affects the penetration of Bioceramic sealers.

Conflict of Interest:

The authors declare no conflict of interest.

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This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry-October 6 university on: 7/9/2020, approval number: (RECO6U/12-2020)

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