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ORIGINAL ARTICLE

Influence of the Remnants of Silicone Oil on Penetration of Three Different Sealers into the Dentinal Tubules: A Confocal Laser Scanning Microscopy Study

Mai RAGAB, Marwa SHARAAN

ABSTRACT

Objective: The aim of this study was to assess penetration of AH Plus, MTA Fillapex and GuttaFlow Bioseal sealers into dentinal tubules after placement and removal of silicone oil using Confocal Laser Scanning Microscopy.

Methods: Sixty single-canaled premolars were instrumented using ProTaper Universal rotary system. Roots were divided into two main groups (n=30) where either Silicone oil was used or not. Subsequently, each main group was subdivided into 3 subgroups according to the investigated sealers. In subgroups where Silicone oil was used, it was placed in canals and then cleared. Obturation was completed utilizing lateral compaction technique using Rhodamine B labeled sealers. Penetration depth of sealer was evaluated by image J software. One way ANOVA, Duncan's test as posthoc test was performed for evaluation of statistical significances among the groups. In each sealer group, Independent -t-test was used to compare between with and without oil. P value was set at <0.05.

Results: Using silicone oil resulted in less dentinal tubule penetration depth with all sealers. Mean dentinal tubule penetration depth was the lowest in apical thirds. AH Plus showed higher penetrability in all thirds compared to MTA Fillapex and GuttaFlow Bioseal despite oil placement and removal. MTA Fillapex displayed higher penetrability in all thirds than GuttaFlow Bioseal.

Conclusion: Remnants of silicone oil has a negative impact on the penetration depth of the tested sealers.

Keywords: AH Plus, confocal laser scanning microscopy, dentinal tubule penetration, GuttaFlow Bioseal, MTA Fillapex, root canal sealer, Silicone oil

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From the Department of Endodontics (M.R., M.S. ✉ marwaelsayedsharaan@gmail.com), College of Dentistry, Suez Canal University, Ismailia, Egypt

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HIGHLIGHTS

- During instrument's retrieval, using silicone oil might affect penetration of the sealer into the dentinal tubules.
- When the oil has to be used during treatment it is better to use AH plus sealer as it displayed a deeper sealer penetration than the tested sealers
- Apical third showed the least depth of sealer penetration irrespective of the type used.

INTRODUCTION

Successful root canal treatment (RCT) is mainly related to optimal cleaning and shaping, and three--dimensional filling of the root canal system. Root canal sealers play a crucial task in the accomplishment of an effective RCT by eliminating spaces between root canal walls and gutta percha (1). Nevertheless, microscopic gaps exist between sealer and dentine as well as between sealer and gutta percha may threaten the outcome of RCT. Because marginal leakage might occur through these gaps leading to failure (2). Dissimilar to gutta percha, sealers can penetrate into hidden areas such as dentinal tubules, fins, isthmuses, and lateral canals (1). The penetration depth of sealers depends on many factors including the substrate (dentine) permeability, smear layer removal, filling technique and other sealer's related factors as the mechanical and physiochemical properties (1). Hence, it is necessary to evaluate the penetrability of different sealers that are used routinely in the endodontic clinic.

AH Plus, an epoxy resin based, is the gold standard sealer in research, it displays acceptable physicochemical properties that have been broadly examined such as flowability, low solubility, biocompatibility, radiopacity, antibacterial action and sealing ability (1-5). Furthermore, it displays a high bond strength to the canal wall and acceptable long term dimensional stability (5).

MTA Fillapex sealer is composed of 13% mineral trioxide aggregate (MTA), silica, bismuth oxide, 38% salicylate resin and natural resin. It demonstrated favourable physicochemical properties to be used in obturation such as acceptable working time, ease of handling and satisfactory radiopacity (3, 6). Apart from that, MTA Fillapex has favourable biocompatibility, bioactivity before setting and does not induce inflammatory responses (6, 7).

GuttaFlow Bioseal sealer has been introduced to the market to foster the penetration of the sealer into the dentinal tubules, thus increasing the bonding to dentine and gutta percha. Guttaflow Bioseal, a formulation of GuttaFlow, is a silicone-based endodontic sealer that blends sealer and gutta-percha in a powder form with a smaller particle size <30 µm (8). It is composed of poly-dimethyl siloxane, platinum catalyst, zirconium dioxide, and micro-silver. GuttaFlow displayed less toxicity to the human gingival fibroblasts cells than AH Plus (9, 10). It has been shown that GuttaFlow Bioseal has adequate physicochemical properties such as setting time, flow, solubility, radiopacity, slight setting expansion which guarantee the maximum sealing ability and high penetration ability into the dentinal tubules (11, 12).

Confocal laser scanning microscopy (CLSM) is a well-known experimental tool used to assess the depth of sealer penetration into the dentinal tubules and has many advantages over other microscopic studies. CLSM yields fewer defects, which helps in picturing up to 10 µm under the surface of the sample and detecting the sealers within the dentinal tubules (11).

Due to the increasing demands towards using NiTi rotary systems, clinicians often face instrument separation. Consequently, searching for an aid to remove the separated instrument is the main concern. Recently, Terauchi and Renton recommended the use of the silicone oil to facilitate the retrieval of the separated instrument (13). Silicone oil has been used in medicine as a vitreous fluid replacement for treating retinal detachment (14).

To the best of knowledge, none of the previous studies investigated the sealer penetration into dentinal tubules after placement and removal of silicone oil.

The aim of this current *in-vitro* study was to examine the penetration of the three various sealers into the dentinal tubules after the placement and removal of the silicone oil using CLSM. The null hypothesis was that there was no significant difference between the sealer penetration after placement and removal of silicone oil.

MATERIALS AND METHODS

Sample size determination

After the study proposal was approved by the Research Ethics Committee at the college of Dentistry (Registration no. 371/2021), a sample size was obtained by using the G* power software statistical analysis (Latest ver. 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Sixty premolars were enough to detect an effect size of 0.57 and a power (1-β) of 92% at a significant level of (α) level of 0.05. Accordingly, 2 main groups (with or without oil) and 3 subgroups (according to the tested sealer) per main group were assigned for the study, resulting in10 samples/ group.

Specimen's preparation

Sixty straight single rooted premolars, extracted because of orthodontic and periodontal reasons, were selected in the study. A dental operating microscope (LABO AMERICA inc., CA, USA) was used to confirm that the included teeth were free of resorption or cracks. Facial and proximal 2D radiographic images were conducted to confirm type I Vertucci's root canal configuration. Included teeth were cleansed of any debris or calculus by an ultrasonic scaler. The teeth were disinfected using 5.25% sodium hypochlorite NaOCl (Clorox, Household Cleaning Products of Egypt, Cairo, Egypt) for 30 mins. After cleansing, the teeth were rinsed under running tap water and stored in 2% thymol solution until use at 37°C. Decoronation of the teeth was completed using a water-cooled, high-speed diamond bur (MANI Dia-Bur, SF-41) to ensure that root canal length was standardized at 15 mm.

Root canal preparation

A K-file # 10 (Dentsply Maillefer, Switzerland) was introduced into each canal until it was visualized through the apical foramen and the length was determined. Working length (WL) was estimated by 0.5 mm from that length. The apical end was sealed with a nail polish. Canal preparation was completed using the ProTaper Universal system (Dentsply-Maillefer, Ballaigues, Switzerland) up to a master apical file # F4 connected to an X-Smart motor (Dentsply-Maillefer, Ballaigues, Switzerland) at 250 rpm and a torque setting of up to 2.5 N/cm. Firstly, SX and S1 were used to 2/3 of WL to flare the coronal and middle 2/3. Later, rotary instrumentation was accomplished using S1, S2, F1, F2, F3 and F4 (size 40/ 0.06 taper) to WL. Between each file change, instrumentation was performed with intermittent irrigation with 5.25% NaOCl for 1 min. followed by sterile distilled water using side vented needle with a 30 gauge (Dentsply Rinn, Elgin, Il). Final irrigation was ended using 5 ml of 17% ethylene diamine tetra acetic acid (EDTA) (MD-Cleanser, META-BIOMED, Korea) for 1 min. followed by 5ml of distilled water.

Specimen's randomization

One author, who was not involved in the clinical procedure, performed the blind allocation of the teeth after running randomization for grouping by Microsoft Excel. Roots were randomly and equally divided into 6 groups and subgroups based on whether oil was placed or not with the 3 tested sealers according to the following:

Group A_1: epoxy resin based sealer (AH Plus; Dentsply Maillefer, Ballaigues, Switzerland): without oil placement (n=10).

Group A_2 : epoxy resin based sealer (AH Plus): with oil placement (n=10).

Group B_1: mineral trioxide aggregate and salicylate resin based root canal sealer (MTA Fillapex; Angelus, Londrina, PR, Brazil): without oil placement (n=10).

Group B_2: mineral trioxide aggregate and salicylate resin based root canal sealer (MTA Fillapex): with oil placement (n=10).

Group C_1: silicone based endodontic sealer (GuttaFlow Bioseal; Coltène/Whaledent AG, Altstatten, Switzerland): without oil placement (n=10).

Group C_2: silicone-based endodontic sealer (GuttaFlow): with oil placement (n=10).

Non-Silicone oil group: (Subgroups A1,B1,C1)
In this group, 30 specimens were prepared according to the previously mentioned protocol without any placement of silicone oil.

Silicone oil group: (Subgroups A2,B2,C2)
In this group, 30 specimens were prepared according to the previously mentioned protocol. Later, 5 ml of silicone oil (Loba Chemie, Mumbai, India) was applied in the canal for 1 minute. Then, irrigation with 6% sodium hypochlorite (Chlor-XTRATM ;Vista Dental Products, Racine, WI) was performed for one minute followed by sterile distilled water using a 5 ml syringe with a 30-gauge side vented needle. Final irrigation was done using 5 ml of 17% EDTA for 1 minute followed by 5ml of distilled water.

Root canal filling
In all groups, dryness of the specimens was completed by paper points # F4 (Dentsply-Maillefer). The canals were coated with the respective sealers that were either mixed (AH Plus and MTA Fillapex) or injected (GuttaFlow Bioseal) according to the manufacturer's instructions, matched with the subgroups, and mixed with 0.1% fluorescent Rhodamine B isothiocyanate (Sigma-Aldrich, St. Louis, MO, USA) to allow visualization under the CLSM (Carl Zeiss LSM 510 Meta; Carl Zeiss Mikroskopie, Jena, Germany). Master apical cone (DiaDent Group International Inc., Korea) # 40/0.02 was used in obturation and to apply the labeled tested sealers. Auxiliary cones # 25/0.02 (DiaDent Group International Inc., Korea) were added and cold-compacted laterally using a finger spreader #25 (Mani, Inc, Japan) 1 mm shorter of the WL next to master cones that were positioned. Auxiliary cones were coated with sealer and placed until no space was available to the spreader. Gutta-percha was severed and compacted vertically with an endodontic plugger (FKG Dentaire, La Chaux-de-Fonds, Switzerland). Mesiodistal and buccolingual radiographic examination was conducted to verify the quality of the obturation. Coronal seal was achieved by glass ionomer restorative material (Riva, Cologne, Germany). Specimens were placed at 37°C and 100% humidity for 1 week to confirm complete setting of the sealers.

Confocal laser scanning microscope investigation
After confirmation of sealer complete setting, the specimen was positioned in cold self-curing resin (Acrostone Dental and Medical Supplies, Egypt). Using Isomet diamond saw (Buehler, Lake Bluff, IL) below continuous water cooling, the specimen was sectioned in a perpendicular direction to the long axis. Three cross sections were taken at 2 mm, 4 mm and 6 mm from the root apex having a thickness of 2 mm. All sections were polished using silicon carbide abrasive papers (Politriz, Struers A/S, Copenhagen, Denmark) and mounted onto glass slides.

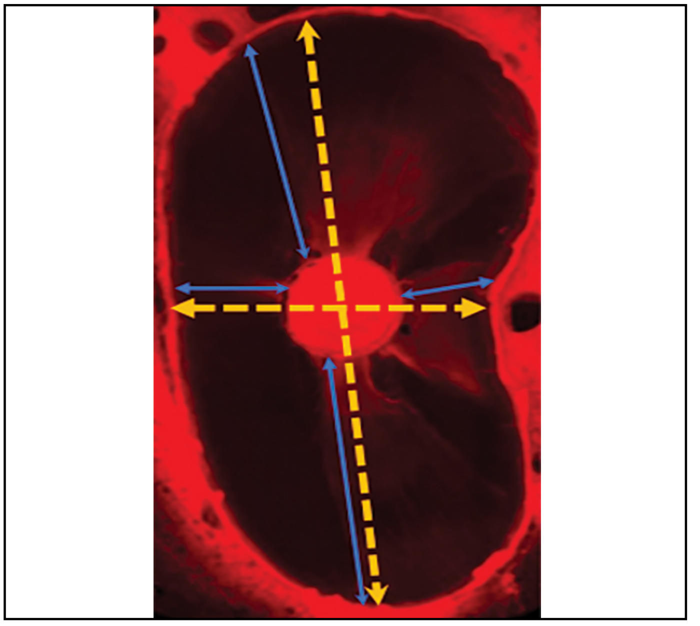

Figure 1. Representative CLSM image, exported to Image J software to calculate the highest depth of sealer penetration, displayed in the blue lines at four predetermined locations in each specimen (beginning with the union of buccolingual and mesiodistal axis lines of the canal wall displayed in the yellow dashed lines)

CLSM: Confocal laser scanning microscopy

Specimens were investigated under a CLSM at 570 nm wavelength, and 10X lens. Management of the specimens was conducted in a dark room setting to avoid any degradation of the Rhodamine B dye. Images were transferred to the ImageJ software (National Institute of Health, Bethesda, MD) for measurement of the tested sealer penetration into dentine. Beginning with the union of buccolingual and mesiodistal axis lines of the canal wall, four locations were pre-determined at the mesial, distal, buccal and lingual parts of each specimen (Fig. 1). The highest depth of sealer penetration was calculated in microns (µm) at the previously mentioned 4 locations after scale calibration in µm. It was measured from the outer surface of the root canal to the periphery of the section. Mean calculation of the four measurements denoted the sealer penetration in each specimen (15). One observer, who was not involved in the study, completed the measurements using the measurement tool and two repeated readings were taken apart from each other to ensure the intra-rater reliability. Furthermore, the two authors evaluated the images repeating the same procedures as the observer to guarantee the inter-rater reliability.

Statistical analysis
Numerical data was examined using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Data displayed normal (parametric) distribution, and were displayed as mean, standard deviation (SD) and 95% Confidence Interval for the

TABLE 1: Means and standard deviation values of the Penetration Depth in µm of the tested sealers with or without Silicone oil (n=10)

Silicone oil	Sealer	Group A AH plus			Group B MTA fillapex			Group C GuttaFlow bioseal			P<0.05
	Thirds	Mean	SD	Min-max	Mean	SD	Min-max	Mean	SD	Min-max	
Without oil	Coronal	414.00^{aA}	32.57	369-480	393.80^{bA}	6.68	378-400	385.60^{bA}	11.08	367-400	0.012*
	Middle	337.00^{aB}	39.87	267-384	348.50^{aB}	28.89	300-394	303.50^{bB}	16.60	270-329	0.001**
	Apical	329.00^{aB}	33.75	301-392	211.70^{bC}	16.37	195-240	190.60^{cC}	11.66	167-201	<0.001**
	Overall	1080^{a}	3.92	1001-1154	954^{b}	11.14	913-990	$879.^{7c}$	3.03	855-919	<0.001**
	P	<0.001**			<0.001**			<0.001**			
With oil	Coronal	361.60^{aA}	39.78	289-420	338.00^{abA}	21.54	309-378	320.00^{bA}	32.19	289-391	0.0252*
	Middle	357.10^{aA}	25.19	321-397	264.20^{bB}	34.33	198-302	276.80^{bB}	27.57	201-290	<0.001**
	Apical	278.00^{aB}	19.67	243-301	214.70^{bC}	25.21	187-261	161.70^{cC}	27.29	123-199	<0.001**
	P	<0.001**			<0.001**			<0.001**			
	Overall	996.7^{a}	10.39	919-1097	816.9^{b}	6.59	746-899	758.5^{c}	2.75	664-835	<0.001**

*: Denotes significance, **: Denotes highly significance. Superscript lowercase letters represent the statistical difference between the different sealers either with or without oil; Superscript uppercase letters represent the statistical difference between the different thirds in each sealer group either with or without oil. MTA: Mineral trioxide aggregate, SD: Standard deviation, Min: Minimum, Max: Maximum

mean values. One way ANOVA was used to compare between sealer groups and different thirds (apical, middle and coronal), Duncan's as posthoc test was performed for the evaluation of statistical significance among the groups. Independent -t-test was used to compare between with and without oil in each sealer group. P value <0.05 is considered to be statistically significant. Statistical analysis was performed using the computer program SPSS software for windows version 25.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp).

RESULTS

Results displayed that the highest depth of sealer penetration into the dentinal tubules was achieved in specimens filled with AH plus sealer and gutta percha, when oil was not used. The lowest depth was obtained in specimens filled with GuttaFlow Bioseal sealer and gutta percha, after placement and removal of oil. AH plus sealer penetrated deeper compared to MTA fillapex and GuttaFlow Bioseal sealers in all thirds either with or without using oil (P<0.05) Table 1, (Fig. 2). MTA fillapex penetrated deeper compared to GuttaFlow Bioseal in all thirds either with or without oil placement and removal (P<0.05) Table 1, (Fig. 2). Using oil lowered the penetration depth of AH plus in all thirds significantly (P<0.05) Table 2, (Fig. 2). Additionally, the penetration depth of MTA Fillapex was significantly decreased in the middle and apical thirds after using oil (P<0.05). However, penetration depth in the coronal third

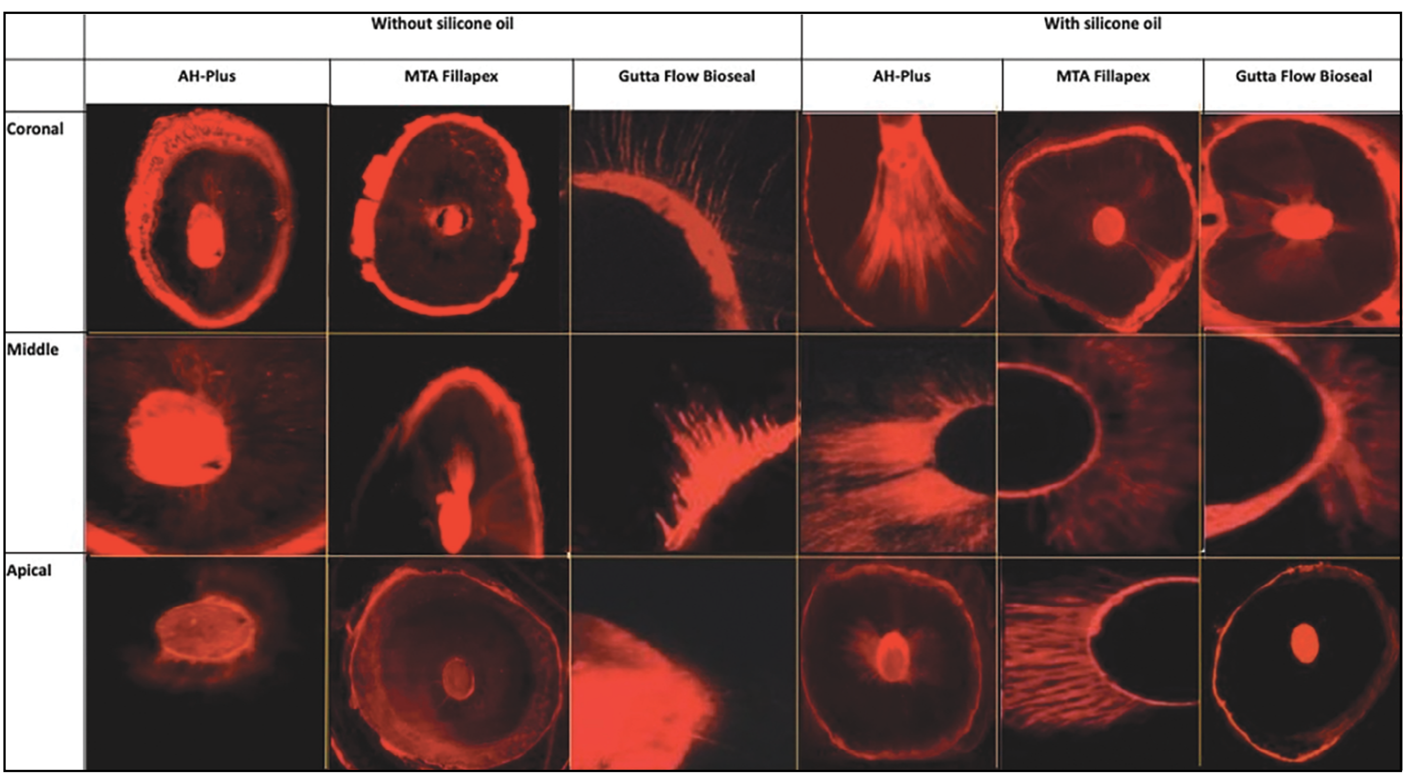

Figure 2. Representative CLSM images displaying images of sealer penetration ability in apical, middle and coronal sections in all tested groups

CLSM: Confocal laser scanning microscopy, MTA: Mineral trioxide aggregate

TABLE 2. Independent -t-test for comparison between with and without oil in each sealer group

		AH plus		MTA fillapex		GuttaFlow bioseal	
P<0.05	Variables	T	P	T	P	T	P
	Coronal	-3.22	0.01**	1.88	0.08 ns	-4.85	<0.001**
	Middle	-7.82	<0.001**	-5.94	0.001**	3.16	0.76 ns
	Apical	-6.09	<0.001**	-2.62	0.02**	-3.07	0.01**

**: Denotes highly significance, MTA: Mineral trioxide aggregate, T: Independent student's test, P: P-value ns: Non-significant

was decreased insignificantly (P>0.05) Table 2, (Fig. 2). On the other hand, using oil lowered the penetration depth of GuttaFlow Bioseal in all thirds significantly except the middle. The penetration depth of all sealers in the coronal thirds of specimens, either with or without oil, was statistically significant from only the apical thirds (P<0.05). The sealer penetration ability increased in an apical-coronal direction. The penetration depth of AH plus in the middle and apical, and the coronal and middle in specimens either treated without or with oil was not statistically significant. However, in specimens either treated with or without oil, MTA fillapex and GuttaFlow Bioseal sealers penetration into the dentinal tubules were statistically significant among the 3 thirds (P<0.05) Table 1, (Fig. 2).

DISCUSSION

Penetration of the sealer into the dentinal tubules might emphasize its retention, which acts as a physical barrier, entombing the remaining microorganisms by depriving them from their nutrients (4, 7, 16). During obturation, the goal is to have a minimum thickness of sealer and maximum body of gutta percha. The composition of each type of sealer would determine its physicochemical properties (5, 17). Three-based sealers, AH Plus , MTA Fillapex and GuttaFlow Bioseal, were used in the study to assess their penetrability.

In this study, canal preparation was utilized using the ProTaper Universal system to master apical file # F4 (18). CLSM was used in this study because it demonstrates that full extension into the dentinal tubules. Additionally, low magnification is possible in horizontal sections to evaluate the depth of sealer penetration. Furthermore, CLSM does not need specimen preparation that might result in defects. Sealer was labelled by Rhodamine B dye during root canal filling. High-contrast points were established when this dye was used;penetration of the sealer into the dentinal tubules could be evaluated with CLSM (11, 16, 17, 19).

To place the sealer inside the root canal, the master cone coating technique was used because it requires no extra equipment. A previous study demonstrated that sealer spreading within root canal was not affected by the technique of sealer application (20). In the present study, all teeth were decoronated at the cementoenamel junction to counteract any variations among coronal, middle and apical sections rising from discrepancy in root length of all teeth (11, 17, 21).

Lateral compaction was the adopted technique for root canal obturation since it is a simple maneuver and does not require any special or many instruments. Further, an earlier study negated the correlation between the obturation technique and depth of sealer penetration (22). In the current study, the Image J software was used to calculate the dentinal tubule penetration area at four locations to confirm the penetration ability of sealers in the whole specimen (15).

Despite the invention of newly released Ni-Ti files, unfortunate separation of files might still occur hindering the completion of the root canal treatment (23). Terauchi and Renton recommended the use of silicone oil as a lubricant during the process of instrument's retrieval (13). A silicone oil is a liquid polymerized siloxane with chains of organic sides. Silicone oil is used in medical purposes such as in penetrating ocular trauma (14). Chlor-XTRATM was used followed by 17% EDTA for 1 to ensure the elimination of the oil from the canal before obturation.

The null hypothesis of this study was rejected: The penetrability characteristics of AH plus were superior compared with MTA Fillapex and GuttaFlow Bioseal, even in case of using oil. The current results were in agreement with previous studies which stated that AH plus sealer was the best sealer regarding adaptability on dentine surface when compared with other sealers (24-27). This can be attributed to formation of the covalent bond between the dentine and sealer due to the action of epoxy resin that can combine with the amine group of collagen (24). Additionally, good flow rate, reduced film thickness, small particle size, and its longer setting time encourage the mechanical interlocking between the sealer and dentine (26).

In the present study, MTA Fillapex showed less significant penetration than AH plus. This result could be explained by the sealer having salicylate in its constituents. This displayed initial shrinkage which caused an overall contraction of the sealer; this could also be a contributing factor in the present study for less tubular penetration by MTA Fillapex, other than its high solubility and no presence of hydrophilic characteristics (28).

On the other hand, Cruz et al. (29) registered higher tubular penetration of MTA Fillapex than AH Plus even in cases where they used calcium hydroxide as a medicament. Different results might be attributed due to methodology such as the medication used. Interestingly, a previous study investigated the dentinal tubule penetration of the AH Plus, MTA Fillapex and GuttaFlow Bioseal sealers and concluded that the dentinal tubule penetration area was significantly affected by the selection of root canal sealer, final irrigation procedure, and root canal third. Use of iRoot with PIPS tip or PUI seems advantageous in dentinal tubule penetration (11).

In this study, GuttaFlow Bioseal showed significantly less depth of penetration than AH plus as well as MTA Fillapex. This might be due to the larger particle size of GuttaFlow Bioseal, poor wetting ability, presence of silicone that might increase the surface tension, or the lower setting time that makes the penetration of sealer inside the canal rather challenging (26).

A recent study showed that GuttaFlow displayed deeper penetration into the dentinal tubules than AH plus and MTA Fillapex (30). They stated that GuttaFlow Bioseal has both osteointegrative and osteoconductive effects and it is said to bond mechanically to bone tissue through the formation of hydroxyapatite crystals. Different results may be achieved because of different methodologies used for root canal filling.

In the current study, using silicone oil lowered the mean values of the penetration depth lengths into the dentinal tubules in all thirds for all sealers. Likewise, this effect was pronounced for GuttaFlow Bioseal. Silicone oil, could have repelled its dissolve and retrieval from the root canal by Chlor-XTRATM and EDTA (31). On the other hand, 7% maleic acid or 10% citric acid were observed to be further efficient than 17% EDTA in removing calcium hydroxide mixed with silicone oil (31). Maleic acid 7% or 10% citric acid may be beneficial in the removal of the silicone oil in further studies.

In this study, the depth of sealer penetration was found to be greater at the coronal than at the middle and at the apical sections, which is in accordance with the study done by Generali et al. (32). This can be attributed to the presence of more sclerotic dentine, fewer number and density of dentinal tubules in the apical area (33). Furthermore, some amount of moisture is left in root canal even after drying, due to capillary action in narrow apical third of canal thus limiting the flow of sealer in the apical third. Moreover, at the coronal thirds, better penetration might be related to the greater lateral compaction during obturation and better action of the irrigants (32, 33).

One of the drawbacks of the current study was that a recent study demonstrated the inadequacy of Rhodamine B dye in sealer detection in dentine (34). They justified that the dye was passively diffused into the dentinal tubules because it was not permanently attached to the sealer. Therefore, further studies on tubular penetration should be considered in combination with other aspects, such as sealing ability and push-out bond strength.

CONCLUSION

Under the limitations of this study, epoxy resin based sealer has a higher dentinal penetration ability than calcium silicate or silicone based sealers either with or without using silicone oil. Additionally, calcium silicate based sealer penetrated significantly more than silicone based sealer even with using oil. It could be of great advantage to obturate the root canal with AH plus instead of MTA Fillapex and GuttaFlow Bioseal especially in case where removal of separated instrument was managed by using silicone oil. Additional studies are required to assess the penetration ability of different based sealers into oil treated dentine after using different irrigants.

Disclosures

Conflict of interest: The authors deny any conflict of interest.

Ethics Committee Approval: This study was approved by The Suez Canal University College of Dentistry Research Ethics Committee (Date: 05/01/2021, Number: 371).

Peer-review: Externally peer-reviewed.

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