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Depth and Percentage of Penetration of Sure Seal Root and AH Plus sealers into Dentinal Tubules with two different obturation techniques

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Abstract
Background: *To evaluate and compare depth and percentage of penetration of Sure Seal Root sealer and AH Plus sealer into dentinal tubules with cold lateral condensation (CLC) and single cone (SC) obturation techniques.*
Materials and methods: *Human freshly extracted 40 maxillary anterior teeth were prepared and assigned to 4 experimental groups (n =10), designated as group I: (AH Plus + CLC), group II: (AH Plus + SC), group III: (Sure Seal Root + CLC) and group IV: (Sure Seal Root +SC). Teeth were sectioned at three root canal levels (coronal, middle, and apical) and examined by confocal laser scanning microscopy. Then, the depth of sealer penetration in dentinal tubules and percentages of the penetrated sealer into dentinal tubules in each section were measured. Data were analyzed using one-way ANOVA in a level of confident at 95% followed by post hoc tukey test for comparisons.*
Results: *Group III showed significantly higher penetration depth at all levels than the other groups. The percentage of sealer penetration around the root canal walls in group III was significantly higher than other groups at all levels.*
Conclusion: *Regardless of the filling technique used, Sure Seal Root achieves a better filling quality and greater tubular penetration than AH Plus. Taking into account the excellent bioactivity of the Sure Seal Root sealer, it can improve the sealing of the root canal system. Although Group III (Sure Seal Root + CLC) displayed deeper penetration into the dentinal tubules there was no difference in the percentage of penetration into the root canal walls among the 4 groups evaluated.*
Keywords: *AH Plus; cold lateral condensation; confocal laser scanning microscopy; Rhodamine B; single cone; Sure Seal Root.*

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I. Introduction

Three-dimensional obturation of the root canal system is crucial for successful endodontic therapy as it eliminates microleakage of periapical exudate into the root canal space, prevents reinfection, and provides a favourable environment to enhance healing[1]. The most widely accepted obturation technique is using core material such as gutta-percha (GP) or resin based material in conjunction with root canal sealer [2]. GP can be adapted well to the root canal walls, but when used alone, it can't fill irregularities occurred in the root canal and dentinal tubules. Therefore, a root canal sealer is needed not only to fill irregular spaces, but also to reduce microleakage and improve penetration of inaccessible dentinal tubules [3,4]. Sealers also have antibacterial activity, through direct contact of the chemical components of sealer with bacteria and indirectly through isolation of residual bacteria into the dentinal tubules and keeping them away from nutrition [5,6].

Therefore, the ability of root canal sealer for penetration into dentinal tubules is considered a desirable outcome as it enhances the sealing and retention of the filling material improved by mechanical interlocking of sealer tags into dentinal tubules [7,8]. The capability of a sealer to penetrate into dentinal tubules effectively is the main determinant for sealer selection and sealer placement techniques during obturation [3] .To gain optimal filling effect, EDTA should be applied before using sealers to remove the smear layer (a layer of organic and inorganic material) from the dentin as this layer easily adheres to the sealing material and the root canal wall, thereby reducing the bond between sealer and dentin [9].

There are two main obturation techniques: cold lateral condensation (CLC) and single cone (SC). CLC, using GP and a root canal sealer, is one of the most widely used obturation technique that has excellent long term results, predictability, controlled placement and relative ease of use, but needs lateral condensation pressure [10,11]. However, SC uses a single GP cone and a sealer, without the need for lateral or vertical pressure, thereby decreasing the risk for root fracture and thermal damage to the periodontal membrane and saving clinicians time [10,12]. However, because the filling process has no condensation pressure, the canal always contains a volume of sealer much more than the one produced by CLC, thereby leading to formations of gaps in the interface between sealers, canal walls and the root canal system which ultimately affect the success of root canal treatment [13].

Various microscopical techniques, including stereo-microscopy, scanning microscopy (SEM) and confocal laser microscopy (CLSM) are currently used to evaluate the sealer dentin interface [14]. Unlike conventional SEM, CLSM provides thorough information about sealers distribution within the dentinal tubules at various depths, with less or no artifacts, and in non-dehydrated samples [15]. Rhodamine B allows identification of sealers within the dentinal tubules at low or high magnification and does not affect the physical properties of the sealers as only small amount of dye (0.1%) is mixed with the sealers. Moreover, CLSM software can create a three-dimensional reconstruction that can efficiently allow calculation of sealer depth and percentage of penetration[3]. For all these advantages, CLSM was used in our study rather than SEM [3].

Most of these previous studies investigated dentinal tubules penetration of resin-based sealers (such as AH Plus) and calcium silicate-based sealers (such as Sure-Seal Root) each alone or in combination using different irrigation methods. However, little is known about the comparison between the penetration depth and percentage of the AH Plus and Sure-Seal Root sealers in combination with different obturation methods (such as CLC and SC). It is important to compare the penetrability of various types of sealers used in routine dental clinic with different obturation methods to improve the quality of endodontic treatment and the success rate. Also, little information is available in publication regarding Rhodamine fluorescence intensity of the sealers, although it is an important parameter for assessment of the degree of penetration using CLSM. Therefore, this study was designed to compare between AH Plus and Sure-Seal Root canal sealers in terms of depth and percentage of penetration into dentinal tubules as well as their fluorescence intensity with two different obturation techniques (CLC and SC) using confocal laser scanning microscopy.

II. Material And Methods

Forty recently extracted human single-rooted anterior teeth with straight single root canal which extracted for periodontic or orthodontic reasons between the ages of 20 to 40 years old will be collected from the outpatient clinic of Oral Surgery department, Faculty of Dentistry, Tanta University.

Study Design:A controlled experimental study was adopted for this work.

Study Location: Faculty of Dentistry, Tanta University.

Study Duration: December 2018 to December 2019.

Sample size: 40 extracted teeth divided into 4groups (3 sample/ each) with total of 120 samples.

Procedure methodology:

Forty recently extracted human single-rooted anterior teeth with straight single root canal which extracted for periodontic or orthodontic reasons between the ages of 20 to 40 years old will be collected from the outpatient clinic of Oral Surgery department, Faculty of Dentistry, Tanta University. Teeth will be thoroughly cleaned by removing the hard deposits using hand scaler[*] and the soft deposits by soaking in 5.25% sodium hypochlorite solution[†] (NaOCl) for 15 minutes. The teeth will be stored in a normal saline solution at room temperature until being used within three months [16].

Root canal preparation and filling:

The crown of each tooth was decoronated perpendicular to the long axis of the tooth by using low-speed diamond disc[‡] under water cooling to reach standardized root length of 15±1 mm [17]. Canal patencyand working length were determined through insertion of hand stainless steel K file [§] #10 until just visible at the apical foramen and then the file was withdrawn and the length was recorded and subtracting 1 mm from this length. Mechanical instrumentation of root canal was performed using a crown down technique with 2 Shape file system according to the manufacturer’s instructions up to master file F35 (#35/0.06). Begins with a

[*] Dentsply/Maillefer, Switzerland.
[†] Clorox Co,10th of Ramadan, Egypt
[‡] Komet; Brasseler, Lemgo, Germany
[§] Dentsply/Maillefer,Switzerland.

progressive movement in 3 waves with upward circumferential filing movement until no resistant felt in the canal [18,19].

The canal will be throughout irrigated using 2 ml of 2.5% NaOCl solution using an irrigation syringe with a 27-gauge needle after every file during the instrumentation procedure. Then, the canal was flushed out with 5 ml distilled water after the last NaOCL irrigation solution to separate it from the following administration of ethylenediamine-tetraacetic acid[**] (EDTA) solution. Afterwards, a total of 1 mL 17% EDTA was used for irrigation which withdraws slowly with 27 gauge needle and leave it in the canal for 1 minute followed by final flush with 5 ml of distilled water then the canal will be finally dried with sterile absorbent paper points[††7].

Samples will be randomly divided into 4 groups, each composed of 10 teeth and were obturated with Sure Seal Root[‡‡] sealer and AH Plus[§§] with both cold lateral condensation and single cone technique:

- **Group I:** Root canals were filled by AH Plus sealer and Gutta-percha master cone[***] (#35/0.04) using lateral condensation technique according to manufacturer instructions.
- **Group II:** Root canals were filled by AH Plus sealer and Gutta-percha master cone (#35/0.06) using single cone technique according to manufacturer instructions.
- **Group III:** Root canals were filled by Sure Seal Root sealer and Gutta-percha master cone (#35/0.04) using lateral condensation technique according to manufacturer instructions.
- **Group IV:** Root canals were filled by Sure Seal Root bioceramic sealer and Gutta-percha master cone (#35/0.06) using single cone technique according to manufacturer instructions

AH Plus sealer were mixed according to manufacturer's directions. While Sure Seal Root required no mixing as it is premixed and stored in air tight syringe ready for injection into root canal. To allow analysis under the CLSM, each sealer was labeled with Rhodamine B[†††] (Sigma-Aldrich, St. Louis, MO) to an approximate concentration of 0.1%. Exact proportions were determined using a precision analytic balance. For the SC technique, the master cone #35/0.06 was used to coat the canal walls with sealer. The cone was recoated with sealer and introduced slowly into the root canal until reaches the working length[7,20]. The cone was sheared off at the level of the orifice and lightly condensed by heated plugger. In Group IV Sure Seal Root / Single cone, the sealer was injected in the canal 2-3 mm shorter than working length and then master GP cone were inserted in the canal until reaches the working length as manufacturer instructed.

For lateral condensation, the master cone gutta-percha[‡‡‡] (#35,0.04) was tried to fit with tug back at the working length. Then, canal walls were coated with the sealer-dye mixture with the master GP cone. Then, the master cone GP (#35,0.04) was introduced slowly into the root canal until reaches the working length. A size 25 endodontic finger spreader[§§§] was inserted 2-3 mm short of the working length, and accessory gutta percha cones #25/0.02 were used. Repeated insertion of accessory gutta-percha were done until complete obturation and the spreaders could not penetrate more than 2 mm in the canals. Excess gutta-percha was sheared off by using a heated plugger[****] and vertical compaction was performed at the orifice level [7].

Radiographs of the samples were taken to confirm the quality of the root canal filling[21]. Samples with inadequate obturation were discarded. Roots of all groups were sealed with a temporary filling material [††††] from the coronal part and stored in jars which kept moist by keeping them in a gauze moistened with sterile saline solution in the incubator and were maintained at 100% humidity for 7 days at 37°C to allow the sealer to fully set [3,7]. Roots were placed vertically and centered in cold cured acrylic resin[‡‡‡‡] blocks[22]. These blocks were fabricated by using a 5 ml plastic syringe as a mold, the coronal surface of the roots was fixed onto a glass slap using a sticky wax to facilitate its centralization within the block without any inclination to any direction. A separator medium was used to cover the internal surface of the syringe for easier removal. Each sample was left to ensure complete setting of acrylic resin[7].

Sectioning and image analysis:

Each root block was sectioned horizontally by using 0.3 mm isomet 5000 precision saw[§§§§] at 200 rpm under continuous water cooling (5°C) to prevent frictional heat [3]. Horizontal sections will be at the 3, 5 and 7

[**] Prevest Denpro, Digiana, Jammu e India.
[††] Dentsply/Maillefer, Switzerland.
[‡‡] Sure Endo, Gyeonggi-do, Korea.
[§§] Dentsply DeTrey GmbH, Konstanz, Germany.
[***] Micromega, France.
[†††] Sigma-Aldrich, St. Louis, MO
[‡‡‡] Micromega, France.
[§§§] Dentsply/Maillefer, Switzerland.
[****] Dentsply/Maillefer, Switzerland.
[††††] Orafil-G, PREVEDT DenPro, Bari Brahmana, India.
[‡‡‡‡] Acrostone Co., industrial zone, 15 km northwest of Cairo, Egypt.
[§§§§] Buehler, 41Waukegan road, Lakebluff, USA.

mm levels from the apex after discarding the last apical 2 mm to represent apical, middle and coronal third respectively with a section of 2 mm in thickness measured with a digital caliper. The coronal, middle and apical sections were colour-coded as red, blue and black respectively.

Confocal imaging of the samples were done using LSM 710[*****] with an image size of 1024 × 1024 pixels, a 16-bit depth, and objectives EC Plan-Neofluor 10x/0.3 M27 oil DIC and EC Plan-Neofluor 40x/1.3 M27 oil DIC. The acquisition was performed using Zen 2.3. software and processing were conducted using Zen 2012 (blue and black edition). CLSM images were captured in the fluorescent mode. A helium-neon laser was used as the light source and the excitation light source had a wavelength of 543 nm. The fluorescent light was collected beyond 560 nm. The laser power settings were kept constant for all root sections [3]. All samples were examined from a coronal view with a confocal laser scanning microscopy. The full sample acquisition was imaged with a 10x objective lens in the format of 1,024 × 1,024 pixels. While the 40x oil lenses confirm the content of the sealer inside the dentinal tubules. Each sample image was imported on the ZEN software[†††††] to be examined [3,7].

Confocal images of all root sections were assessed by two examiners. A full image view of the distribution of sealers at the dentin-sealer interface was examined for a uniform fluorescent ring around the canal wall. The fluorescence, which has been traced from the sealer-dentin interface into the maximum depth, revealed the extent of the sealer's penetration in the dentinal tubules. The method used by Gharib et al.[14] and Bitter et al.[23] was applied to evaluate the images using the digital measuring ruler tool that present in the ZEN software, to measure the depthof sealer penetration at four standardized starting points in each wall of the root canal. The canal wall served as the start point and the penetration of the sealer was measured to maximum depth. These data points will be averaged in order to obtain one measure for each section as shown in Fig-1.

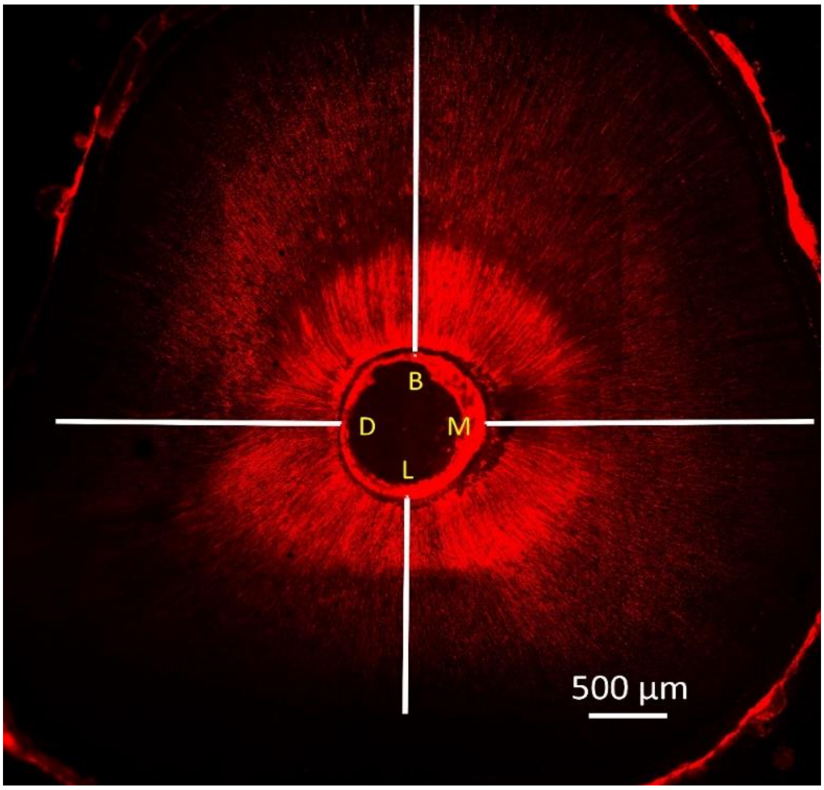

Fig-1 CLSM image 10x shows 4 standardized points on each wall of root canal (buccal, mesial, Distal and lingual). These point will be averaged to get the mean of depth of penetration for the section.

To obtain the percentage of sealer penetration around the root canal in each section, the circumference of the root canal wall was obtained by drawing with spline contour tool in ZEN software. Next, areas along the canal walls which covered by the sealer and the area of the sealer which penetrated inside dentinal tubules from the whole circumference of the root canal with any distance were outlined and measured using the same method as shown in Fig-2.The outlined lengths where sealer was penetrated will be divided by the canal circumferences and multiplying the result by 100. Subsequently, the percentage of the root canal wall with sealer penetration in

[*****] Carl Zeiss (ZEISS), LSM 710,Jena, Germany.
[†††††] Carl Zeiss (ZEISS), ZEN 2.3, Jena, Germany.

that section was calculated [3,7]. The 63x oil lens with additional Zoom 3 was used to check the contents of dentinal tubules to assess whether dentinal tubules with fluorescence are consistent with rhodamine marked sealers [3].

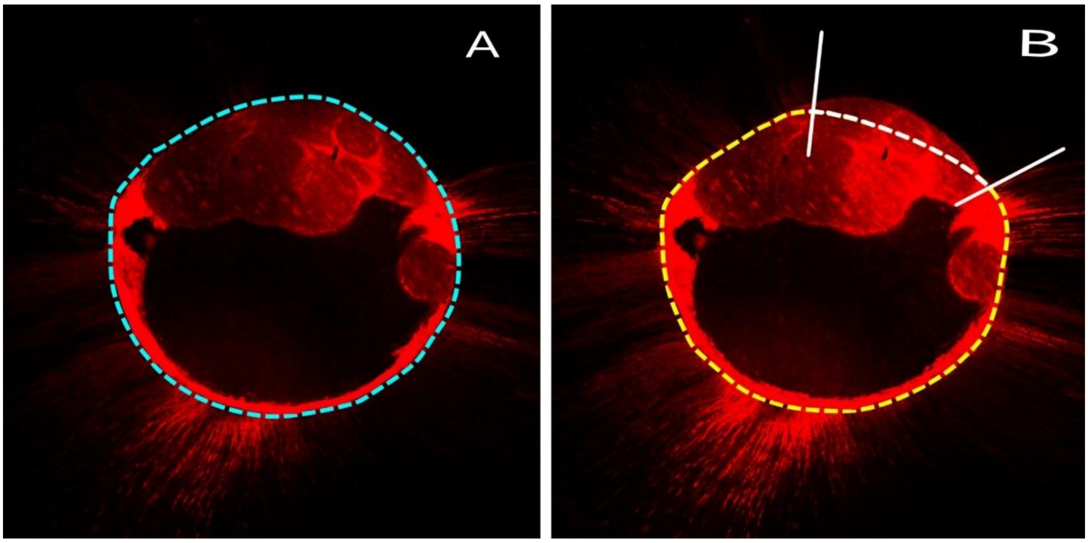

Fig-2: CLSM images 10x where A: circumference of root canal. B: Selected area of the root canal where the sealer penetrated to any distance into the dentinal tubules (yellow line).

Statistical analysis

Statistical significance for the depth and percentage of root canal sealer penetration was determined for each root canal level and evaluated using one-way ANOVA in a level of confident at 95%. Whenever a statistically significant difference was observed between different tested groups or sections of the root canal, the Tukey-Karmer post-hoc test was performed to compare each significant difference group or section with each other. The level of significance was set at $P < 0.05$. The analyses of the tests were performed using SPSS software version 23.0 ‡‡‡‡‡.

III. Result

Effect of AH Plus and Sure-Seal Root sealers and obturation techniques on penetration depth

Examination of CLSM images revealed varying amounts of dentinal tubules penetration around the root canal walls for the two sealers. Both sealers showed a continuous fluorescent ring of sealer around root canal and almost total adaption to the canal wall in both CLC and SC techniques (Fig.3). Based on CLSM data, group III (Sure-Seal Root +CLC) showed significantly ($P < 0.05$) higher penetration depth at the three root canal levels (coronal, middle, apical) as compared to group I (AH Plus +CLC), and group II (Sure-Seal Root +SC) (Table 1, Fig.4). Group III also had significant ($P < 0.05$) higher penetration depths at the middle and apical levels than group IV (Sure-Seal Root +SC). However, at the coronal level group III displayed insignificant ($P > 0.05$) higher penetration than group IV (Table 1, Fig. 4). Additionally, group IV showed significant ($P < 0.05$) higher penetration depth at all root levels than groups I and II. However, group I had insignificant ($P > 0.05$) higher penetration depth than group II at the three root levels (Table 1, Fig. 4).

Regarding comparison of penetration depths within each group, all groups (except group III) exhibited significant ($P < 0.05$) differences between any of the coronal or the middle level and the apical level, with highest penetration at the coronal level and lowest penetration at the apical level (Table 1, Fig. 4). However, no statistical significance ($P > 0.05$) found in penetration depth between the coronal and the middle levels in all groups.

Effect of AH Plus and Sure-Seal Root sealers and obturation techniques on percentage of penetration

The sealer penetration around the root canal walls in group III was significantly ($P < 0.05$) higher than group II at all root levels (Table 2). However, no statistical significance ($P > 0.05$) in percentage of penetration was noticed between other groups at all levels or among the three levels (Table 2).

‡‡‡‡‡ IBM, Armonk, NY.

Effect of AH Plus and Sure-Seal Root sealers on rhodamine fluorescence intensity

Upon examination with CLSM at 40x and 63x oil lenses, we found a difference in the intensity of rhodamine fluorescence in dentinal tubules between the Sure-Seal Root groups and AH Plus groups (Fig. 5). Sure-Seal Root had lower fluorescence as revealed by incomplete or partial obturation of dentinal tubules with the sealer. In contrast, AH Plus sealer showed more fluorescence as indicated by completely obturated dentinal tubules.

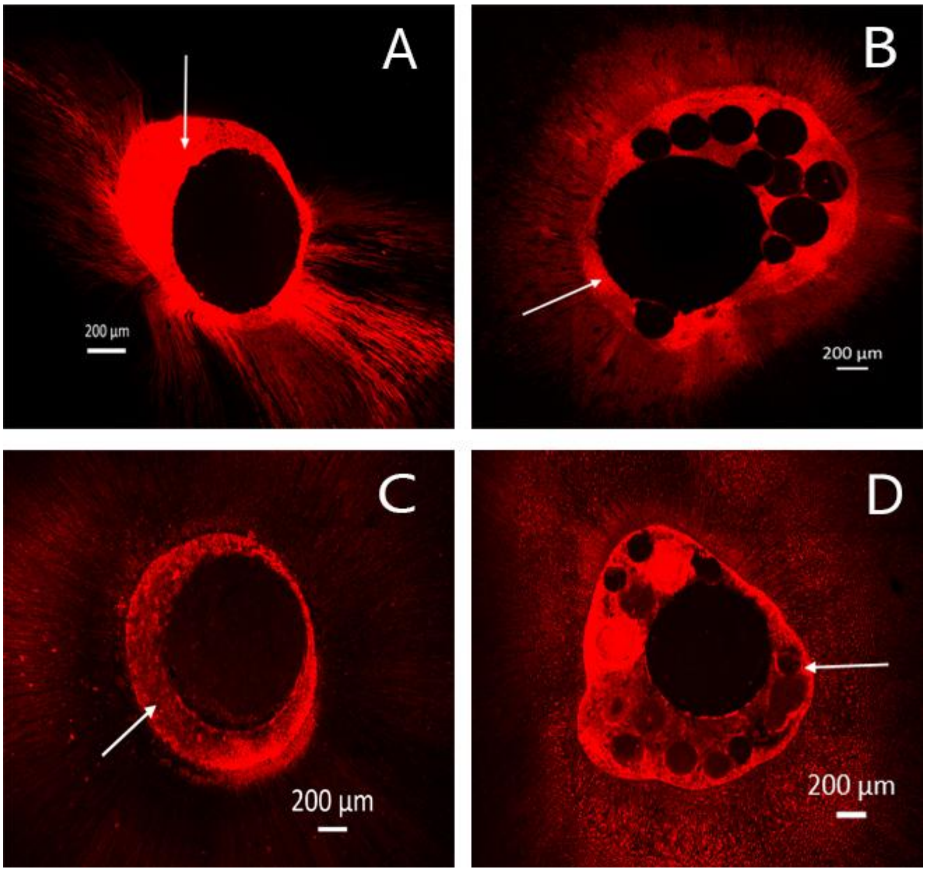

Fig-3 CLSM images (x10) shows continuous fluorescent ring of sealer around root canals (arrows). A: Group I, B: Group II, C: Group III, D: Group IV.

Table 1. Depth of sealer penetration (µm) into dentinal tubules.

Root level	Group I (AH Plus+CLC)	Group II (AH Plus+SC)	Group III (Sure Seal Root+CLC)	Group IV (Sure Seal Root+SC)
Coronal	629.55 ± 109.57 ^{aB}	586.40 ± 40.29 ^{aB}	1828.75 ± 133.18 ^{A}	1682.75 ± 219.25 ^{aA}
Middle	583.65 ± 82.56 ^{abC}	537.32 ± 52.97 ^{aC}	1753.44 ± 144.87 ^{A}	1549.23 ± 259.71 ^{aB}
Apical	525.01 ± 70.99 ^{bC}	469.84 ± 81.14 ^{bC}	1627.89 ± 253.76 ^{A}	1317.11 ± 151.59 ^{bB}

Data was presented as mean ± SD (n = 10/root level/group). The different uppercase superscript letters (A, B and C) indicate significance between all groups at every root level (coronal, middle, apical). The different lowercase superscript letters (a, b and c) indicate significance between root canal levels in each group at $P \leq 0.05$.

The mean, standard deviation and statistical analysis of the percentage of sealer penetration are presented in Table 2. ANOVA-Tukey post hoc tests indicated significant difference in the percentage of sealer penetration around the root canal walls among the 4 groups regardless root canal level ($P < 0.05$). Also, a statistical significant difference was found between all root canal levels regardless groups ($P < 0.05$; ANOVA-Tukey).

Table 2. Percentage of sealers penetration around the root canal wall.

Root sections	Group I (AH Plus+CLC)	Group II (AH Plus+SC)	Group III (Sure Seal Root+CLC)	Group IV (Sure Seal Root+SC)
Coronal	95.3 ± 8.2 ^{AB}	92.5 ± 7.5 ^{B}	99.3 ± 1.1^{A}	99.1 ± 1.1 ^{AB}
Middle	91.8 ± 8.6 ^{AB}	89.9 ± 8.2 ^{B}	99.0 ± 1.4 ^{A}	93.8 ± 6 ^{AB}
Apical	86.1 ± 13.7 ^{AB}	85 ± 8^{B}	96.7 ± 4.3 ^{A}	93.4 ± 7.7 ^{AB}

Data was presented as mean ± SD (n = 10/root canal level/group). The different uppercase superscript letters (A, B and C) indicate significance between all groups at in each root canal level (coronal, middle, apical) at P ≤ 0.05.

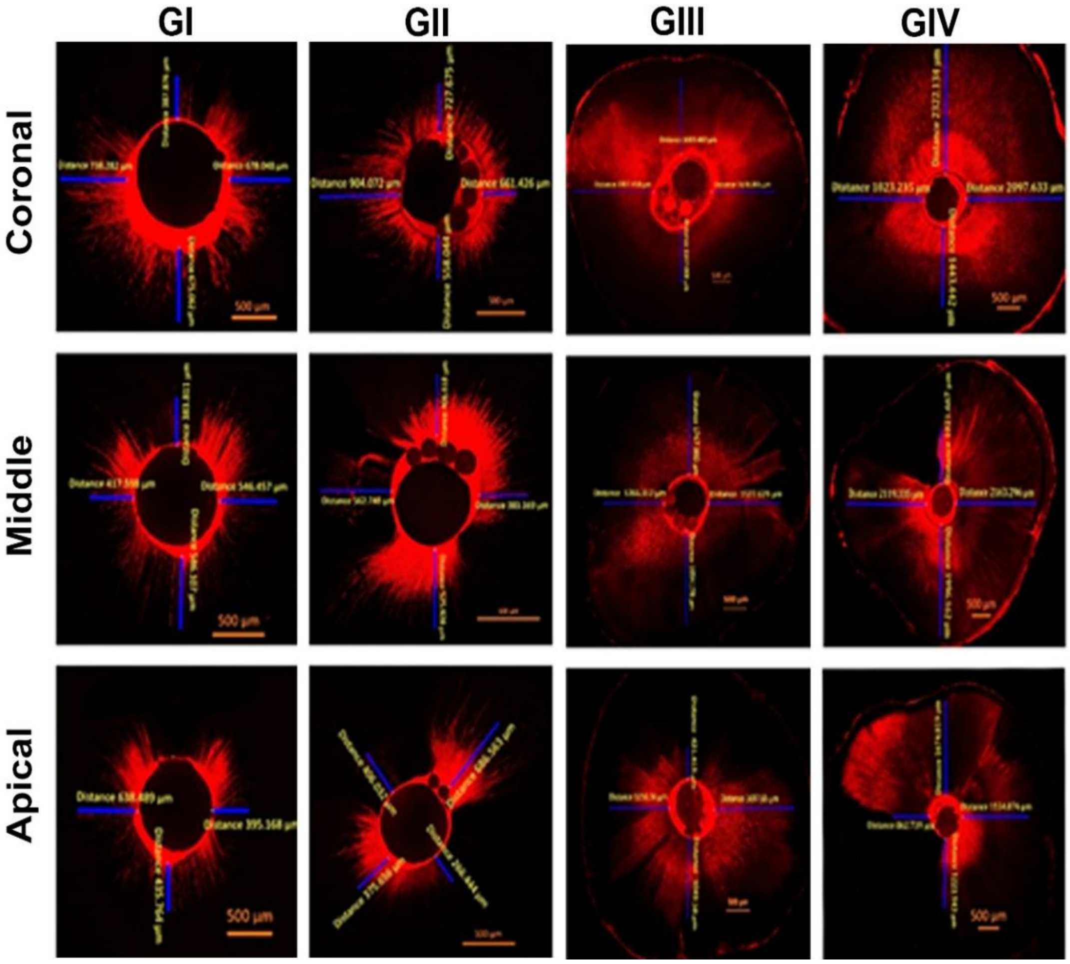

Fig.4. CLSM representative images show sealer penetration depth inside dentinal tubules in all groups at the three root canal levels.

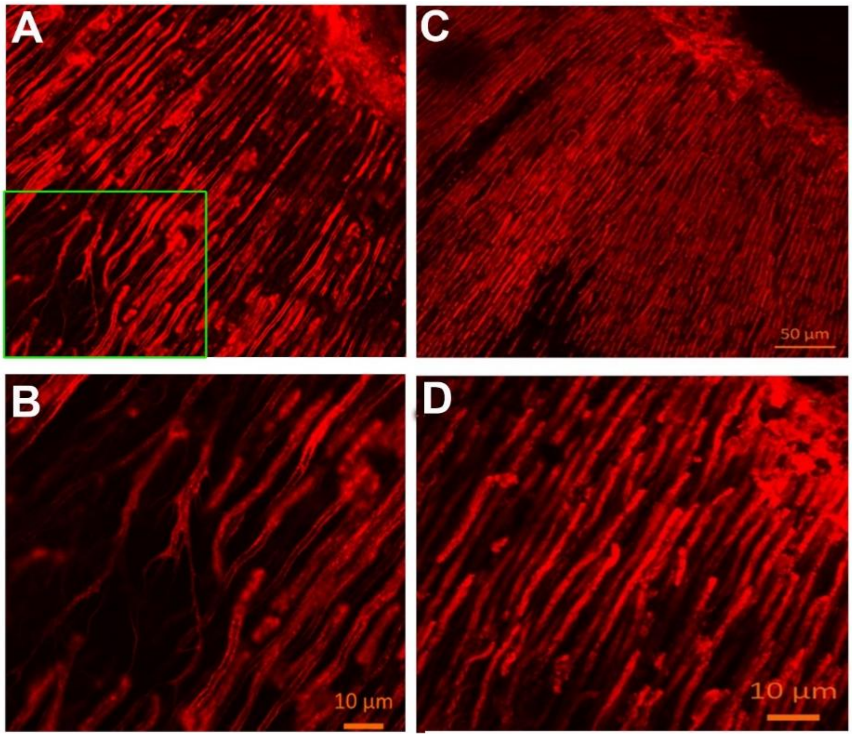

Fig 5. CLSM representative images show: (A, B) Sure Seal Root sealer penetration dentinal tubules (A, x40 oil lens) and partial obturation of dentinal tubules lumen (B, x63 oil lens), and (C, D) AH Plus sealer penetration inside dentinal tubules (C, x40 oil lens) and nearly complete obturation of dentinal tubules lumen (D, x63 oil lens). The green border square in A indicates the higher magnified image in B.

IV. Discussion

Sealer penetration into the dentinal tubules depends onmany factors including smear layer removal, variation in tubular density and the size of root canal. Also, the physio-chemical properties of the sealer [24–27]. For that, it is obvious that if a proper agitation technique and a sealer with a high capacity to penetrate into the dentinal tubules is used, it will be possible to minimize leakage and the incidence of reinfection in the root canal system. Therefore, using proper irrigation method, suitable obturation technique and a sealer with superior penetration capability is necessary to improve penetration, and minimize leakage and infection in the root canal[4]. For their wide uses and excellent physical properties and biocompatibility, the resin-based AH Plus sealer and the bioceramic sealers such as Sure-Seal Root were used in the present study to examine their penetration depth into dentinal tubules and the percentage of penetration around the root canal wall when used with two different obturation techniques (CLC and SC)[4,28,29].

As previously stated, sealer physio-chemical property is an essential factor for efficacy of sealer penetration. Regardless the type of the obturation technique, we found higher penetration depth for Sure-Seal Root sealer inside the dentinal tubules than AH Plus sealer. Indeed, we found that group III (Sure-Seal Root +CLC) exhibited significantly highest depth and percentage of penetration, followed by group IV (Sure-Seal Root +SC), group I (AH Plus+CLC), and group II (AH Plus+SC) at the three root canal levels (coronal, middle, and apical). Consistent with our findings,Akcay et al.[30]and Toursavadkohi et al. [22]found better penetration ability for the two bioceramic sealers iRoot SP and Sure-Seal Root than the resin-based sealers AH Plus and AH26, respectively.Other studies also reported excellent flow quality and deeper tubular penetration into dentin for bioceramic sealers[4].This better penetration effect could be attributed to higher flowability, small particle sizes, low film thickness and high level of viscosity of the bioceramic sealers[31,32]. Moreover, bioceramic sealers exhibit a minimum or no shrinkage during the setting phase due to their calcium silicate components have the ability to utilize the moisture in dentinal tubules to begin and complete the setting reaction[33]. In addition, Sure Seal Root sealer exhibits 0.2% expansion during the setting period. These characteristics also support the spread of the sealer over the dentin walls of the root canal and filling of the lateral canals [34].

In contrast, some other studies reported better penetration ability for AH Plus than other sealers and attributed this to its pseudoplastic behaviour which decreases viscosity and increases flow during filling procedures[7,35,36]. This physical property is important as it enables the sealer to effectively adapt to the root canal wall and penetrate dentinal tubules. These contradictory results may be due to variation in irrigation and obturation techniques in addition to the physico-chemical properties of the used sealers.Moreover, the variation in the penetration results between different sealers may be influenced by powder/liquid or paste/paste ratio of the mixed material. Even small alterations to this ratio may cause a change in thickness and flow of the material [37]. It is important for manufacturers to provide measuring equipment for clinicians to achieve ideal powder/liquid or paste/paste ratio of root canal sealers. In the present study, Sure Seal Root sealer were provided as premixed injectable syringe providing standardizing mixtures. While, AH Plus sealer were mixed according to manufacture instruction in an equal ratio pate to paste.

Sealers penetration varied according to the levels of the root canal. Our results revealed that the coronal level followed by the middle levels showed significantly higher depth of sealer penetration than the apical level in groups I, II and IV. This may be as a result of the efficient removal of smear layer in coronal and middle thirds of the canals and the number of dentinal tubules and the size of their lumens in the coronal area is well known to be significantly bigger than in the apical area, they allow for better adhesion of the sealer to the root canal walls [8,38,39]. Calcium silicate-based sealers showed greater than 80% sealer penetration circumferentially at the coronal level for both techniques [12]. However, we did not find any statistical difference between the three levels in group III. This might be due to the ability of bioceramic sealers to penetrate dentinal tubules even in the presence or absence of smear layer [40].

The second main determinant for sealer penetration capability is the obturation technique. Regardless the type of sealer used, groups involved CLC technique give better penetration than those used SC techniques. For example, Sure-Seal Root +CLC and AH Plus +CLC groups had higher penetration depth than Sure-Seal Root +SC and AH Plus +SC groups, respectively. In agreement, Macedo et al. [41] reported that CLC and GP-induced vertical condensation had higher penetration and bond strength than SC. Also, McMichael et al.[12] reported that Fillapex MTA had significantly higher tubule penetration with warm vertical condensation technique than SC. Additionally, Souza et al.[42]investigated the effect of obturation techniques on sealer distribution and stated that the sealer-coated root canal wall area was significantly higher when CLC was used. Whereas another suggests that bioceramic sealers showed inferior bond strength when used with the continuous wave technique [43]. Better sealer penetration associated with CLC could be due to the greater flow of the sealer under compaction pressure[40]. Taken together, we and Macedo et al. [41] concluded that the compression forces of CLC with the excellent physical properties of the bioceramic sealer Sure-Seal Root resulted in a greater sealer penetration. In support, Kuci et al. [40]also found that the bioceramic MTA Fillapex sealer had greater penetration than AH Plus when used with CLC technique.

Even though most of the groups, especially groups III, IV, I (in order), showed higher penetration capability, their penetration pattern around the circumference of the root canal walls was interrupted in some areas, thereby leading to penetration percentage less than 100%. Among the four groups, only group III showed significantly higher sealer penetration percentage around the root canal walls than group II at all levels. However, no statistical significance was noticed between other groups at all levels. All penetration percentages were ranged from 85 ± 8% (in group II at the apical level) to 99.3 ± 1.1% (group III at the coronal level). This means that in group II only 85 ± 8% of the root canal walls at the apical level contained sealers within the dentinal tubes. Hence, at least 15% of the root canal wall did not contain sealer. These negative areas may be formed by the compact forces of the spreader during lateral compaction technique[3]. We did not find significant differences among the three levels in each group. In contrast, Ordinola Zapataet al.[3]and Weis et al. [27] reported higher penetration percentage at the coronal level than at the apical level and attributed these changes to the presence of larger number of dentinal tubules with higher density or occluded tubules in apical dentin than in coronal dentin.

Previous CLSM studies have found that it is essential to include Rhodamine B in the sealer to determine the extent of sealer adaptation and penetration [3,7,23]. As it allows for the identification of sealers within the dentinal tubules [44] and does not affect the physical properties of the sealers, as long as a small amount of dye (less than 0.2%) is mixed with the sealers [45]. Hence for, it renders the penetration depth and percentage of sealer to dentinal tubules easily evaluated at low magnification. Moreover, at higher magnifications, a panoramic view of sealer adaptation into the root canal and dentinal tubules can be easily confirmed [3].Although the advantages of confocal microscopy, in evaluation of the sealer-dentine interface, it is necessary to determine whether the dye leached from the sealer or not because this fact would interfere with the evaluation of microscopic images [46]. Patel et al. [47] confirmed the fact that the dentine is labelled with Rhodamine B in the absence of endodontic sealer. As it was conducted in a pilot test prior to the investigation of the penetration of the two root filling materials not labelled with Rhodamine B was found to be similar to that of the Rhodamine-labelled sealers. The possibility of false results due to leaching of Rhodamine from the sealers was therefore excluded [47]. The orthogonal section (X-Y-Z optical section) has been used to confirm this fact. If the red fluorescence is limited to the sealer layer and to the lumen of dentinal tubules or lateral branches, an image should be considered appropriate. As observed, intensity of the fluorescence in dentinal tubules is related to the quantity of sealer inside the dentinal tubule (Fig. 5). Higher fluorescence was correlated to complete obturation of dentinal tubules, while less fluorescence attributed to partial or incomplete obturation of dentinal tubular lumen [3]. Another consideration is the ability of the dye to influence the polymerization of the sealer and to minimize the bond strength of the materials being examined to their substrate [15].

Upon examination with CLSM at 40x and 63x oil lenses, there was a difference in the intensity of fluorescence in dentinal tubules between the bioceramic sealer groups and AH plus groups. The lumen of dentinal tubules was observed and evaluated. The finding was that the Sure Seal Root sealer had lower fluorescence which means incomplete or partial of obturation of dentinal tubules with the sealer but with a higher depth of penetration. While AH Plus sealer showed a lesser depth of penetration but with more fluorescence in the lumen of dentinal tubules. Therefore, the dentinal tubules almost completely obturated with sealers as shown in Fig.5. This fact supported by the previous studies regarding flowability of both sealers, the difference in viscosity of the sealer cement, the particle size and film thickness of the sealers [31,48–50]. Future studies should be done to evaluated the extent of sealer penetration and its relation with the complete or partial obturation of dentinal tubules lumens and which is better in elimination of residual bacteria inside dentinal tubules.

In contrast to our study, Jeong et al. [51] examined the use of Fluo-3 dye as a fluorescent marker for the bioceramic sealer instead of Rhodamine B dye because Rhodamine B shows a powerful sensitivity to moisture and a lower calcium affinity in the sealer composition. In this way, it can separate from its mixtures with the sealer, following any small degrees of moistures in the dentine and emit fluorescence independent of the sealer, and show deeper tubular penetration into the dentinal tubules, producing inaccurate results. Fluo-3 has been used to trace calcium ions under CLSM because fluorescence increases by 100-times in the proximity of calcium ions. As it contains calcium that is dissociated in the moisture within root canals and dentinal tubules, and in the absence of calcium ions it does not emit fluorescence under CLSM [31,52]. So, it is considered a suitable fluorescent dye when using a bioceramic sealer that contains a calcium silicate-based material. Yet, other studies had used rhodamine B dye as a fluorescent marker in evaluating the depth of penetration of calcium silicate-based sealer and did not report any leaching out [12,30,40,53,54]. Although none of the investigated sealers affected the fluorescence of rhodamine B in the present study, Fluo-3 could be used in future studies to label calcium silicate based sealers.

Although, this study did not examine the interface between the gutta-percha and dentin wall. We found that all groups showed a uniform distribution of sealer around the root canal in the coronal, middle and apical third of the root canal with both obturation techniques. Further studies are necessary to analyze the interfacial adaptation of these sealers to root canal walls.

V. Conclusion

Combination of Sure-Seal Root sealer and CLC technique gave better effect in terms of higher penetration depth for Sure-Seal Root into the dentinal tubules and higher percentage of penetration around the root canal walls as compared to using AH Plus sealer with SC technique. However, AH Plus -SC showed higher fluorescence in the lumen of dentinal tubules, thereby suggesting almost completely obturation of tubules with large amount of sealers than Sure-Seal Root -CLC.

Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

The authors declare (i) that all authors have contributed significantly and (ii) that all authors are in agreement with the manuscript.

Ethics statement

The protocol for collection and investigation of extracted teeth has been approved by the Tanta University Research Ethics Committee.

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