

Age-Related Changes in The Penetration of Calcium Silicate-Based Sealer into Dentinal Tubules: A Confocal Laser Scanning Microscopy Evaluation

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Abstract

Objective: The purpose of this study was to compare dentinal penetration of calcium silicate-based sealers according to different ages of a tooth.

Materials and Methods: A total of 32 extracted human single-rooted teeth were decoronated to standardize with a root length of 12 mm. A root canal was prepared using Protaper Next rotary nickel-titanium system and obturation was done by single cone technique. The teeth were randomly divided into four groups (n = 8); based on the Tooth age and types of sealer, used as follows: (1) Tooth age <25 years/AH plus (2) Tooth age <25 years/iRoot SP (3) Tooth age >40 years/AH plus and (4) Tooth age >40 years/iRoot SP. The sealers were labelled with fluorescent dye. Transverse sections at 2, 5 and 8 mm from the apex were analyzed through CLSM. The mean of the maximum sealer penetration depth and circumferential sealer penetration depth were evaluated by the ImageJ software. The data was analyzed with Two-way ANOVA and Tukey's HSD test with a 95% confidence level.

Results: The Tooth age <25 years/iRoot SP had a mean of the maximum sealer penetration depth which was significantly higher than the age of the tooth >40 years/iRoot SP at middle and apical third while Tooth age <25 years/AH plus was significantly higher than Tooth age >40 years/AH plus at all root levels (p < 0.05). Tooth age <25 years with iRoot SP or AH plus also had circumferential sealer penetration depth significantly higher than the Tooth age >40 years with iRoot SP or AH plus at all root levels (p < 0.05). Additionally, Tooth age <25 years/iRoot SP showed significantly higher circumferential sealer penetration depth than the Tooth age <25 years/AH plus at apical level (p < 0.05).

Conclusion: Tooth age and types of sealer affected the mean of the maximum sealer penetration depth and circumferential sealer penetration depth, especially in apical part of root.

Keywords: Calcium silicate-based sealer, Tooth age, Dentinal tubule penetration, Confocal laser scanning microscopy

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Introduction

The success of root canal therapy relies on the effective elimination of microorganisms within the root canal, as well as the three-dimensional filling of the canal using gutta-percha combined with a sealer (1). The root canal sealer plays a critical role in effectively filling the irregular spaces and voids present within the root canal system. It has the capacity to penetrate dentinal tubules, which enhances the bond between the sealer and the dentin. This penetration improves the sealing ability, promotes micromechanical locking, and increases the retention of the root canal filling (2). Besides, the sealer can form a physical barrier and entomb any residual bacteria within the dentinal tubules, thereby reducing the risk of microbial leakage and preventing reinfection of the root canal (3).

AH Plus (Dentsply DeTrey, Konstanz, Germany) is a well-established root canal sealer that is considered the gold standard for using as a control endodontic sealer because of its efficient physicochemical and sealing ability (4). Recently developed calcium silicate-based sealers have been introduced with their sufficient sealing ability, good biocompatibility and bioactivity (5). iRoot SP (Innovative Bioceramix, Vancouver, BC, Canada) which is also one of the silicate-based sealers demonstrated excellent physicochemical properties (6) and demonstrates lower cytotoxic and genotoxic effects compared to traditional epoxy resin-based sealers (7). In addition, it has favorable flowability and small particle size which enable the sealer to penetrate deeper into dentinal tubules compared to AH Plus (8).

However, the depth and consistency of sealer penetration were influenced by the physical and chemical properties of the sealer such as flowability, particle size, solubility and viscosity (3,9) as well as the size and number of dentinal tubules (10). According to Carrigan et al (11), the number of dentinal tubules diminishes with advancing age and towards the apical region. Besides, the diameter of the dentinal tubules narrows with advancing age because of the formation of peritubular dentine or calcification (12). This formation of dentine occurred due to physiological changes (10,12,13) hence there might be a correlation between the tooth age and the sealer penetration of the root dentine (14).

The aim of this study was to compare dentinal penetration of iRoot SP and AH plus according to different ages of a tooth at cervical, middle and apical thirds of the root.

Materials and Methods

Tooth selection

Under ethical approval (SWUEC-661012), Single-rooted premolars extracted from <25 and >40-year-old patients were selected (n = 32). The extracted teeth were stored in a 0.12% thymol solution and were utilized within 3 months. All specimens were free of root caries, no cracks or fractures, closed apices and a curvature of less than 5 degrees (15).

Digital periapical radiographs were obtained in 2 orientations (buccolingual and mesiodistal) to confirm the existence of a single root canal. Only teeth with an IAF ranging from size #20 to #25 were included to standardize the initial canal dimensions prior to preparation. Selecting

root canals with a buccolingual to mesiodistal width ratio ranging from 1.5 to 2.0 at 5 mm from the root apex ensures consistency in the canal morphology of the selected specimens (16).

Tooth Preparation

All teeth were decoronated to achieve a standardized root length of 12 mm using a slow-speed diamond saw (Isomet 1000; Buhler, Lake Bluff, NY). The root canals were then prepared for their working lengths to be shorter than the root apex by 1 mm, using the ProTaper Next rotary nickel-titanium system (Dentsply Sirona, York, PA) up to size 40/.06. During the preparation of the root canals, a 2.5% sodium hypochlorite solution (M Dent, Bangkok, Thailand) was used for irrigation. The final irrigation involved 3 mL of 17% EDTA (Endo Clean; M Dent, Bangkok, Thailand), followed by 5 mL of 2.5% sodium hypochlorite. The prepared canal was subsequently dried using three size 40/.06 paper points (Dentsply Sirona, York, PA).

Treatment Procedures

The specimens were randomly assigned to 4 groups (n = 8) according to Tooth age and types of sealer as follows: (1) Tooth age <25 years/AH plus (2) Tooth age <25 years/iRoot SP (3) Tooth age >40 years/AH plus and (4) Tooth age >40 years/iRoot SP. Each of the sealers was labelled with 0.1% rhodamine B (Loba Chemie, Mumbai, India). The canal was filled using the single cone technique with ProTaper Next 40/.06

gutta-percha cones (Dentsply Sirona, York, PA). The specimens were kept at 37°C and 100% humidity for 7 days to ensure that the sealer is completely set.

Evaluation by Confocal Laser Scanning Microscopy Evaluation (CLSM)

The specimen was cross-sectioned in 1 mm at 2, 5 and 8 mm from the root apex using a slow-speed diamond saw. Each section was then polished for 10 seconds on wet abrasive papers with grits of 800, 1200, 1500, 2000, respectively. The samples were examined with CLSM (FV10i-DOC; Olympus, Tokyo, Japan) at 10x magnification. The mean of the maximum sealer penetration depth (μm) and circumferential sealer penetration depth (%) were measured and evaluated using the ImageJ software (National Institutes of Health, Bethesda, MD). After 7 days, 20% of the measurements were reanalyzed using the intraclass correlation coefficient (ICC) to assess intra-examiner reliability. Good agreement was achieved, with the correlation coefficient scoring 0.89.

Analysis of Sealer Penetrability

To evaluate the mean of the maximum sealer penetration depth (μm), the maximum penetration lengths of the sealers were measured at 8 points at 45° angles. The total value of the maximum penetration lengths was then divided by eight for each sample.

$$\begin{aligned} & \text{The mean of the maximum sealer penetration depth } (\mu\text{m}) \\ & = \frac{(\text{Length (Buccal+Mesiobuccal+Mesial+Mesiolingual+Lingual+Distolingual+Distal+Distobuccal)})}{8} \end{aligned}$$

The percentage of circumferential sealer penetration depth was determined by dividing the canal perimeter with sealer penetration by

the total canal perimeter, and then multiplying the result by 100 (17).

$$\begin{aligned} \text{Percentage of circumferential sealer} &= \frac{(\text{The canal perimeter with sealer penetration}) \times 100}{(\text{The total canal perimeter})} \\ \text{penetration depth (\%)} & \end{aligned}$$

Statistical Analysis.

Statistical analyses were conducted using SPSS (Statistics 20; SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was utilized to evaluate normal distribution, while the Levene test was applied to assess homogeneity of variance.

A two-way analysis of variance was performed to analyze the mean maximum sealer penetration depth and circumferential sealer penetration depth. To identify significant differences between groups, a Tukey's HSD test was carried out.

Results

The study shows a mean of the maximum sealer penetration depth and the percentage of circumferential sealer penetration depth (Table 1). Fig 1 demonstrate representative samples of confocal images of the different groups and different levels of root.

Table 1 the mean of the maximum sealer penetration depth and the percentage of circumferential sealer penetration depth.

Group		The mean of the maximum sealer penetration depth (µm, mean ± SD)			The circumferential sealer penetration depth (% , mean ± SD)		
		C	M	A	C	M	A
< 25 years	AH plus	1072.39±260.32 ^{Aa}	677.94±168.73 ^{Ab}	208.72±49.04 ^{Ac}	98.28±2.83 ^{Aa}	86.98±7.80 ^{Aa}	40.73±17.02 ^{Ab}
	iRoot SP	1493.10±279.41 ^{Ba}	1153.48±236.61 ^{Bb}	527.17±122.95 ^{Bc}	98.75±2.74 ^{Aa}	88.08±9.13 ^{Aa}	66.89±17.87 ^{Bb}
> 40 years	AH plus	782.05±154.82 ^{Ca}	437.33±107.43 ^{Cb}	62.27±14.82 ^{Cc}	89.61±13.12 ^{Ba}	70.80±16.36 ^{Bb}	21.81±8.45 ^{Cc}
	iRoot SP	1351.30±299.49 ^{Ba}	741.10±109.40 ^{Db}	87.70±20.92 ^{Cc}	89.53±9.95 ^{Ba}	74.08±15.70 ^{Bb}	26.17±9.30 ^{Cc}

A, apical level; C, coronal level; M, middle level

Different uppercase letters (A–D) within each root level (coronal, middle, apical) indicates statistically significant differences between the experimental groups ($p < 0.05$).

Different lowercase letters (a–c) within each experimental group indicates statistically significant differences among root levels (coronal vs. middle vs. apical) ($p < 0.05$).

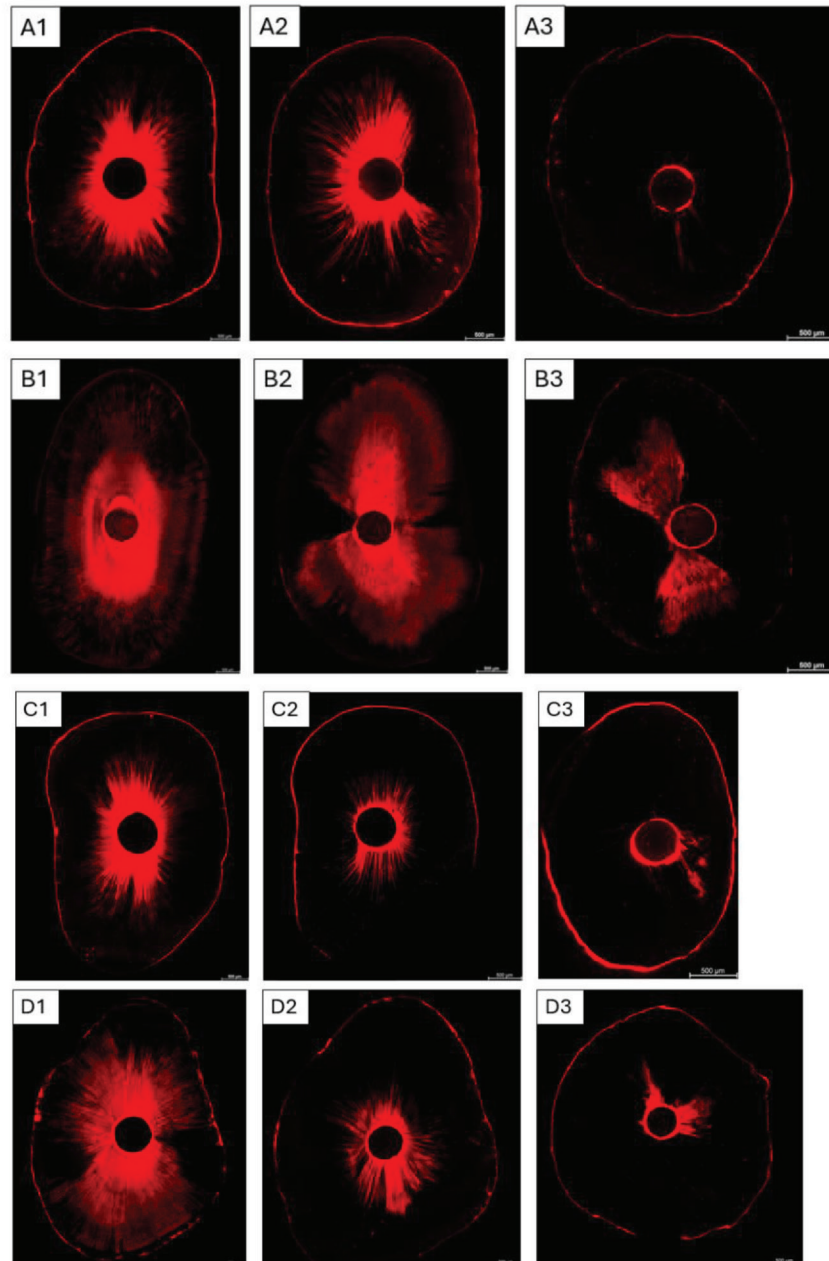


Fig 1. Representative CLSM images of sealer penetration of each of the experimental groups and levels of root (10x magnification). <25 years/AH plus: coronal (A1), middle (A2), and apical (A3) thirds; <25 years/iRoot SP: coronal (B1), middle (B2), and apical (B3) thirds; >40 years/AH plus: coronal (C1), middle (C2), and apical (C3) thirds; >40 years/iRoot SP: coronal (D1), middle (D2), and apical (D3) thirds.

Regarding results on different Tooth ages in this study, the mean of the maximum sealer penetration depth in Tooth age <25 years with iRoot SP sealer was significantly higher than Tooth age >40 years with iRoot SP sealer at middle and apical third of root. While Tooth age <25 years with AH plus sealer was significantly higher than Tooth age >40 years with AH plus sealer at all root levels ($p < 0.05$). Moreover, Tooth age <25 years with iRoot SP or AH plus had the circumferential sealer penetration depth significantly higher than the Tooth age >40 years with iRoot SP or AH plus at all root levels ($p < 0.05$).

Regarding the results on the same Tooth age groups, iRoot SP and AH plus have significantly different on the mean of the maximum sealer penetration depth for all the root levels except apical third of tooth age >40 years ($p < 0.05$). While the circumferential sealer penetration depth in iRoot SP and AH plus have significantly difference on only apical third of tooth age <25 years ($p < 0.05$). However, iRoot SP group had a mean of the maximum sealer penetration depth and the circumferential sealer penetration depth higher than AH plus at all root levels for each of the age groups.

Discussion

In this study, confocal laser scanning microscopy (CLSM) was used to evaluate sealer penetration, because of its several advantages over scanning electron microscopy (SEM), including non-destructive sample, optical sectioning, and 3D reconstruction capabilities (18,19). Additionally, this CLSM analysis was performed from the surface of the samples to a depth of 20–30 μm to avoid surface artifacts and smear layer (20). The acquired depth layers were

subsequently superimposed to generate a 3D image that represents the sealer's penetration pathway. While CLSM offers excellent resolution and suitability for sealer penetration analysis, it does have limitations, such as a relatively small field of view and the need for fluorescent labeling. However, these drawbacks were unlikely to influence the current results, as the study's primary objective is quantifying the depth of sealer penetration into dentinal tubules which was well aligned with CLSM's strengths.

This study used 0.1% rhodamine B as a fluorophore to identify sealers within the dentinal tubules (21). This concentration was adequate for creating the clarity of the CLSM images, while not altering the physical properties of the sealers (22). In our pilot test, we observed that rhodamine B did not penetrate beyond the sealer. The reason might be because tooth preparation in this study did not remain enough moisture in the dentinal tubule, which lead the rhodamine B affinized to the sealer instead of moisture in the tubules (23). This finding is in agreement with the pilot test conducted by Patel et al (24), which reported that rhodamine B-non-labeled sealers exhibited penetration depths comparable to those of rhodamine B-labeled sealers.

Measuring sealer penetration is a surrogate measure of sealing ability, as it provides insight into the effectiveness of the sealer in filling voids and irregularities within the root canal system. This evaluation assesses the sealer's effectiveness in preventing bacterial ingress, which is crucial for the long-term success of endodontic treatment. Several techniques have been used to assess sealer penetration. However, there is no standard technique yet. In previous studies, the researchers usually measured the mean sealer penetration

depth by using the average of the deepest 4 points at the buccal, lingual, mesial and distal directions (25,26). Whereas, in teeth exhibiting the butterfly effect, there is a significantly greater number of dentinal tubules in the buccolingual direction compared to the mesiodistal direction. This variation in tubule orientation may influence the behavior of sealers within root canals, resulting in a significantly greater depth of sealer penetration in the buccolingual direction (27,28). To have more reliability on the results, more than 4 points should be measured. Thus, in this study, the sealer penetration depth was measured at 8 points. This study also measured the percentage of circumferential depth for every sample to confirm the results of the mean sealer penetration depth and demonstrate the penetration and distribution of sealer around the root canal. This measurement indicated the sealing effectiveness against microorganisms within the dentinal tubules, independent of the depth of sealer penetration (29).

Several factors may affect both the mean of the maximum penetration depth and percentage of circumferential sealer penetration. These factors include the physical and chemical properties of the sealer, including particle size, flowability, viscosity, and film thickness; the characteristics of the dentinal tubules; the effective elimination of the smear layer; and the technique used for obturation. This current study focused on sealer penetration into dentinal tubules. Obviously, age influences the changing diameter and density of dentinal tubules. Sclerotic dentin begins to develop in the apical root region during the third decade of life and progressively advances coronally with age (12,30). This phenomenon also increases with advancing age, even in impacted teeth that

are not exposed to pathological or functional stimuli (31,32). Therefore, in the present study, age of tooth <25 and >40 years old were chosen to represent before and after sclerotic dentin occurring in the tooth.

This study demonstrated that Tooth age >40 years had mean of the maximum sealer penetration depth and circumferential sealer penetration depth lower than the age of the tooth <25 years. This reduction in the elderly tooth group is primarily attributed to the presence of advanced sclerotic dentin and calcification. This type of dentin, characterized by its dense and mineralized structure, acts as a barrier to sealer infiltration, making it more difficult for materials to effectively penetrate into the dentinal tubules and contributing to decreased dentin permeability (12,14). Besides, the distribution pattern of sclerosis was consistent across all teeth. In the transverse plane of the root, sclerosis changes first appeared on the mesial and distal sides, leading to the formation of sclerotic zones with a characteristic butterfly shape. This sclerotic zone widened with increasing age, further limiting the pathways available for sealer penetration (12). This result is in agreement with Thaler et al (14) which reported that the dye penetration significantly decreased with increasing the age of the patient. This suggests that the physiological changes associated with aging not only affect the structural integrity of the dentin but also impede the efficacy of root canal sealers, thereby impacting overall treatment outcomes. The implications of these results highlight the importance of considering patient age and associated dental changes when evaluating the effectiveness of endodontic treatments.

Our findings revealed that iRoot SP had sealer penetration depth higher than AH plus at both age groups and all root levels in agreement with Wang et al (8) and Akcay et al (33). Moreover, This was consistent with the findings of Muedra et al (34), which compared sealer penetration into dentinal tubule using tooth age 50–70 years old, and reported that EndoSequence BC sealer exhibited a significantly greater penetration compared to AH Plus. These results showed that iRoot SP still had better penetration in the elderly tooth age group. This probably related to a small particle size of iRoot SP ($<1\text{ }\mu\text{m}$) (35) which was smaller than AH plus ($1.5\text{--}8\text{ }\mu\text{m}$) (25) so iRoot SP had deeper penetration into the dentinal tubules (ranging between $2\text{--}3\text{ }\mu\text{m}$) (17)) than the other. Furthermore, the flow properties and viscosity of endodontic sealers might determine effectiveness of the sealer penetration into dentinal tubules. Zhou et al (36) showed that BC sealer exhibited a higher flow than AH plus.

Regarding root levels, we found that sealer penetration at the coronal and middle levels were significantly deeper than the apical level. This penetration decreased from the coronal to the apical third, regardless of the type of sealer used. This result was consistent with the findings of the previous studies (29,37,38). One explanation could be due to decreasing on diameter and density of dentinal tubule in apical levels and apical level occurred sclerosis more than coronal and middle levels (12). Moreover, the coronal level had more efficiency in delivery of irrigation and better on removal of smear layer. Hence, these explain sealer penetration trend decreasing from the coronal region towards the apical region. Although sealer penetration into dentinal tubules plays a role in improving the seal and entombing

residual microorganisms, it is only one of several properties that contribute to the long-term success of root canal treatment. Other factors such as bond strength to dentin, sealing ability under functional conditions, and material stability are also critical. Future studies should consider evaluating these properties in parallel to determine their relative contributions to clinical outcomes. This will provide a more comprehensive understanding of the overall performance of endodontic sealers.

These findings suggest that tooth age is an important factor affecting the clinical performance of root canal sealers. In older patients, reduced sealer penetration due to dentinal sclerosis may compromise long-term sealing, especially in the apical region (12,14). Therefore, clinicians should carefully consider age-related dentin changes when selecting obturation techniques or materials. Calcium silicate-based sealers such as iRoot SP, which exhibit superior penetration even in sclerotic dentin, may be more advantageous in older populations.

Within the limitations of the present study, results indicate that calcium silicate-based sealers penetrated dentinal tubules better than AH plus. However, their effectiveness was diminished in the elderly group compared to the younger group. Therefore, future research should focus on comparing various calcium silicate-based sealers to determine which one provides better penetration into the dentinal tubules of elderly teeth. This will facilitate the selection of appropriate materials and techniques, ultimately enhancing treatment outcomes in endodontic procedures for older individuals.

Conclusion

Tooth age and types of sealer affected the mean of the maximum sealer penetration depth and circumferential sealer penetration depth.

Conflicts of Interest

The authors deny any conflicts of interest related to this study

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Reference

1. Schilder H. Filling root canals in three dimensions. 1967. J Endod. 2006;32(4):281-90.
2. White RR, Goldman M, Lin PS. The influence of the smeared layer upon dentinal tubule penetration by plastic filling materials. J Endod. 1984;10(12):558-62.
3. Mamootil K, Messer HH. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. Int Endod J. 2007; 40(11):873-81.
4. Garrido AD, Lia RC, França SC, da Silva JF, Astolfi-Filho S, Sousa-Neto MD. Laboratory evaluation of the physicochemical properties of a new root canal sealer based on Copaifera multijuga oil-resin. Int Endod J. 2010;43(4):283-91.
5. Ersahan S, Aydin C. Solubility and apical sealing characteristics of a new calcium silicate-based root canal sealer in comparison to calcium hydroxide-, methacrylate resin- and epoxy resin-based sealers. Acta Odontol Scand. 2013;71(3-4):857-62.
6. Mendes AT, Silva PBD, Só BB, Hashizume LN, Vivan RR, Rosa RAD, et al. Evaluation of Physicochemical Properties of New Calcium Silicate-Based Sealer. Braz Dent J. 2018; 29(6):536-40.
7. Candeiro GT, Correia FC, Duarte MA, Ribeiro-Siqueira DC, Gavini G. Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. J Endod. 2012; 38(6):842-5.
8. Wang Y, Liu S, Dong Y. In vitro study of dentinal tubule penetration and filling quality of bioceramic sealer. PLoS One. 2018;13(2): e0192248. doi: 10.1371/journal.pone.0192248.
9. Grossman LI. Physical properties of root canal cements. J Endod. 1976;2(6):166-75.
10. Porter AE, Nalla RK, Minor A, Jinschek JR, Kisielowski C, Radmilovic V, et al. A transmission electron microscopy study of mineralization in age-induced transparent dentin. Biomaterials. 2005;26(36):7650-60.
11. Carrigan PJ, Morse DR, Furst ML, Sinai IH. A scanning electron microscopic evaluation of human dentinal tubules according to age and location. J Endod. 1984;10(8):359-63.
12. Vasiliadis L, Darling AI, Levers BG. The amount and distribution of sclerotic human root dentine. Arch Oral Biol. 1983;28(7):645-9.
13. Stavrianos C, Stavrianou I, Kafas P, Vasiliadis L, Diedrich MJ. Assessment of morphological changes on the pulpal wall of root dentine during growth. Research Journal of Medical Sciences. 2008;2(6):269-74.
14. Thaler A, Ebert J, Petschelt A, Pelka M. Influence of tooth age and root section on root dentine dye penetration. Int Endod J. 2008; 41(12):1115-22.

15. Schneider SW. A comparison of canal preparations in straight and curved root canals. *Oral Surg Oral Med Oral Pathol.* 1971;32(2):271-5.
16. Jou YT, Karabucak B, Levin J, Liu D. Endodontic working width: current concepts and techniques. *Dent Clin North Am.* 2004;48(1):323-35.
17. Eymirli A, Sungur DD, Uyanik O, Purali N, Nagas E, Cehreli ZC. Dentinal Tubule Penetration and Retreatability of a Calcium Silicate-based Sealer Tested in Bulk or with Different Main Core Material. *J Endod.* 2019;45(8):1036-40.
18. Tedesco M, Chain MC, Bortoluzzi EA, da Fonseca Roberti Garcia L, Alves AMH, Teixeira CS. Comparison of two observational methods, scanning electron and confocal laser scanning microscopies, in the adhesive interface analysis of endodontic sealers to root dentine. *Clin Oral Investig.* 2018;22(6):2353-61.
19. Tedesco M, Felipe MC, Felipe WT, Alves AM, Bortoluzzi EA, Teixeira CS. Adhesive interface and bond strength of endodontic sealers to root canal dentine after immersion in phosphate-buffered saline. *Microsc Res Tech.* 2014;77(12):1015-22.
20. Bitter K, Paris S, Martus P, Scharner R, Kielbassa AM. A Confocal Laser Scanning Microscope investigation of different dental adhesives bonded to root canal dentine. *Int Endod J.* 2004;37(12):840-8.
21. Al-Haddad A, Abu Kasim NH, Che Ab Aziz ZA. Interfacial adaptation and thickness of bioceramic-based root canal sealers. *Dent Mater J.* 2015;34(4):516-21.
22. Thota MM, Sudha K, Malini DL, Madhavi SB. Effect of Different Irrigating Solutions on Depth of Penetration of Sealer into Dentinal Tubules: A Confocal Microscopic Study. *Contemp Clin Dent.* 2017;8(3):391-4.
23. Furtado TC, de Bem IA, Machado LS, Pereira JR, SÓ MVR, da Rosa RA. Intratubular penetration of endodontic sealers depends on the fluorophore used for CLSM assessment. *Microsc Res Tech.* 2021;84(2):305-12.
24. Patel DV, Sherriff M, Ford TR, Watson TF, Mannocci F. The penetration of RealSeal primer and Tubliseal into root canal dentinal tubules: a confocal microscopic study. *Int Endod J.* 2007;40(1):67-71.
25. El Hachem R, Khalil I, Le Brun G, Pellen F, Le Jeune B, Daou M, et al. Dentinal tubule penetration of AH Plus, BC Sealer and a novel tricalcium silicate sealer: a confocal laser scanning microscopy study. *Clin Oral Investig.* 2019;23(4):1871-6.
26. Jeong JW, DeGraft-Johnson A, Dorn SO, Di Fiore PM. Dentinal Tubule Penetration of a Calcium Silicate-based Root Canal Sealer with Different Obturation Methods. *J Endod.* 2017;43(4):633-7.
27. Russell AA, Chandler NP, Hauman C, Siddiqui AY, Tompkins GR. The butterfly effect: an investigation of sectioned roots. *J Endod.* 2013;39(2):208-10.
28. Russell A, Friedlander L, Chandler N. Sealer penetration and adaptation in root canals with the butterfly effect. *Aust Endod J.* 2018;44(3):225-34.
29. McMichael GE, Primus CM, Opperman LA. Dentinal Tubule Penetration of Tricalcium Silicate Sealers. *J Endod.* 2016;42(4):632-6.

30. Paqué F, Luder HU, Sener B, Zehnder M. Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals. *Int Endod J*. 2006;39(1):18-25.

31. Azaz B, Michaeli Y, Nitzan D. Aging of tissues of the roots of nonfunctional human teeth (impacted canines). *Oral Surgery Oral Medicine Oral Pathology*. 1977;43(4):572-8.

32. Singhal A, Ramesh V, Balamurali P. A comparative analysis of root dentin transparency with known age. *J Forensic Dent Sci*. 2010;2(1):18-21.

33. Akcay M, Arslan H, Durmus N, Mese M, Capar ID. Dentinal tubule penetration of AH Plus, iRoot SP, MTA fillapex, and guttaflow bioseal root canal sealers after different final irrigation procedures: A confocal microscopic study. *Lasers Surg Med*. 2016;48(1):70-6.

34. Muedra P, Forner L, Lozano A, Sanz JL, Rodríguez-Lozano FJ, Guerrero-Gironés J, et al. Could the Calcium Silicate-Based Sealer Presentation Form Influence Dentinal Sealing? An In Vitro Confocal Laser Study on Tubular Penetration. *Materials (Basel)*.2021;14(3):659. doi: 10.3390/ma14030659.

35. Chang SW, Lee YK, Zhu Q, Shon WJ, Lee WC, Kum KY, et al. Comparison of the rheological properties of four root canal sealers. *Int J Oral Sci*. 2015;7(1):56-61.

36. Zhou H, Peng B, Zheng Y. An overview of the mechanical properties of nickel–titanium endodontic instruments. *Endodontic Topics*.2013; 29(1):42-54.

37. Osiri S, Banomyong D, Sattabanasuk V, Yanpiset K. Root Reinforcement after Obturation with Calcium Silicate-based Sealer and Modified Gutta-percha Cone. *J Endod*. 2018;44(12):1843-8.

38. Reynolds JZ, Augsburger RA, Svoboda KKH, Jalali P. Comparing dentinal tubule penetration of conventional and ‘HiFlow’ bioceramic sealers with resin-based sealer: An in vitro study. *Aust Endod J*. 2020;46(3):387-93.

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