

DENTINAL TUBULE PENETRATION OF DIFFERENT ENDODONTIC SEALERS: A CONFOCAL MICROSCOPE STUDY

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ABSTRACT

Background and Aim: The purpose of this in vitro study was to evaluate the penetration of four different endodontic sealers into radicular dentinal tubules via confocal microscopy.

Materials and Methods: Forty single-rooted teeth were prepared using MTwo rotary files. 2.5% sodium hypochlorite (NaOCl) and 17% EDTA were used as irrigation solutions. Teeth were then allocated into four groups, containing 10 teeth in each. Before the obturation, sealers were labeled with 0.1% Rhodamine B. After that, the teeth were obturated with AH Plus, iRoot SP, MTA Fillapex or MetaSEAL using with gutta-percha as a core material. The roots were sectioned horizontally and examined under confocal laser scanning microscope to determine the depth of penetration, penetration area and percentage of penetration of the sealers into the dentinal tubules. Data were analyzed using Kruskal Wallis and Bonferroni tests.

Results: MetaSEAL did not show any significant difference than other sealers.

Conclusion: The findings showed that there was no significant difference among the groups regarding the depth of penetration, penetration area and percentage of penetration of the sealers.

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FARKLI ENDODONTİK PATLARIN DENTİN TÜBÜL PENETRASYONU: KONFOKAL MİKROSKOP ÇALIŞMASI

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ÖZ

Amaç: Dört farklı endodontik kanal dolgu patının radiküler dentin tübüllerine penetrasyonunu konfokal mikroskop yardımıyla değerlendirmek.

Gereç ve Yöntem: Bu çalışmada kırk adet tek köklü dişler kullanılmıştır. MTwo döner ege sistemleri ile kemomekanik preparasyon tamamlanmıştır. İrrigasyon solüsyonu olarak % 2.5 sodyum hipoklorit (NaOCl) ve % 17 EDTA kullanılmıştır. Dişler daha sonra her birinde 10 diş içeren dört gruba ayrılmış, kanal dolgu işleminden önce, patlara % 0.1 Rodamin B karıştırılmıştır. Daha sonra dişlerin kanal dolguları sırasıyla AH Plus, iRoot SP, MTA Fillapex veya MetaSEAL patları kullanılarak tamamlanmıştır. Kökler yatay olarak kesilmiş ve penetrasyon derinliği, penetrasyon alanı ve dolguların penetran yüzdesinin dentin tübüllerine olan penetrasyon yüzdeleri belirlemek için konfokal lazer tarama mikroskobu altında incelenmiştir. Veriler Kruskal Wallis ve Bonferroni testleri kullanılarak analiz edilmiştir.

Bulgular: MetaSEAL, diğer patlardan anlamlı bir farklılık göstermedi.

Sonuç: Penetrasyon derinliği, penetrasyon alanı ve penetrasyon yüzdesi açısından gruplar arasında anlamlı bir fark olmadığını bulunmuştur.

Sorumlu Yazar

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Anahtar Kelimeler: Dentin tübül penetrasyonu, konfokal lazer tarama mikroskobu, kök kanal patı

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INTRODUCTION

The success of root canal treatment depends largely on the root canal system being thoroughly cleansed and disinfected, followed by three-dimensional obturation of the prepared canal space with a biocompatible and inert material.¹ Since the first description of the smear layer in instrumented root canals,² accumulating evidence has demonstrated the importance of smear layer removal, which results in a more thorough disinfection of the root canal system and the dentinal tubules that would ensure a better dentinal tubule penetration of root canal sealers.³ Sealer penetration into dentinal tubules increases the interface between the filling material and dentin, thus improves the sealing ability of filling material and prevents bacterial colonization and re-infection by entombing any residual bacteria within the tubules.⁴⁻⁵

Today, most root canal filling methods continue to utilize different formulations of gutta-percha in conjunction with a minimum amount of sealer. Numerous materials have been developed to improve the sealing ability and stability of the root canal filling. Despite not presenting ideal tissue response, AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany), still be considered as the gold standard in terms of its biocompatibility.⁶ iRoot SP (Innovative Bioceramics, Vancouver, Canada) and MTA Fillapex (Angelus Soluções Odontológicas, Londrina, PR, Brazil) are contemporary bioceramic sealers, that are biocompatible, bioactive and osseointegrative.⁷ iRoot SP is an injectable, calcium silicate based bioceramic sealer that hardens with water.⁸ MTA Fillapex is paste-paste MTA-based bioceramic sealer, it has proper working time and proper biocompatibility.⁹

MetaSEAL (Parkell Inc, Farmington, NY) is a new dual-curable fourth generation methacrylate resin-based, 4-methacryloyloxyethyl trimellitate anhydride (4-META) containing polymethylmethacrylate-based (PMMA) sealer. According to manufacturers, it can both be used either resilon (Resilon Research LLC, Madison, CT) or gutta percha and should not be used with hot obturation techniques. Additionally, it has been reported that MetaSEAL bonds to dentin via hybrid layer and creates monoblock formation.¹⁰ According to recent studies MetaSEAL shows greater bond strength¹¹⁻¹⁴ and higher fracture resistance¹⁵ when compared with the methacrylate resin-based, epoxy resin-based and bioceramic based sealers. To date there are no reported studies that have investigated the dentinal tubule penetration of MetaSEAL.

In the light of these observations, the purpose of this in-vitro study was to compare the dentinal tubule penetration of different root canal sealers. The null hypothesis tested was that there is no difference in the dentinal tubule penetration of root canal sealers.

MATERIALS AND METHODS

Forty periodontally involved, freshly extracted single-rooted human teeth with straight roots were selected for this study after Ethics Committee approval (GO 14/305 - 04/06/2014). The crowns of all teeth were removed using a water-cooled, slow-speed diamond precision saw (Isomet 1000; Buehler, Lake Bluff, IL), so as to adjust the length of the roots to a standardized length of 11mm.

Specimen preparation

A size 10 K-file was introduced into each canal until it was seen through the apical foramen and glide path was verified. Working length was established by subtracting 0.5 mm from apical foramen. Then, the root canals were prepared using MTwo rotary files (VDW, Munich, Germany) up to master apical rotary size 25.06 in conjunction with 2 ml of 2.5% NaOCl (sodium hypochlorite) irrigation between each file. After preparation, the root canals were irrigated with 5 ml 17% EDTA for 1 minute to remove smear layer, followed by a final rinse of 2 ml distilled water. The specimens were randomly divided into four groups (n= 10 each) according to root canal sealer used: 1. AH Plus; 2. iRoot SP; 3. MTA Fillapex; 4. MetaSEAL. Each root was dried with paper points. All sealers were mixed according to manufacturer instructions. Before mixing, 0.1% Rhodamin B added to all sealers, in order to get fluorescent view. Thereafter, the roots were obturated with 25.06 taper gutta-percha cone-sealer combinations by using the single-cone obturation technique. Following root filling procedures, the specimens were stored at 37°C and 100% humidity for 1 week to allow complete set of the test materials.

Sectioning and image analysis

The roots were embedded in a block of acrylic resin and left until the resin was set. Each root was sectioned horizontally at the distance of 6 mm from the apical tip with a diamond saw rotating at 800 rpm and under constant water-cooling. Segments were mounted onto glass slides and were assessed under a confocal laser scanning microscopy (CLSM) (LSM Pascal, Carl Zeiss, Jena, Germany) by using the 543 nm wavelength of helium laser under $\times 2.5$ magnification (Figure 1). Each image was imported into LSM

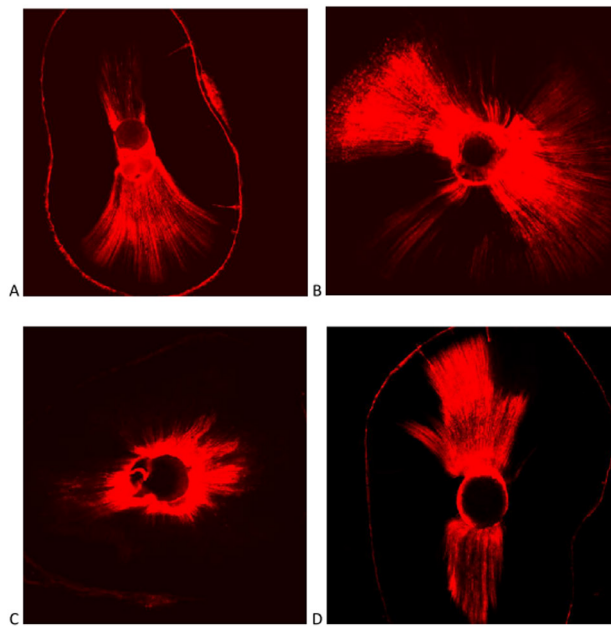


Figure 1. Representative confocal laser scanning microscopic images. A: AH Plus B: iRoot SP C: MetaSEAL D: MTA Fillapex (2,5X)

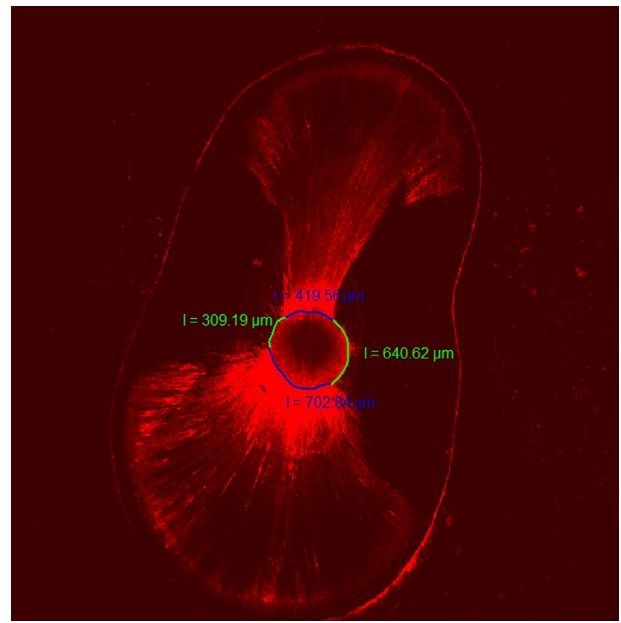


Figure 2. Measurement of percentage of penetration of sealer using LSM Image Examiner Software (Carl Zeiss)

image Examiner (Carl Zeiss) software and the percentage of the sealers was obtained in a method similar to that used by Gharib et al.¹⁶ In each image, the circumference of the root canal wall was outlined and measured with “closed free shape curve drawing” measuring tool. Next, areas along the canal walls, which the sealer impregnated into dentinal tubules were outlined and measured using the “open free shape curve drawing” tool. Outlined distances were divided by the canal circumference to calculate the percentage of sealer (Figure 2). The maximum penetration depth was measured by using “open arrow drawing” tool, from the root canal wall to the point of deepest penetration (Figure 3). Penetration area was measured by using “closed free shape curve drawing” tool. First, sealer impregnated areas were outlined and measured, then circumference of the root canal wall was outlined and area of that chamber measured. Area of the root canal was then subtracted from the total sealer penetration area in each section (Figure 4).

Statistical Analysis

The data were analysed statistically using Kruskal-Wallis test with Bonferroni correction at $p = 0.05$. (IBM SPSS for Windows Version 22.0).

RESULTS

The percentages of sealer penetration to the canal walls in micrometers are shown in Table 1. Based on that, iRoot SP

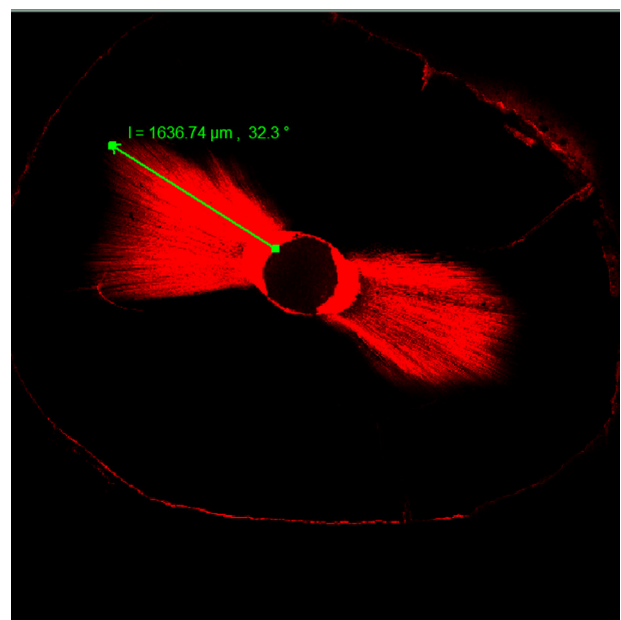


Figure 3. Measurement of maximum depth of penetration of sealer using LSM Image Examiner Software (Carl Zeiss).

group showed the highest percentage of penetration, while AH Plus group showed the lowest percentage but there was no significant difference among the groups ($p > 0.05$). Results also demonstrated that MTA Fillapex, iRoot SP, AH Plus, MetaSEAL groups showed maximum penetration depths respectively without any significant difference

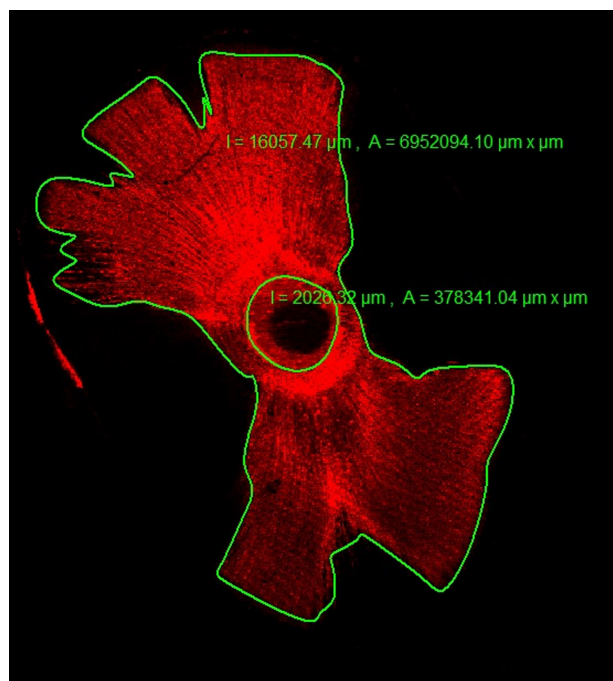


Figure 4. Measurement of sealer penetration area using LSM Image Examiner Software (Carl Zeiss).

($p > 0.05$), showed in Table 2. In terms of penetration area, iRoot SP group showed highest values, followed by MTA Fillapex, AH Plus and MetaSEAL Groups (Table 3). There were no significant differences among all groups ($p > 0.05$) regarding all investigated parameters.

DISCUSSION

In the present study, we compared penetration of four different root canal sealers into radicular dentinal tubules via CLSM. So far, investigators have used different methods to evaluate the penetration of sealers into the dentinal tubules including light microscope¹⁷⁻¹⁸ and scanning electron microscope (SEM).^{3,5,19} These methods have some disadvantages over CLSM; the difficulty with light

microscope is the inability to distinguish the sealer from the radicular dentin. De Deus et al.¹⁷ tried to use a digital software and Weis et al.¹⁸ used a histologic stain in order to overcome this disadvantage. The main disadvantages of SEM are to be inability to obtain a total view at low magnification and producing artifacts during the sample preparation. On the contrary, CLSM analysis can provide 3-dimensional interpretations without special specimen processing which leads to produce artifacts.²⁰ Furthermore, it allowed the quantitative evaluation and interpretation of the penetration that was directly related to the penetration of the fluorescently labeled materials.²¹

Labeling of a sealer with Rhodamine B is mandatory for obtaining fluorescence view, and observing sealer penetration via CLSM. According to American Dental Association specifications, 0.1% Rhodamine B has no effect on sealers flow properties and any concentration greater than 0.1% resulted in excessive fluorescence that made the specimens difficult to visualize.¹⁶ Therefore, in this study, sealers were labeled with 0.1% Rhodamine B prior to CLSM evaluation.

According to the literature, sections in the sealer penetration studies were performed and compared apically, middle and coronally.^{16,18,22-23} Some of these studies showed that greater sealer penetration was found in the middle third of the root canals, and they stated that it might have been due to large diameter of the tubule orifices and greater forces might be applied during lateral condensation at this part of the root.²²⁻²³ Therefore, in this study, middle sections of the roots were selected.

The previous studies showed that the penetration differences of root canal sealers might be influenced by different physical and chemical properties of the sealers.^{3,5,16} However, in the present study, CLSM evaluation showed that there was no significant difference between four

Table 1. Percentage (%) of sealer penetration into the canal wall.

	N	Median	Std. deviation	Lower Bound	Upper Bound
AH Plus	10	75.57	18.88	62.06	89.06
iRoot SP	10	89.68	15.49	78.59	100.75
MTA Fillapex	10	85.52	12.69	76.44	94.60
MetaSEAL	10	84.31	26.23	65.55	103.07

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Table 2. Maximum depth of penetration into the dentinal tubules (µm)

	N	Median	Std. deviation	Lower Bound	Upper Bound
AH Plus	10	1856.00	204.39	1709.79	2002.21
iRoot SP	10	2003.26	254.97	1820.86	2185.65
MTA Fillapex	10	2168.95	381.90	1895.76	2442.15
MetaSEAL	10	1742.72	633.65	1289.43	2196.00

Table 3. Penetration Area (µm²)

	N	Median	Std. deviation	Lower Bound	Upper Bound
AH Plus	10	7419642.20	3143322.66	5171044.62	9668239.78
iRoot SP	10	10179975.16	3549544.73	7640783.83	12719166.50
MTA Fillapex	10	9964246.42	3357623.82	7562347.03	12366145.82
MetaSEAL	10	7344835.39	4159714.13	4369155.16	10320515.63

different sealers in terms of maximum penetration depth. Based on the results, the null hypothesis was accepted. The difference between the present finding and previous studies might have been related to the type of the methodology. In all these studies sealer penetrations evaluated with SEM. In our study, AH Plus displayed higher penetration values than MetaSEAL without any significant difference. This finding confirms the results of previous study that reported that AH plus showed similar penetration results when compared with an another methacrylate based sealer.^{21,24} On the other hand, Kara Tuncer et al.²⁵ revealed that AH Plus and MTA Fillapex showed greater percentage of penetration to the canal wall than iRoot SP. In the present study, there was no significant difference amongst AH Plus, iRoot SP and MTA Fillapex sealers. Also, Kara Tuncer et al.²⁵ used only maxiller incisors in their study, but in the present study, all single rooted teeth were used as similar. Gharib et al.¹⁶ It is difficult to standardize the amount and distribution of sclerotic dentin and irregular secondary dentin which may have influence on the sealer penetration. Even a careful sample selection does not guarantee a homogeneous penetration pattern among the specimens. Possible differences might be attributed to teeth selection. Although evaluated sealers showed ability to penetrate

into dentinal tubules in these studies, the penetration was not continuous along the entire canal perimeter. There is two possible reasons of this finding. First, compaction forces of the spreader might leave areas without sealer after the filling.²⁶ Another reason is a phenomenon called "butterfly effect".²⁷ Sealer penetration was found to be greater in the buccolingual direction as compared with that of mesiobuccal direction. This findings are in line with that of previous studies.^{18,28} Butterfly-like appearance seen on the root cross-sections that occurs as a result of increased sclerosis along the tubules located on the mesio-distal direction of the canal lumen.²⁹

The depth of penetration on previous studies has shown great variation. De Deus et al.¹⁷ reported between 79-136 µm, Kokkas et al.¹⁹ reported 17-59 µm and Kouvas et al.³ reported between 1-77 µm penetration values. In these studies longitudinal sectioning techniques were used. Mamootil & Messer⁵ on the other hand, reported the maximum penetration as 1337 µm. They used horizontal sections in their study and samples being observed with SEM. In the present study, we found higher maximum penetration depth values up to 3101 µm. Mamootil & Messer⁵ stated that the disadvantage of the longitudinal sectioning technique is that it could not provide a complete

observation of all of the dentine surrounding the canal and there is potential to miss areas of deep penetration. Also, it should be kept in mind that one of the main disadvantages of SEM is inadequate observation of sample area in low magnifications.

CONCLUSION

Under the limitations of this study, it can be concluded that MetaSEAL did not show better results in terms of dentin penetration from other groups.

CONFLICT OF INTEREST

The authors have stated that there are no conflict of interests in connection with this article.

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REFERENCES

1. Epley SR, Fleischman J, Hartwell G, Cicalese C. Completeness of root canal obturations: Epiphany techniques versus gutta-percha techniques. *J Endod* 2006; 32: 541-544.
2. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. *J Endod* 1975; 1: 238-242.
3. Kouvas V, Liolios E, Vassiliadis I, Parissis-Messimeris S, Boutsoukis A. Influence of smear layer on depth of penetration of three endodontic sealers: a SEM study. *Dent Traumatol* 1998; 14: 191-195.
4. Nikhil V, Singh R. Confocal laser scanning microscopic investigation of ultrasonic, sonic, and rotary sealer placement techniques. *J Conserv Dent* 2013; 16: 294-299.
5. Mamootil K, Messer HH. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. *Int Endod J* 2007; 40: 873-881.
6. Tavares CO, Böttcher DE, Assmann E, Kopper PMP, de Figueiredo JAP, Grecca FS et al. Tissue reactions to a new mineral trioxide aggregate-containing endodontic sealer. *J Endod* 2013; 39: 653-657.
7. Chang SW, Lee SY, Kang SK, Kum KY, Kim EC. In vitro biocompatibility, inflammatory response, and osteogenic potential of 4 root canal sealers: Sealapex, Sankin apatite root sealer, MTA Fillapex, and iRoot SP root canal sealer. *J Endod* 2014; 40: 1642-1648.
8. Nagas E, Uyanik MO, Eymirli A, Cehreli ZC, Vallittu PK, Lassila LV et al. Dentin moisture conditions affect the adhesion of root canal sealers. *J Endod* 2012; 38: 240-244.
9. Gomes-Filho JE, Watanabe S, Lodi CS, Cintra LTA, Nery MJ, Dezan E et al. Rat tissue reaction to MTA FILLAPEX®. *Dent Traumatol* 2012; 28: 452-456.
10. Kim YK, Grandini S, Ames JM, Gu LS, Kim SK, Pashley DH et al. Critical review on methacrylate resin-based root canal sealers. *J Endod* 2010; 36: 383-399.
11. Stiegemeier D, Baumgartner JC, Ferracane J. Comparison of push-out bond strengths of Resilon with three different sealers. *J Endod* 2010; 36: 318-321.
12. Sun Y, Li YH, Fan MW. Push-out bond strength of self-adhesive methacrylate resin-based sealers to root dentin. *J Huazhong Univ Sci Technol Med Sci* 2014; 34: 108-113.
13. Babb BR, Loushine RJ, Bryan TE, Ames JM, Causey MS, Kim J et al. Bonding of self-adhesive (self-etching) root canal sealers to radicular dentin. *J Endod* 2009; 35: 578-582.
14. Onay EO, Ungor M, Ari H, Belli S, Ogus E. Push-out bond strength and SEM evaluation of new polymeric root canal fillings. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 107: 879-885.
15. Ulusoy OI, Nayir Y, Darendeliler Yaman S. Effect of different root canal sealers on fracture strength of simulated immature roots. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; 112: 544-547.
16. Gharib SR, Tordik PA, Imamura GM, Baginski TA, Goodell GG. A confocal laser scanning microscope investigation of the epiphany obturation system. *J Endod* 2007; 33: 957-961.
17. De Deus G, Gurgel-Filho E, Maniglia-Ferreira C, Coutinho-Filho T. The influence of filling technique on depth of tubule penetration by root canal sealer: a study using light microscopy and digital image processing. *Aust Endod J* 2004; 30: 23-28.
18. Weis MV, Parashos P, Messer HH. Effect of obturation technique on sealer cement thickness and dentinal tubule penetration. *Int Endod J* 2004; 37: 653-663.
19. Kokkas A, Boutsoukis A, Vassiliadis L, Stavrianos C. The Influence of the Smear Layer on Dentinal Tubule Penetration Depth by Three Different Root Canal Sealers: An In Vitro Study. *J Endod* 2004; 30: 100-102.
20. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Perdigao J, Lambrechts P et al. Microscopy investigations. Techniques, results, limitations. *Am J Dent* 2000; 13: 3-18.

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21. Patel D, Sherriff M, Ford T, Watson T, Mannocci F. The penetration of RealSeal primer and Tubliseal into root canal dentinal tubules: a confocal microscopic study. *Int Endod J* 2007; 40: 67-71.
22. Vassiliadis LP, Sklavounos SA, Stavrianos CK. Depth of penetration and appearance of Grossman sealer in the dentinal tubules: an in vivo study. *J Endod* 1994; 20: 373-376.
23. Çaltı S, Serper A. Dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide. *J Endod* 1999; 25: 431-433.
24. Chandra SS, Shankar P, Indira R. Depth of penetration of four resin sealers into radicular dentinal tubules: a confocal microscopic study. *J Endod* 2012; 38: 1412-1416.
25. Kara Tuncer A, Tuncer S, Gökyay SS. Correlation between sealer penetration into dentinal tubules and bond strength of two new calcium silicate-based and an epoxy resin-based, endodontic sealer. *J Adhes Sci Technol* 2013; 28: 702-710.
26. Wu MK, Özok A, Wesselink P. Sealer distribution in root canals obturated by three techniques. *Int Endod J* 2000; 33: 340-345.
27. Russell AA, Chandler NP, Hauman C, Siddiqui AY, Tompkins GR. The butterfly effect: an investigation of sectioned roots. *J Endod* 2013; 39: 208-210.
28. Kuçi A, Alaçam T, Yavaş Ö, Ergül-Ulger Z, Kayaoglu G. Sealer Penetration into Dentinal Tubules in the Presence or Absence of Smear Layer: A Confocal Laser Scanning Microscopic Study. *J Endod* 2014; 40: 1627-1631.
29. Vasiliadis L, Darling A, Levers B. The amount and distribution of sclerotic human root dentine. *Arch Oral Biol* 1983; 28: 645-649.