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ORIGINAL RESEARCH

Confocal laser scanning microscopic analysis of the penetration of an epoxy resin-based sealer into dentinal tubules after calcium hydroxide dressing

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Abstract

This study assessed the penetration of an epoxy resin-based sealer into dentinal tubules of root canals previously medicated with calcium hydroxide. Sixteen palatal root canals of upper molars were instrumented and distributed into two groups: (G1) root canals medicated with calcium hydroxide and obturated after its removal; (G2) root canals obturated without the use of dressing. Sealer mixed with rhodamine B provided fluorescence for confocal laser scanning microscopy. Measurements of area and linear penetration of the sealer were assessed in three thirds by ImageJ software. The data were statistically compared by Kruskal–Wallis, Dunn's and Mann–Whitney U tests ($P < 0.01$). The calcium hydroxide decreased the penetration of the sealer. The coronal and middle thirds had similar areas and linear penetrations in both groups, whereas the apical third had less penetration. In conclusion, the penetration of the epoxy resin-based sealer is influenced by the calcium hydroxide dressing used between appointments.

Introduction

The disinfection of the root canal system is an important step during endodontic treatments as bacteria are able to invade dentinal tubules and survive in poor-nutrient environment for long time (1). These bacteria have been identified as the main factor in the development of apical periodontitis (2). Although conventional endodontic treatments can significantly reduce the bacterial load in the root canal, the remaining bacteria in dentinal tubules can re-infect the main canal (3).

Calcium hydroxide has been widely used as an intracanal dressing when it is not possible to perform one-visit endodontic treatment and to increase the antibacterial ability of teeth with pulp necrosis (4). However, its removal is important for root canal filling because the penetration of the sealer into dentinal tubules helps in the intratubular antibacterial activity (5) and entombment of remaining bacteria (1). Some calcium hydroxide removal techniques have been proposed, but the literature shows that all of them are ineffective (6), and that the residual dressing can influence the penetration of endodontic sealers (7,8).

Among the various endodontic sealers, the epoxy resin-based sealers such as AH Plus (Dentsply), Sealer Plus (MKLife), Adseal (Meta Biomed America) and Sealer 26 (Dentsply) have been used due to low solubility, flow and antibacterial property (9–12). Although they are all epoxy resin-based sealers and similar to AH Plus, sealer Plus and Sealer 26 have calcium hydroxide (9,12), and the Adseal has calcium phosphate (11). Among them, the AH Plus has been highlighted because of the highest radiopacity (11,12). Considering that it is important to observe the influence of the remaining intracanal dressing on the root canal filling, the aim of this study was to assess the penetration of the epoxy resin-based sealer (AH Plus) into dentinal tubules of root canals previously medicated with calcium hydroxide. The null hypothesis was that the remaining of calcium hydroxide dressing does not interfere in the penetration of the epoxy resin-based sealer (AH Plus) into dentinal tubules.

Materials and methods

The sample size was calculated by using the G*Power software version 3.1.9.2 (Heinrich Heine, Universität Düsseldorf, Düsseldorf, Germany) and Mann–Whitney test. The mean and standard deviation of the data obtained in a pilot study were used, and the effect size was established in 3.3. Alpha-type error and beta power were stipulated as 0.05 and 0.80, respectively. A total of six roots *per* group were indicated as the ideal size for no significant differences. Two additional roots *per* group were used for any processing errors. Thus, after approval by the local research ethics committee (006-FO-PG-2019), 16 palatal root canals of upper molars were standardised in 12 mm and explored with a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until the foramen for confirmation of patency. Next, the apex was sealed with composite resin. The root canals were instrumented to a working length of 11 mm up to a #20 K-file (Dentsply Maillefer) and then with a WaveOne Large file (40/0.08, Dentsply Maillefer) by using a motor in reciprocating motion (X-Smart Plus, Dentsply Maillerfer). Irrigation with 5 mL of 5.25% sodium hypochlorite was used between each instrumentation, and final irrigation with 10 mL of 5.25% sodium hypochlorite was ultrasonically activated (IRRIS, VDW, Munich, Deutschland) for 2 cycles of 30 s each, followed by 5 mL of EDTA 17% (MD-Cleanser, Meta Biomed, Chungcheongbuk-do, Korea) for 3 min and 10 mL of distilled water. The root canals were dried by using aspiration and WaveOne Large paper points (Dentsply Maillefer) before being fixed on a cell culture plate (VWR, Amadora, Portugal) with condensation silicone (Speedex Putty, Coltene, Altstätten, Switzerland), and then randomly distributed into two groups:

Group 1

Root canals were filled with a paste of calcium hydroxide (Eufar, Bogotá, Colombia) with propylene glycol (0.25 g/0.1 mL) by using a #35 lentulo spiral carrier (Dentsply Maillefer) and compacted with a #3 finger plugger (Dentsply Maillefer). The roots were coronally sealed with temporary cement (Coltosol, Coltene) and kept at 37°C and 100% humidity for 15 days. After this period, the paste was removed by using a re-instrumentation with WaveOne Large file and irrigation with 15 mL of sodium hypochlorite 5.25%, followed by 5 mL of EDTA 17% for 3 min and 10 mL of distilled water. Syringe and a 29 gauge NaviTip (Ultradent Products, South Jordan, USA) positioned 2 mm from the working length were used for irrigation with in-and-out motion. The root canals were dried by aspiration and paper points before being obturated. The AH Plus sealer (Dentsply, De Trey, Konstanz, Germany) was manipulated according to the manufacturer's instructions and weighed by using an analytical precision balance scale (Sartorius, Goettingen, Germany). Rhodamine B powder (Sigma Aldrich, Saint Luis, USA) was weighed and mixed with the sealer to a proportion of 1 : 100 (w/w). Then, the root canal was obturated with rhodamine-sealer mixture and WaveOne Large gutta-percha cone by using the single-cone technique. The obturation was cut at the entrance of the root canal by using a thermal condenser (Gutta cut, Surident Mart, Arumbakkam, Chennai), followed by cold vertical compaction. The root canal entrance was cleaned with 70% alcohol and sealed with temporary cement (Coltosol, Coltene).

Group 2 (control group)

Root canals were obturated by using the single-cone technique as described in the previous group, but without using previous intracanal dressing with calcium hydroxide.

After obturation, the roots were kept at 37°C and 100% humidity for 7 days before being transversely sectioned with a precision metallographic saw (IsoMet 1000 Buehler, Illinois, USA) at 9, 6 and 3 mm from the apex. The resulting sections were polished (Ecomet - Politriz, Buehler, IL, USA) by using a double speed polisher and then observed with a confocal laser scanning microscope (Leica, Mannheim, Germany) at 4× magnification for scanning at 10 μm from the surface with an absorption wavelength of 540 nm and emission of 590 nm. The photomicrographs were analysed with the ImageJ software (National Institutes of Health, Maryland, USA) to determine area and maximum linear penetration of the endodontic sealer into the dentinal tubules according to Deniz Sungur (13): The sealer penetration area (in μm^2) was assessed by outlining the circumference of the root canal and of the sealer inside the dentinal tubule. Then, a line was traced from the root canal wall to the deepest point of penetration to measure the linear penetration of the sealer (in μm).

Data normality was analysed by using the Lilliefors test and homoscedasticity of the variances by using the Levene test. Data on area and linear penetration were assessed with Kruskal–Wallis test and Dunn's *post hoc* test for intra-group comparison, whereas Mann–Whitney *U* test was used for comparison between groups per thirds. All statistical analyses were performed at a significance level of 1%.

Results

The comparison between the groups showed that calcium hydroxide decreased the penetration of the sealer into the root canals in terms of both area and maximum linear depth ($P < 0.01$). The sealer penetrated a larger area (range 77.8%–89.9%) and deeper (range 56.2%–76.3%) when calcium hydroxide was not used. Figure 1 shows the percentage differences between calcium hydroxide group and control group as 100% regarding the different thirds.

Intra-group analysis showed that the coronal and middle thirds have similar areas and linear penetrations in both groups ($P > 0.01$). The apical third had less penetration of the sealer in both groups, but the middle third had a linear penetration similar to that of the control group.

Statistical comparison of the area and maximum linear penetration of the sealer can be seen in Tables 1 and 2, respectively, and Figure 2 shows a representative image of the sealer penetration in the different thirds in both groups.

Discussion

Calcium hydroxide has been widely used in worldwide, but its removal prior to root canal filling is crucial (6). The present study assessed the influence of the remaining calcium hydroxide on the obturation of the root canal system as it was demonstrated that this dressing decreased the sealer penetration into the dentinal tubules.

Over the years, methodologies have been proposed for assessment of sealer penetration into dentinal tubules, such as stereomicroscopy (13), scanning electron microscopy (7,15,16) and more currently, confocal laser scanning microscopy (8,13,16,17). The latter is justified because it improves the contrast between dentin and sealer, thus allowing a panoramic image at lower magnifications with less artefacts and using a conservative process. Bitter *et al.* (18) compared the scanning electron microscopy to confocal laser scanning microscopy, and observed that the latter shows greater details of the sealer penetration into the dentin (18). In addition, Tedesco *et al.* (19) observed a great number of long tags when confocal laser scanning microscopy was used, but the correlation between the methodologies was low despite the latter being more reliable. For this reason, confocal laser scanning microscopy was the method used in this study. However, sealer penetration was assessed by using linear penetration as a parameter (8,16,17). Additionally, the present study also analysed the area penetrated by the sealer as proposed by Deniz Sungur *et al.* (13). This methodology allows a better interpretation of the sealer penetration in the entire circumferential area of the dentin as well as its depth. The endodontic sealer was mixed with rhodamine B dye before evaluation under confocal laser scanning microscope. This dye has been used in various studies evaluating sealer penetration into dentinal tubules (8,13,16,17,19). However, Wang *et al.* (20) found that rhodamine B dye can influence the physical–mechanical properties of dental adhesive resins, and Bim Júnior *et al.* (21) observed that the concentration of this dye influences the image labelling in confocal laser scanning microscopy. The present study used a concentration of 0.01%, thus following studies by Russel *et al.* (16) and Veintimilla Lozada *et al.* (17) on penetration of endodontic sealers.

Various techniques and irrigation substances for calcium hydroxide removal have been proposed (6). However, all of them are ineffective and result in different levels of medication remnants (6). Therefore, any removal technique could be used in this study and re-instrumentation of the root canal was performed because it is very commonly used by clinicians (17).

Calcium hydroxide influenced the sealer penetration in terms of area and linear depth in all root thirds. The

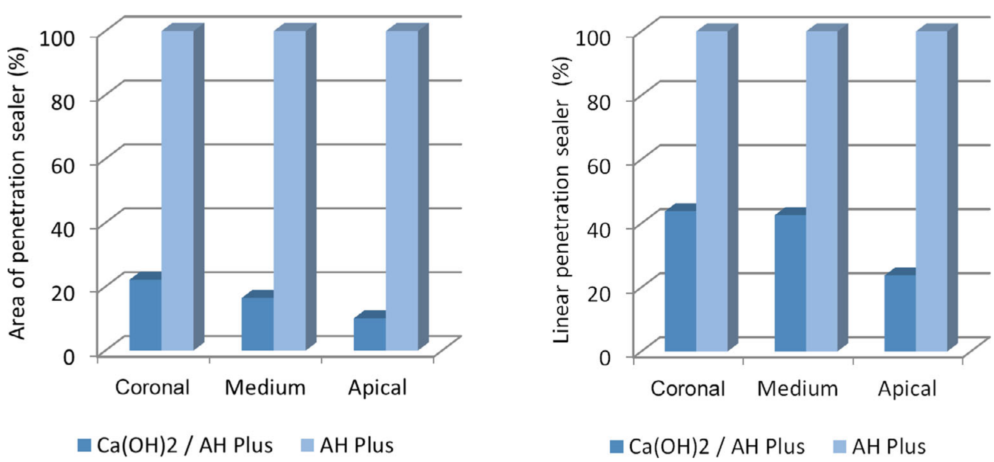

Figure 1 Percentage difference between calcium hydroxide group and control group as 100%

Table 1 Comparisons of penetration area (μm^2) of the epoxy resin-based sealer AH Plus into dentinal tubules

	$Ca(OH)_2$ use			No $Ca(OH)_2$ use		
Third	Median (max–min)	Q_1–Q_3	Confidence interval (95%)	Median (max – min)	Q_1 – Q_3	Confidence interval (95%)
Coronal	235 199 (163 994–320 361)$^{a,¥}$	205 818.25–264 874.75	190 558–280 924	1 130 051.5 (470 702 –1 578 417)$^{a,℧}$	955 313.25–1 201 023.25	77 6514–1 350 777
Middle	119 545 (61 872–250217)$^{a,b,¥}$	74 605.25–188 450.75	74 770–203 914	942 405.5 (425 198–1 037 112)$^{a,℧}$	760 222.75–988 989.5	666 806–1 027 142
Apical	12 540.5 (2064 - 81943)$^{b,¥}$	6379.75–15 426.75	2383–41 116	123 352 (50 135–475 960)$^{b,℧}$	85 332.25–225 917.5	48 076–335 499

Different letters mean difference between thirds. Kruskal–Wallis and Dunn's test ($P < 0.01$). Different symbols mean difference between groups. Mann–Whitney U test ($P < 0.01$).

Table 2 Comparisons of maximum linear penetration (μm) of the epoxy resin-based sealer AH Plus into dentinal tubules

	$Ca(OH)_2$ use			No $Ca(OH)_2$ use		
Third	Median (max–min)	Q_1–Q_3	Confidence interval (95%)	Median (max–min)	Q_1–Q_3	Confidence interval (95%)
Coronal	200.5 (176–250)$^{a,¥}$	192.75–215	186–224	496 (264–562)$^{a,℧}$	421,5–538.75	386–551
Middle	179.5 (97–267)$^{a,¥}$	156.25–204.75	138–227	427.5 (332–500)$^{a,b,℧}$	419–450.25	388–469
Apical	57 (36–129)$^{b,¥}$	44.75–87.5	42–95	305.5 (166–485)$^{b,℧}$	234.25–414.5	225–420

Different letters mean difference between thirds. Kruskal–Wallis and Dunn's test ($P < 0.01$). Different symbols mean difference between groups. Mann–Whitney U test ($P < 0.01$).

comparison of these results with the literature needs to consider important topics. Mamootil and Messer (15) observed that the type of sealer promotes different penetrations into the dentinal tubules, and that calcium hydroxide can alter the physical–chemical properties according to the type of sealer. In the present study, the AH Plus sealer was used because it is considered the gold standard in the literature and is mostly used worldwide (14). However, there are other epoxy resin-based sealers such as Sealer Plus and Sealer 26, both containing calcium hydroxide (9,12). Siqueira Jr. *et al*. (9) found that AH Plus had flow values significantly superior to those of Sealer 26. According to Duarte *et al*. (10), although AH Plus has high flow ability, calcium hydroxide can affect its film thickness and flow properties. This would explain the low penetration of the sealer in the experimental group, as also observed by Veintimilla Lozada *et al*. (17) in the assessment of the linear penetration of AH Plus into dentinal tubules containing calcium hydroxide. Besides the physical–chemical properties of the sealer, changes in the dentin permeability may have occurred due to the presence of dressing. Porkaew *et al*. (22) reported that the residual calcium hydroxide can reach the dentinal tubules, decreasing the dentin permeability. Camargo *et al*. (8) confirmed that the remaining calcium hydroxide can penetrate the dentinal tubules together with the sealer. In addition, Yassen *et al*. (23) observed that intracanal dressing with calcium hydroxide reduces dentin wettability. On the other hand, Çalt and Selper (7) reported that the residual dressing does not penetrate into the dentinal tubules, but it forms a thin layer preventing sealer penetration. In the present study, areas penetrated and not penetrated by the sealer suggest that a combination of the two situations may occur.

The area penetrated by the sealer without the use of calcium hydroxide dressing was reduced in the apical third. The literature shows that the apical region is physiologically obliterated and has less dentinal tubules than in the upper thirds (24). This could explain the results obtained in this group, which are in agreement with study of Camargo *et al*. (8). In the calcium hydroxide group, the worst results in the apical third could be explained by the fact that it is the most difficult region to be cleaned by irrigation, and consequently, there could be a greater amount of remaining dressing (25).

Analysis of linear penetration showed that calcium hydroxide also influenced the penetration depth of sealer into the dentinal tubules, which may have caused an obstruction of these tubules. Additionally, the apical region may have had lower values due to its smaller diameter in relation to the upper thirds (24). The similar penetration of the sealer into the cervical and middle thirds in terms of linear penetration and area may be due to the similarity in the density and diameter of the dentinal tubules in these thirds (24). The linear penetration of

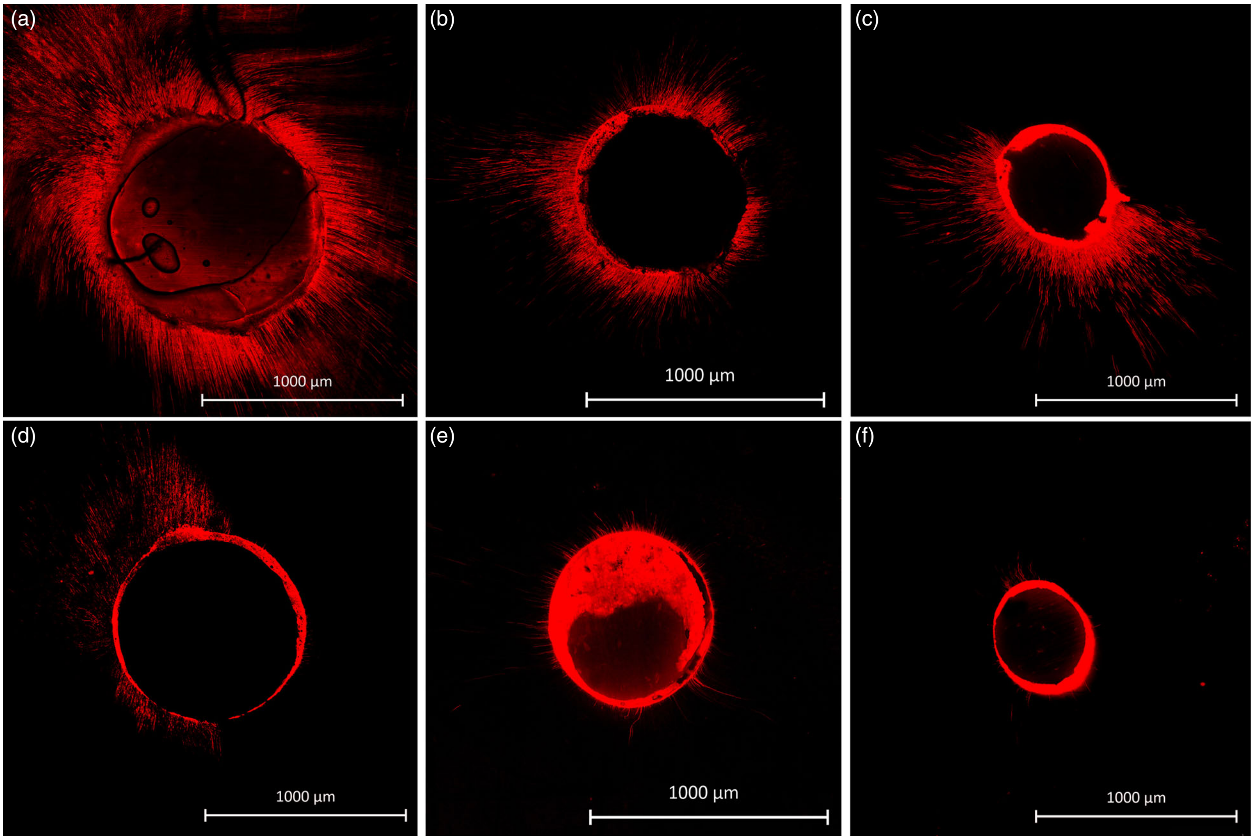

Figure 2 Confocal laser scanning microscopy (CLSM). Representative photomicrographs of the penetration of the epoxy resin-based sealer AH Plus into dentinal tubules of different thirds. (a) Coronal, (b) middle and (c) apical thirds of the root canal filling without use of calcium hydroxide dressing. (d) Coronal, (e) middle and (f) apical thirds of the root canals filling with previous use of calcium hydroxide dressing

the sealer into the cervical and middle thirds without dressing use was 496 and 427 μm, respectively. These values were near of the penetration of resin epoxy-based sealer observed (552 and 304 μm) by Russel *et al*. (16).

It is important to seek a relationship between the results of in vitro studies and the clinic ones. Sealer penetration has been related to the quality of the sealing at the root canal filling-dentin interface, but an in vitro study by Moinzadeh *et al*. (26) demonstrated that the presence of remaining calcium hydroxide does not affect the quality of obturation. On the other hand, Ricucci and Langeland (27) reported that incomplete removal of intracanal calcium hydroxide dressing from the apical third prevented the complete repair of apical periodontitis. De-Deus *et al*. (28) showed that there is no correlation between sealer penetration and sealing ability. However, Rechenberg *et al*. (29) mapped the bacterial infiltration during obturation and found that bacteria can penetrate the dentinal tubule network instead of the root canal filling-dentin interface. Therefore, bacterial penetration occurs not only at the root canal filling-dentin interface, but also at the dentinal tubule network (28). Mjor and Nordhal (30) observed that tubular dentin is complex as it has several branches and interconnections. In this respect, the deep penetration of sealer would be important to prevent remaining bacteria to reach the periapical region. Therefore, further studies focused on the filling of the dentinal complex and its relationship with bacteria should be encouraged.

The results of this study showed a significant penetration of the sealer into the dentinal tubules when calcium hydroxide dressing was not used. This result is preliminary and may be related to the filling adhesion to the root canal, meaning that further studies using the push-out bond strength analysis and/or adhesion interface analysis with scanning electron microscopy should be encouraged to complement these findings.

Disclosure

The authors deny any conflicts of interest related to this study.

Authorship declaration

We declare that all authors have contributed significantly in this project, and that all authors an agreement with the manuscript. Each contribution is listed below: Dr. Machado was responsible by project administration, supervision, design of the project, interpretation of the results and final review of the manuscript; Dr. Guillén, Lozada and Rengifo participated in the literature review, execution of the experimental essay and final review of the manuscript; Dr. Caballero-Flores worked in methodological design, image analysis, statistical analysis and final review of the manuscript; and Dr. Nabeshima was responsible for writing, literature review, data interpretation and manuscript editing.

References

1. Sedgley CM, Lennan SL, Appelbe OK. Survival of *Enterococcus faecalis* in root canals ex vivo. Int Endod J 2005; 38: 735–2.
2. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965; 20: 340–9.
3. Machado MEL, Nabeshima CK, Caballero-Flores H *et al*. Instrument design may influence bacterial reduction during root canal preparation. Braz Dent J 2017; 28: 587–91.
4. Vera J, Siqueira JF Jr, Ricucci D *et al*. One- versus two-visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. J Endod 2012; 38: 1040–52.
5. Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. Survival of Enterococcus faecalis in infected dentinal tubules after root canal filling with different root canal sealers in vitro. Int Endod J 2004; 37: 193–8.
6. Kuga MC, Tanomaru-Filho M, Faria G, Só MV, Galletti T, Bavello JR. Calcium hydroxide intracanal dressing removal with different rotary instruments and irrigating solutions: a scanning electron microscopy study. Braz Dent J 2010; 21: 310–4.
7. Çalt S, Serper A. Dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide. J Endod 1999; 25: 431–3.
8. Camargo EJ, Vivan RR, Bramante CM *et al*. The inluence of calcium hydroxide on adaptation and root canal penetration in teeth filled with methacrylate-based resin sealer. Dental Press Endod 2015; 5: 21–7.
9. Siqueira JF Jr, Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP. Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod 2000; 26: 274–7.
10. Duarte MA, Ordinola-Zapata R, Bernardes RA *et al*. Influence of calcium hydroxide association on the physical properties of AH Plus. J Endod 2010; 36: 1048–51.
11. Marciano MA, Guimarães BM, Ordinola-Zapata R *et al*. Physical properties and interfacial adaptation of three epoxy resin-based sealers. J Endod 2011; 37: 1417–21.
12. Silva EJ, Hecksher F, Vieira VT *et al*. Cytotoxicity, antibacterial and physicochemical properties of a new epoxy resin-based endodontic sealer containing calcium hydroxide. J Clin Exp Dent 2020; 12: e533–e539.
13. Deniz Sungur D, Aksel H, Purali N. Effect of a low surface tension vehicle on the dentinal tubule penetration of calcium hydroxide and triple antibiotic paste. J Endod 2017; 43: 452–5.
14. De Deus GA, Gurgel-Filho ED, 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: 23–8.
15. Mamootil K, Messer HH. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. Int Endod J 2007; 40: 873–81.
16. Russell A, Friedlander L, Chandler N. Sealer penetration and adaptation in root canals with the butterfly effect. Aust Endod J 2018; 44: 225–34.
17. Veintimilla Lozada VN, Guillén Guillén R, Caballero Flores HV, Machado MEL. Influencia de la medicación intracanal con pasta de hidróxido de calcio en la penetración del cemento obturador. Rev Odontología 2019; 21: 5–18.
18. Bitter K, Paris S, Mueller J, Neumann K, Kielbassa AM. Correlation of scanning electron and confocal laser scanning microscopic analyses for visualization of dentin/adhesive interfaces in the root canal. J Adhes Dent 2009; 11: 7–14.
19. 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: 2353–61.
20. Wang L, Júnior BO, Lopes AC *et al*. Water interaction and bond strength to dentin of dye-labelled adhesive as a function of the addition of rhodamine B. J Appl Oral Sci 2016; 24: 317–24.
21. Bim Júnior O, Cebim MA, Atta MT, Machado CM, Francisconi-Dos-Rios LF, Wang L. Determining optimal fluorescent agent concentrations in dental adhesive resins for imaging the tooth/restoration interface. Microsc Microanal 2017; 23: 122–30.
22. Porkaew P, Retief DH, Barfield RD, Lacefield WR, Soong SJ. Effects of calcium hydroxide paste as an intracanal medicament on apical seal. J Endod 1990; 16: 369–74.
23. Yassen GH, Sabrah AH, Eckert GJ, Platt JA. Effect of different endodontic regeneration protocols on wettability, roughness, and chemical composition of surface dentin. J Endod 2015; 41: 956–60.

24. Marion D, Jean A, Hamel H, Kerebel LM, Kerebel B. Scanning electron microscopic study of odontoblasts and circumpulpal dentin in a human tooth. Oral Surg Oral Med Oral Pathol 1991; 72: 473–8.
25. Yaylali IE, Kececi AD, Ureyen KB. Ultrasonically activated irrigation to remove calcium hydroxide from apical third of human root canal system: a systematic review of in vitro studies. J Endod 2015; 41: 1589–99.
26. Moinzadeh AT, De Moor RJG, De Bruyne MAA. Influence of a calcium hydroxide-based intracanal dressing on the quality of the root canal filling assessed by capillary flow porometry. Clin Oral Investig 2018; 22: 1733–9.
27. Ricucci D, Langeland K. Incomplete calcium hydroxide removal from the root canal: a case report. Int Endod J 1997; 30: 418–21.
28. De-Deus G, Brandão MC, Leal F *et al*. Lack of correlation between sealer penetration into dentinal tubules and sealability in nonbonded root fillings. Int Endod J 2012; 45: 642–51.
29. Rechenberg DK, Thurnheer T, Zehnder M. Potential systematic error in laboratory experiments on microbial leakage through filled root canals: an experimental study. Int Endod J 2011; 44: 827–35.
30. Mjör IA, Nordahl I. The density and branching of dentinal tubules in human teeth. Arch Oral Biol 1996; 41: 401–12.

