

The effect of final irrigation on the penetrability of an epoxy resin-based sealer into dentinal tubules: a confocal microscopy study

Alexander Pompermayer Jardine¹ · Ricardo Abreu da Rosa¹ ·
Manuela Favarin Santini¹ · Márcia Wagner¹ · Marcus Vinícius Reis Só¹ ·
Milton Carlos Kuga² · Jefferson Ricardo Pereira³ · Patrícia Maria Poli Kopper¹

Received: 22 November 2013 / Accepted: 9 April 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract

Introduction The aim of this study was to compare the effect of QMix, BioPure MTAD, 17 % EDTA, and saline on the penetrability of a resin-based sealer into dentinal tubules using a confocal laser scanning microscope (CLSM) and to describe the cleaning of root canal walls by SEM.

Methods Eighty distobuccal roots from upper molars were selected and randomly divided into four groups ($n=20$) before root canal preparation according to the solution used in the final rinse protocol (FRP): QG (QMix), MG (BioPure MTAD), EG (17 % EDTA), and CG (control group: saline). Ten roots of each group were prepared for SEM, and images ($\times 2000$) from the canal walls were acquired. The remaining canals were filled with a single gutta-percha cone and AH Plus with 0.1 % Rhodamine B. The specimens were horizontally sectioned at 4 mm from the apex, and the slices were analyzed in CLSM ($\times 10$). Sealer penetration was analyzed with Adobe Photoshop software.

Results QG and EG presented similar amounts of sealer penetration ($P > .05$). MG and CG presented the lowest penetrability values ($P < .05$). The best results for smear layer removal

of the apical third of the root canal were achieved by the QG and EG groups when compared with MG and CG ($P < .05$).

Conclusions Seventeen percent EDTA and QMix promoted sealer penetration superior to that achieved by BioPure MTAD and saline.

Clinical relevance Despite studies have not confirmed the relationship between sealing ability of endodontic sealers and their penetration in dentinal tubules, sealer penetration assumes importance, since endodontic sealers, unlike gutta-percha, are able to penetrate in dentinal tubules, isthmus, and accessory canals, filling the root canal system.

Keywords Endodontics · Confocal laser scanning microscopy · Scanning electron microscopy · Smear layer · Root canal irrigant · Final irrigation solution

Electronic supplementary material The online version of this article (doi:10.1007/s00784-015-1474-8) contains supplementary material, which is available to authorized users.

✉ Marcus Vinícius Reis Só
endo-so@hotmail.com

¹ Conservative Dentistry Department, Rio Grande do Sul Federal University, Porto Alegre, RS, Brazil

² Department of Endodontics, School of Dentistry, Sao Paulo State University, UNESP, Araraquara, SP, Brazil

³ Department of Prosthodontics, Dental School, University of Southern Santa Catarina, Tubarão, SC, Brazil

Introduction

It is impossible to create a sterile environment in infected teeth after chemomechanical preparation [1]. The complexity of root canal systems leads to remaining pulp tissue and inorganic debris, mainly in isthmuses or in areas that instruments did not reach [2, 3]. In this context, irrigants play an indispensable role. They must present antibacterial activity and an ability to dissolve organic and inorganic tissues. However, none of the current irrigants individually meet all these requirements. Therefore, associations have been proposed [4–8].

Sodium hypochlorite (NaOCl) is the most common irrigant used during root canal therapy. However, despite its great antibacterial activity, NaOCl has no effect on the inorganic portion of the smear layer [9]. The presence of the smear layer attached to the canal wall impairs irrigants, medications, and endodontic sealers from accessing dentinal tubules [10]. Thus,

preceding the placement of canal dressings or the root filling, additional solutions have been used to act on the inorganic portion of the smear layer [11–14].

The association of NaOCl and EDTA has been shown to be effective in removing the smear layer and pulpal remnants within the root canal system [15, 16]. Recently, researchers have focused on developing new irrigants and establishing alternative irrigation protocols to better remove the smear layer and debris from the root canal, especially at the apical third [4, 17].

BioPure MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA) and QMix (Dentsply Tulsa Dental) are relatively new solutions used during the final rinse procedure. Both products aim at removing the inorganic smear layer and disinfect the root canal system [6, 12, 18]. QMix associates the chelating properties of EDTA with the substantivity and antimicrobial properties of chlorhexidine gluconate [18]. QMix consists of a mixture of a bisbiguanide antimicrobial agent, a polyaminocarboxylic acid calcium-chelating agent, saline, and a surfactant [6]. BioPure MTAD, in turn, consists of a mixture of doxycycline, citric acid, and a detergent (Tween 80).

Many studies have investigated the action of QMix and BioPure MTAD on the removal of the smear layer and debris [6, 8, 12]. Some results have pointed to an equivalent efficacy of QMix, EDTA, and BioPure MTAD on smear layer removal [4, 5, 8, 19]. However, no study has assessed their influence on the sealer's penetration into dentinal tubules. The main goal of filling procedures is to adequately fill the root canal system, including accessory canals, isthmus, apical delta, and dentinal tubules. From a microbiological standpoint, sealer penetration is desirable because it might entomb residual debris and microorganisms and it may maintain them far from nutrient sources [20]. Moreover, deep endodontic sealer penetration is notably important because it decreases the interface between gutta-percha and root dentin and it may improve the retention of the filling mass by mechanical locking [21].

The aims of the present study were to compare the effect of QMix, BioPure MTAD, 17 % EDTA, and saline on the penetrability of a resin-based sealer into dentinal tubules using a confocal laser scanning microscope and to describe the cleaning of root canal walls by SEM analyses. The null hypothesis was that there would be no differences in sealer penetrability after the final rinse using QMix, BioPure MTAD, 17 % EDTA, or saline.

Materials and methods

This study was approved by the Ethics Committee of the Federal University of Rio Grande do Sul. Eighty distobuccal roots from maxillary molars were selected and transversely sectioned using a diamond disc (KG Sorensen, Cotia, SP, Brazil) at the cemento-enamel junction. Next, the presence of a single canal was radiographically verified. Only straight roots at least

12 mm in length with completely formed root apices and the absence of root reabsorption were included in this study.

The canals were accessed and the working length (WL) was established 1 mm short of the total canal length via the insertion of a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until it was just visible at the apical foramen. Before root canal preparation, all roots were randomly divided into four groups ($n=20$) according to the solution used in the final rinse protocol (FRP): QG (QMix), MG (BioPure MTAD), EG (17 % EDTA), and CG (control group: saline).

The root canals were prepared at WL using the ProTaper system (Dentsply Maillefer) up to F3 file. After each instrument change, 2 mL of 2.5 % NaOCl were delivered into the root canals. According to the manufacturer's recommendation, in MG, the roots were irrigated with 2 mL of 1.3 % NaOCl. Five-milliliter disposable plastic syringes (Ultradent Products Inc., South Jordan, UT, USA) with 30-gauge needles (Endo Easy Tip, Ultradent Products Inc.) inserted 1 mm shorter than the WL were used for irrigation. The FRP for the experimental groups was 5 mL of the test solution for 2 min followed by 2 mL of saline. In the CG, 5 mL of saline was delivered for 2 min followed by 2 mL of the same solution. Finally, the canals were dried with paper points (Dentsply Maillefer).

Two roots of each group were prepared for scanning with an electron microscopy (SEM) (JEOL 6060, JEOL, Tokyo, Japan) operated at 10 kV. Specimens were split by applying slight pressure to an enamel chisel into the longitudinal groove, and one half of the specimen was randomly chosen. Each sample was dehydrated in graded series of ethanol solutions, mounted on stubs, gold-sputtered, and examined on SEM. The apical third was used to illustrate the amount of debris and the smear layer after FRP ($\times 2000$). Each micrograph was evaluated using five score index [22] as follows: score 1: no smear layer, dentinal tubules open; score 2: small amount of smear layer, some dentinal tubules open; score 3: homogenous smear layer covering the root canal wall, only few dentinal tubules open; score 4: complete root canal wall covered by a homogenous smear layer, no open dentinal tubule; and score 5: heavy, non-homogenous smear layer covering the complete root canal wall. The scoring procedure was performed by two calibrated examiners who could not identify the experimental group of each specimen. Statistical analysis was performed using Bioestat 3.0 software (Bioestat, Instituto Mamirauá, Belém, PA, Brazil). The data was analyzed using the Kruskal-Wallis test and the Dunn post hoc test. The significance levels were set at 5 %.

The remaining 40 roots were filled using the single cone technique. Equal parts of paste A and paste B of an epoxy resin-based sealer (AH Plus, Dentsply Maillefer) were mixed with 0.1 % Rhodamine B (RITC; Sigma-Aldrich, St. Louis, MO). The endodontic sealer was placed in the canal 1 mm shorter than the WL using a 400-rpm lentulo spiral (Dentsply Maillefer) for 5 s [23], and a ProTaper F3 gutta-percha cone (Dentsply Maillefer) was immediately positioned. The

specimens were stored at 37 °C and 100 % humidity for 7 days to allow the sealer to set.

Each specimen was horizontally sectioned under water cooling at 4 mm from the apex using a cut machine (Extac Labcut 1010, Enfield, CT) to obtain a 2-mm-thick slice. Next, the coronal surfaces of the slices were polished with Arotec paste (Arotec, Cotia, SP, Brazil) to eliminate dentin debris generated during the cutting procedures and to produce a highly reflective surface.

The slices were examined using an Olympus Fluoview 1000 confocal laser scanning microscope (CLSM) (Olympus Corporation, Tokyo, Japan) with $\times 10$ magnification. The image settings were 70- μm depth with 800×800 pixels. Using Adobe Photoshop software (Adobe Systems, San Jose, CA, USA), one calibrated examiner ($R=0.86$) analyzed the images. Initially, the size of all images was standardized with 512×512 pixels with root canal centered. Then, with rectangular marquee tool, the whole image was selected and the total number of pixels was registered in the histogram tool. Next, the magnetic lasso tool was used to outline the canal area, and the number of pixels was obtained. The canal area has been colored black to hide the sealer inside the canal. By subtracting these values (pixels of total area and pixels of canal area), the number of pixels of dentin area was obtained. Afterward, the sealer impregnated dentin area, colored red, was outlined with the same tool, and in the color range tool, red was selected, and the number of red pixels, corresponding to the sealer penetration, was registered. Finally, the percentage of sealer penetration in the dentin wall was calculated. Statistical analyses were performed using SPSS 16.0 software (SPSS Co. Chicago, IL, USA). The data was analyzed using the Shapiro-Wilk normality test, the Kruskal-Wallis test, and the Dunn post hoc test. The significance levels were set at 5 %.

Results

The Shapiro-Wilk normality test indicated a non-normal distribution of the data ($P=.11$). The percentage of sealer penetration into dentinal tubules is shown in Table 1 by the median and by the 25th and 75th percentiles. Kruskal-Wallis analysis

Table 1 Median and 25th and 75th percentile of sealer impregnated dentin area after using QMix (QG), BioPure MTAD (MG), 17 % EDTA (EG), and saline—control group (CG) at the FRP

	GQ	GM	GE	CG
Median	4.14 % ^a	0.32 % ^b	5.97 % ^a	0.36 % ^b
25th percentile	2.0 %	0.0 %	4.4 %	0.0 %
75th percentile	6.5 %	1.1 %	9.5 %	0.6 %

Different letters denote significant differences after the Kruskal-Wallis and the Dunn's post hoc tests ($\alpha < .05$)

showed a significant difference in the sealer penetration among the groups ($P < .05$). QMix promoted sealer penetration that was similar to that achieved after using 17 % EDTA ($P > .05$). MG and CG presented the lowest penetrability values ($P < .05$). Figure 1 shows representative images of sealer penetration according to the irrigant used at the FRP.

The results for the evaluation of the smear layer are summarized in Table 2. The lowest scores for smear layer removal of the apical third of the root canal were achieved by the QG and EG groups when compared with MG and CG ($P < 0.05$).

In the SEM images, QG (Fig. 2a) and EG (Fig. 2c) presented the lowest scores of smear layer attached to the root canal walls. However, higher scores of smear layer were observed in MG (Fig. 2b) and CG (Fig. 2d).

Discussion

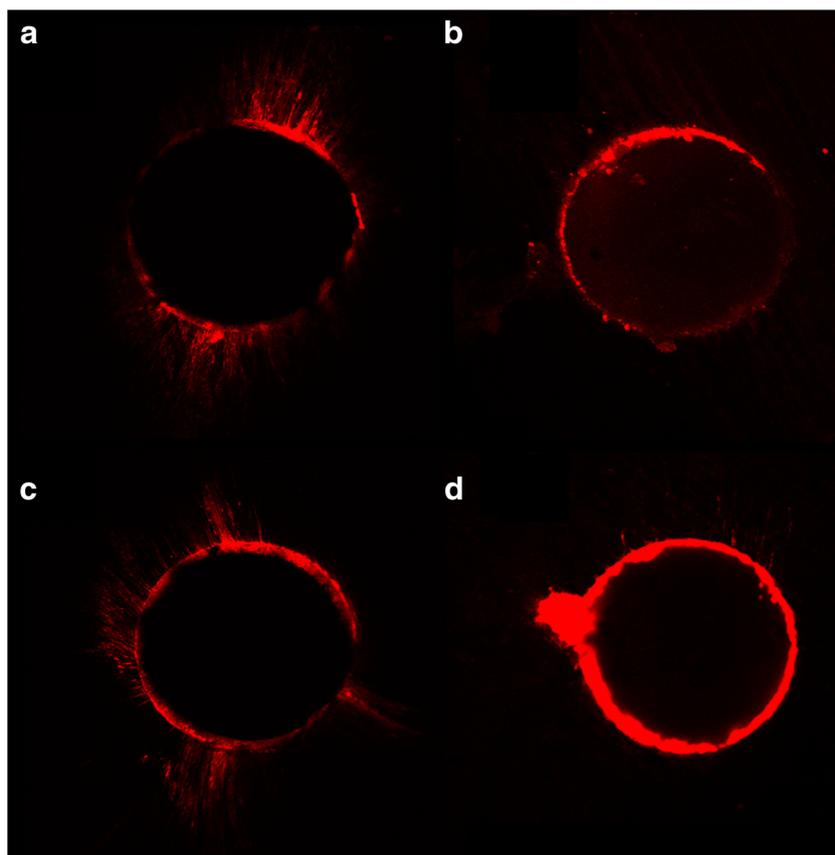
The presence of a smear layer on the canal walls may reduce dentin permeability and may hinder sealer penetration into dentinal tubules [24]. Therefore, its removal is essential to improve the quality of the root canal filling. Recently, DeDeus et al. [25] did not find correlation between sealer penetration and the sealability of an epoxy resin-based sealer. However, this study did not aim to establish any correlation between these aspects. Sealer penetration into accessory canals, isthmus, apical delta, and dentinal tubules is particularly important because the sealer's antibacterial activity and its blockade effect can be considered beneficial to prevent reinfection [24].

According to the literature, it is harder to remove the smear layer from the apical third than from the other thirds [21, 26, 27]. At this third, the delivery of the irrigant is impaired, and dentin exhibits less tubule density or even areas with absent of tubules [28]. Because of these characteristics, sealer penetration after FRP with different solutions was assessed at this portion of the root canal. Slices were obtained 4 mm from the root apex in order to minimize the inclusion of apical deltas and anatomical irregularities which must impair the analysis of sealer penetration.

The single cone technique was performed due to its wide use in endodontics and because sealer penetration does not depend on the filling technique [29, 30]. The tubule penetration of resin-based sealers is not dependent on the hydraulic forces created during filling; instead, the sealer is drawn into the tubules by capillary action [27].

Some authors evaluated the maximum penetration of the sealer by tracing a straight line from the canal wall up to the outer limit of the visible field in the microscope [20, 27]. The method used in the present study allows the assessment of the overall penetration related to the visible field. In many cases, the sealer penetration is not homogenous along the entire perimeter of the canal;

Fig. 1 CLSM images ($\times 10$) to illustrate the sealer penetration: **a** QG, **b** MG, **c** EG, and **d** CG



therefore, the maximal penetration must not be representative. For this reason, this study measured the percentage of sealer impregnated dentin area.

Light microscopy [31], SEM [24], and CLSM [32, 33] have been used to assess smear layer removal and sealer penetration into dentinal tubules. The main disadvantage of light microscopy was the inability to distinguish the sealer from the radicular dentin [27]. CLSM presents certain advantages over other techniques because it does not require any special specimen processing [30]. To evaluate the samples by SEM, the specimens must undergo gold sputtering, and because SEM works under a vacuum, the procedure is time-consuming. Additionally, CLSM produces fewer artifacts and does not promote sample dehydration when compared to SEM [34].

Table 2 Median and 25th and 75th percentile of smear layer scores after using QMix (QG), BioPure MTAD (MG), 17 % EDTA (EG), and saline—control group (CG) at the FRP

	QG	MG	EG	CG
Median	1.0 ^a	2.0 ^{ab}	1.0 ^a	5.0 ^b
25th percentile	1.0	2.0	1.0	4.25
75th percentile	2.0	3.0	1.75	5.0

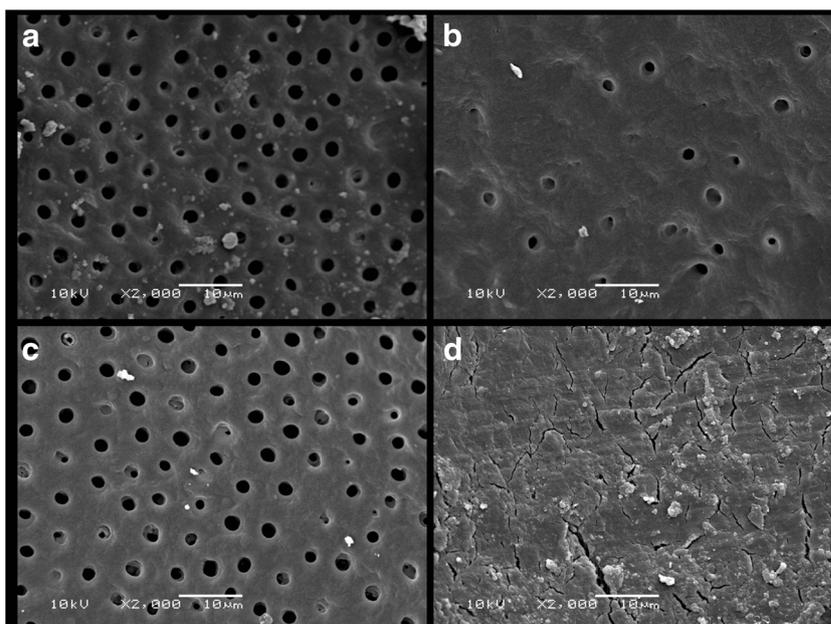
Different letters denote significant differences after the Kruskal-Wallis and the Dunn's post hoc tests ($\alpha < .05$)

CLSM permits image acquisition from several optical sections, even from thick specimens, which are further reconstructed to achieve the final image. However, SEM permits the visualization of only one plane. Rhodamine B dye was used to promote the fluorescence of the sealer because CLSM works with high contrast points to identify the sealers within the dentinal tubules [35]. Patel et al. [36] did not observe an influence of Rhodamine B dye on the physical properties of the sealer, most likely because of the small amount of the dye (0.01 %) mixed with the sealer.

Based on the results of the present study, the null hypothesis was rejected. Sealer penetration was influenced by the irrigant used at the FRP. It is important to note that sealer penetration into dentinal tubules can be used as an indicator for smear layer removal; if the smear layer has closed the tubule openings, the sealer would not penetrate into them.

Despite the presence of surfactant in the QMix formulation that decreases surface tension and increases the wettability, the SEM images showed smear layer removal in QG and EG. In line with these findings, Stojicic et al. [8] verified that QMix and 17 % EDTA presented a similar ability to remove the smear layer in 5 min. The percentages of opened tubules were 88 and 85 %, respectively. Adding these results to the promising results concerning the antibacterial activity of QMix [8, 18], the chelating effects of QMix seem to be effective in tubule opening and also in killing bacteria.

Fig. 2 SEM images ($\times 2000$) to illustrate the cleanness of the dentin walls: **a** QG, **b** MG, **c** EG, and **d** CG



Several studies evaluated the ability of different chelating agents to remove the smear layer and debris [5–8, 19]. However, the literature does not present any study that has evaluated the effect of these relatively new solutions (QMix and BioPure MTAD) on sealer penetrability.

In this investigation, sealer penetration was higher in EG and QG when compared with MG and CG ($P < .05$). These findings are coherent with the results obtained of smear layer removal. SEM illustrative micrographs showed the smear layer attached to the canal walls in groups that presented the lowest sealer penetration (MG and CG), and almost all tubules were opened in groups where the sealer penetration was higher (EG and QG). According to De-Deus et al. [25], root canal walls that were completely covered by a smear layer did not exhibit sealer penetration into dentinal tubules. So, despite the main goal of the present study being to investigate the sealer penetration into dentinal tubes after different FRP, our results can be correlated with the ones that evaluated smear layer removal.

In contrast with findings of the present investigation, Torabinejad et al. [4] showed that BioPure MTAD was effective to remove smear layer. The contradictory results may be due to differences in the volume of solution and time spent for irrigation. Whereas Torabinejad et al. [4] used 1 mL of MTAD per 5 min and, then, a final flush with 4 mL, in the current study, to standardize the volume and time for all tested solutions, 5 mL of MTAD was used per 2 min.

In accordance with our results, comparing cleaning ability of BioPure MTAD with 17 % EDTA, Wu et al. [37] showed better results for 17 % EDTA and Mancini et al. [5] find no differences in smear layer removal between 17 % EDTA, BioPure MTAD, and 42 % citric acid.

Some CLSM images showed the absence of a homogeneous layer of sealer in the dentinal tubules along the entire canal perimeter. The presence of sclerotic dentin, the small number of dentinal tubules relative to the cervical and middle thirds, and the difficulty in removing the smear layer from the apical third constitute possible reasons that could explain such results [20]. It is difficult to standardize the amount and distribution of sclerotic dentin and irregular secondary dentin which may have influence on the sealer penetration or smear layer patterns. Even a careful sample selection does not guarantee a homogeneous dentin pattern among the specimens. Thus, at the moment, it consists in a drawback inherent to this methodology. Compaction loads during root canal filling may lead to a lack of sealer between the gutta-percha and the root canal wall and may also contribute to the irregular patterns of sealer penetration [20].

Current methods to evaluate the smear layer removal have been contested [25]. SEM does not allow a longitudinal observational character, in which a given dentin area can be observed at different times. Additionally, the development of computational routines able to automatically extract quantitative data of dentin morphology, thus minimizing human bias, is needed [25]. However, SEM has been widely used for this purpose.

Conclusions

Within the limitations of this study, it can be concluded that 17 % EDTA and QMix promoted sealer penetration superior to that achieved by BioPure MTAD. When saline and BioPure MTAD were used for final rinse, higher scores of smear layer removal were obtained. However, QMix and 17 % EDTA promote the lowest scores to open the dentinal tubules.

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

References

- Pataky L, Ivanyi I, Grigar A, Fazekas A (2002) Antimicrobial efficacy of various root canal preparation techniques: an in vitro comparative study. *J Endod* 28:6–3
- Davis SR, Brayton SM, Goldman M (1972) The morphology of the prepared root canal: a study utilizing infectable silicone. *Oral Surg Oral Med Oral Pathol* 34:642–8
- Fomari VJ, Silva-Sousa YT, Vanni JR, Pécora JD, Versiani MA, Sousa-Neto MD (2010) Histological evaluation of the effectiveness of increased apical enlargement for cleaning the apical third of curved canals. *Int Endod J* 43:988–94
- Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, Kim J, Shabahang S (2003) A new solution for the removal of the smear layer. *J Endod* 29:170–5
- Mancini M, Armellini E, Casaglia A, Cerroni L, Cianconi L (2009) A comparative study of smear layer removal and erosion in apical intraradicular dentine with three irrigating solutions: a scanning electron microscopy evaluation. *J Endod* 35:900–3
- Dai L, Khechen K, Khan S, Gillen B, Loushine BA, Wimmer CE, Gutmann JL, Pashley D, Tay FR (2011) The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris. *J Endod* 37:80–4
- Lotfi M, Vosoughhosseini S, Saghiri MA, Zand V, Ranjkesh B, Ghasemi N (2012) Effect of MTAD as a final rinse on removal of smear layer in ten-minute preparation time. *J Endod* 38:1391–4
- Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M (2012) Antibacterial and smear layer removal ability of a novel irrigant, QMiX. *Int Endod J* 45:363–71
- Violich DR, Chandler NP (2010) The smear layer in endodontics—a review. *Int Endod J* 43:2–15
- Torabinejad M, Handysides R, Khademi AA, Bakland LK (2002) Clinical implications of the smear layer in endodontics: a review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 94:658–66
- Foster KH, Kulild JC, Weller RN (1993) Effect of smear layer removal on the diffusion of calcium hydroxide through radicular dentin. *J Endod* 19:136–40
- Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S (2003) The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. *J Endod* 29:233–9
- Zehnder M, Schmidlin P, Sener B, Waltimo T (2005) Chelation in root canal therapy reconsidered. *J Endod* 31:817–20
- Shahravan A, Haghdoost AA, Adl A et al (2007) Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis. *J Endod* 33:96–105
- Baumgartner JC, Ibay AC (1987) The chemical reactions of irrigants used for root canal debridement. *J Endod* 13:47–51
- Khedmat S, Shokouhinejad N (2008) Comparison of the efficacy of three chelating agents in smear layer removal. *J Endod* 34:599–602
- Blank-Gonçalves LM, Nabeshima CK, Martins GH, Machado ME (2011) Qualitative analysis of the removal of the smear layer in the apical third of curved roots: conventional irrigation versus activation systems. *J Endod* 37:1268–71
- Morgental RD, Singh A, Sappal H, Kopper PM, Vier-Pelisser FV, Peters OA (2013) Dentin inhibits the antibacterial effect of new and conventional endodontic irrigants. *J Endod* 39:406–10
- Aranda-Garcia AJ, Kuga MC, Vitorino KR, Chávez-Andrade GM, Duarte MA, Bonetti-Filho I, Faria G, Só MV (2013) Effect of the root canal final rinse protocols on the debris and smear layer removal and on the push-out strength of an epoxy-based sealer. *Microsc Res Tech* 76:533–7
- Ordinola-Zapata R, Bramante CM, Graeff MS, del Carpio PA, Vivan RR, Camargo EJ, Garcia RB, Bernardineli N, Gutmann JL, de Moraes IG (2009) Depth and percentage of penetration of endodontic sealers into dentinal tubules after root canal obturation using a lateral compaction technique: a confocal laser scanning microscopy study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 108:450–7
- Mamootil K, Messer HH (2007) Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. *Int Endod J* 40:873–81
- Hülsmann M, Rummelin C, Schäfers F (1997) Root canal cleanliness after preparation with different endodontic handpieces and hand instruments: a comparative SEM investigation. *J Endod* 23:301–306
- Barreto MS, Moraes Rdo A, Rosa RA, Moreira CH, Só MV, Bier CA (2012) Vertical root fractures and dentin defects: effects of root canal preparation, filling, and mechanical cycling. *J Endod* 38:1135–9
- Kokkas AB, Boutsioukis AC, Vassiliadis LP, Stavrianos CK (2004) The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. *J Endod* 30:100–2
- De-Deus G, Brandão MC, Leal F, Reis C, Souza EM, Luna AS, Paciornik S, Fidel S (2012) Lack of correlation between sealer penetration into dentinal tubules and sealability in nonbonded root fillings. *Int Endod J* 45:642–51
- Eliot C, Hatton JF, Stewart GP, Hildebolt CF, Jane Gillespie M, Gutmann JL. The effect of the irrigant QMix on removal of canal wall smear layer: an ex vivo study. *Odontology* 2013;19. [Epub ahead of print]
- Chandra SS, Shankar P, Indira R (2012) Depth of penetration of four resin sealers into radicular dentinal tubules: a confocal microscopic study. *J Endod* 38:1412–6
- Carrigan PJ, Morse DR, Furst L, Sinai IH (1984) A scanning electron microscopic evaluation of human dentinal tubules according to age and location. *J Endod* 10:359–63
- Weis MV, Parashos P, Messer HH (2004) Effect of obturation technique on sealer cement thickness and dentinal tubule penetration. *Int Endod J* 37:653–63
- Kok D, Húngaro Duarte MA, Abreu Da Rosa R, Wagner MH, Pereira JR, Só MV (2012) Evaluation of epoxy resin sealer after three root canal filling techniques by confocal laser scanning microscopy. *Microsc Res Tech* 75:1277–80
- De Deus GA, Gurgel-Filho ED, Maniglia-Ferreira C, Coutinho-Filho T (2004) 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* 30:23–8
- Kara Tuncer A, Tuncer S (2012) Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. *J Endod* 38:860–3
- Bolles JA, He J, Svoboda KK, Schneiderman E, Glickman GN (2013) Comparison of Vibringe, EndoActivator, and needle irrigation on sealer penetration in extracted human teeth. *J Endod* 39:708–11
- Hammad M, Qualtrough A, Silikas N (2008) Three-dimensional evaluation of effectiveness of hand and rotary instrumentation for retreatment of canals filled with different materials. *J Endod* 34:1370–3
- D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, Pashley DH (2006) Use of fluorescent compounds in assessing bonded resin-based restorations: a literature review. *J Dent* 34:623–34

36. Patel DV, Sherriff M, Ford TR et al (2007) The penetration of RealSeal primer and Tubliseal into root canal dentinal tubules: a confocal microscopic study. *Int Endod J* 40:67–71
37. Wu L, Mu Y, Deng X et al (2012) Comparison of the effect of four decalcifying agents combined with 608C 3 % sodium hypochlorite on smear layer removal. *J Endod* 38:381–384