ACTA ODONTOLOGICA SCANDINAVICA, 2018
VOL. 76, NO. 3, 199–203
https://doi.org/10.1080/00016357.2017.1402208

ORIGINAL ARTICLE

Evaluation of final irrigation regimens with maleic acid for smear layer removal and wettability of root canal sealer

Nidambur Vasudev Ballal[a], Carmen Maria Ferrer-Luque[b], Mrunali Sona[c], K. Narayan Prabhu[c], Teresa Arias-Moliz[b] and Pilar Baca[b]

[a]Department of Conservative Dentistry & Endodontics, Manipal College of Dental Sciences, Manipal University, Manipal, India; [b]Department of Stomatology, School of Dentistry, University of Granada, Granada, Spain; [c]Department of Metallurgical & Materials Engineering, National Institute of Technology Karnataka, Surathkal, India

ABSTRACT

Objective: To evaluate the smear layer removal and wettability of AH Plus sealer on root canal dentin treated with MA (maleic acid), MA + CTR (cetrimide) and MA + CTR + CHX (chlorhexidine) as final irrigating regimens.

Material and methods: For smear layer removal, 40 teeth were instrumented to size F4 and divided into four groups: (1) 7% MA, (2) 7% MA + 0.2% CTR, (3) 7% MA + 0.2% CTR + 2% CHX, (4) distilled water (control). After irrigation, teeth were subjected to SEM analysis. For contact angle analysis, 20 teeth were split longitudinally and divided into four groups similar to smear layer analysis. AH plus sealer was placed on each specimen and contact angle was analysed.

Results: In both smear layer ($p = .393$) and contact angle analysis ($p = .961$), there was no significant difference between the groups MA and MA + CTR. However, MA + CTR + CHX removed smear layer less effectively ($p = .023$) and increased the contact angle of the sealer ($p = .005$). In smear layer analysis, specimens in negative control group were heavily smeared. In case of contact angle analysis, samples in the control group had least contact angle.

Conclusion: MA alone or in combination with CTR removed smear layer effectively and increased the wettability of AH plus sealer to root canal dentin.

ARTICLE HISTORY
Received 23 May 2017
Revised 24 September 2017
Accepted 1 November 2017

KEYWORDS
Chlorhexidine; contact angle; cetrimide; maleic acid; root canal sealer; mear layer

Introduction

Bacteria and their by-products play an essential role in the development of pulp and periapical diseases [1] and are determinant factors in the success of endodontic therapy [2]. Eradication of these microorganisms is accomplished by a combination of mechanical instrumentation, irrigation and placement of intracanal medicaments [3]. Mechanical instrumentation of the root canal produces a smear layer that prevents sufficient penetration of irrigants and intracanal medicaments into the dentinal tubules. It may also reduce adaptation between root filling materials and dentin [4,5]. Although the smear layer has not been directly implicated on the outcome of root canal treatment, incomplete canal debridement can lead to decrease in success [6,7]. The combined application of ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl) is commonly used for the effective removal of the smear layer from the root canal system [8]. Even though EDTA is the most frequently used chelator in endodontics, it possess several disadvantages like reduced smear layer removal efficiency from the apical third of the root canal system [9,10], cytotoxicity [11], reduces the bond strength of resin cements [12], reduces the active chlorine when combined with NaOCl [13] and forms precipitate with chlorhexidine gluconate [14]. Maleic acid (MA) is a mild organic acid which has shown to remove the smear layer more effectively than 17% EDTA especially at the apical third of root canal system [9,10]. It is also shown to be less cytotoxic when compared to 17% EDTA [11]. Studies have demonstrated that, the combination of MA with chlorhexidine gluconate (CHX) and cetrimide (CTR) has effective antimicrobial activity against *Enterococcus faecalis* biofilm when compared to the use of MA alone [15–17]. Irrigating solutions when used might change the wettability of root canal dentin, which might influence the adhesion of bacteria [18] and affect the interaction between the root canal dentin and restorative materials [19]. Wettability can be expressed in terms of contact angle (θ), which is formed between the drop of a liquid and the plane surface of the solid. The contact angle has an inverse relationship with the surface free energy (wettability), that is, the lower the contact angle, the greater the surface free energy and hence improved adhesion [20]. Adhesion of root canal sealers is mainly influenced by the relative surface free energy (wetting ability) of the intraradicular dentin surface [21,22]. Ballal et al. [23] have demonstrated that, 7% MA improved the wettability of resin sealers. Because wettability of the surface is a crucial factor for adhesion, of sealers, it is of interest to examine the effect of different final irrigation regimens on the wettability of

CONTACT Vasudev Ballal drballal@yahoo.com Department of Conservative Dentistry & Endodontics, Manipal College of Dental Sciences, Manipal University, Manipal 576104, Karnataka, India

© 2017 Acta Odontologica Scandinavica Society

resin sealers. To date, there are no studies in literature evaluating the smear layer removal efficacy and measurement of the contact angle of resin based sealers to root canal dentin treated with final irrigation regimens with MA. Hence, the purpose of this study was to investigate the smear layer removal and wettability of AH Plus, an epoxy resin-based sealer on root canal dentin treated with MA, MA + CTR and MA + CTR + CHX final irrigating regimens. The null hypotheses tested were: (1) there are no differences in the ability of MA, MA + CTR and MA + CTR + CHX combination to remove canal wall smear layer when these solutions are used as final root canal irrigants and (2) there is no difference in the wettability of AH Plus sealer to root canal dentin after irrigating with MA, MA + CTR and MA + CTR + CHX combination.

Materials and methods

Root canal irrigants

The irrigating solutions used in the present study were: 2.5% NaOCl, 7% MA (Panreac, Castellar del Valles, Spain), 0.2% CTR (Sigma-Aldrich Chemie, Steinheim, Germany) and 2% CHX (Guinama, Alboraya, Spain). The MA was tested alone, combined with CTR (1:1) and combined with CTR and CHX (1:1:1).

Smear layer evaluation

Specimen preparation

The protocol followed in this study was approved by the Ethics Committee of the University of Granada, Spain (UGR-438). Forty extracted human anterior teeth with single straight canals and fully formed roots were selected. Superficial soft tissues were removed with a brush and the teeth were stored in 0.1% thymol solution at 4 °C until use. The teeth were decoronated at cemento-enamel junction to obtain a standardized root length of 12 mm by using a diamond disk in an Accutom 50 cutting machine (Struers, Ballerup, Denmark). The external surface of roots was sealed with nail polish and the apices were sealed with sticky wax to prevent the extrusion of irrigants through the apical foramen and simulate the closed end system. The samples were then divided randomly into three experimental groups and a control group ($n = 10$).

Root canal preparation

The working length (WL) was established by inserting a No. 10K file (Mani Inc- Tochigi Ken, Japan) into each root canal until it was just visible at the apical foramen (observed under magnifying loupes) and by subtracting 1 mm from this point. Root canal instrumentation was performed by the same operator using nickel-titanium ProTaper Universal files (Dentsply Maillefer, Switzerland) up to F4 size. In all the groups, irrigation was performed with 2 mL of 2.5% of NaOCl for 1 min between each instrument change. Irrigation was carried out using in-and-out motion by using a 30-G side-vented needle (NaviTip, Ultradent, South Jordan, UT) placed 1–2 mm short of WL. The final irrigation was as follows: Group 1: 5 mL 7% MA; Group 2: 5 mL 7% MA + 0.2% CTR; Group 3: 5 mL 7% MA + 0.2% CTR + 2% CHX, Group 4: distilled water (control group).

In all cases, the final irrigation was performed using a 30-G side-vented placed 1–2 mm short of working length for 1 min. Finally the canals were flushed with 5 mL of distilled water to remove any precipitate if formed. After preparation, the root canals were dried with absorbent paper points (Dentsply Maillefer, Switzerland).

Scanning electron microscopy (SEM) evaluation

Longitudinal grooves were prepared in buccolingual direction of each root by using a diamond disc at a slow speed without penetrating the canal. The roots were then split into two halves using a straight chisel. For each root, the half containing the most visible part of the root canal wall was selected. The samples were then dehydrated using ascending grades of ethyl alcohol (25%, 50%, 75% and 100%) for 15 min. Samples were then mounted on metallic stubs, gold sputtered using an ion sputter and examined under field emission scanning electron microscopy (LEO 1430 VP; Carl Zeiss NTS GmbH, Oberkochen, Germany). Photomicrographs from the approximate centre of the canal walls were taken at the coronal (12 mm), middle (6 mm) and apical (2 mm) thirds, respectively, from the apex. The presence or absence of smear layer was evaluated from images at 2000× and 10 KV applying the criteria given by Dai et al. [24]:

1. Smear layer covering less than 25% of the canal wall; most tubules were clean and patent (coronal third and middle third) or occluded with sclerotic casts (apical third).
2. Smear layer evident in more than 25% of the canal surface. Tubules contained debris.
3. Smear layer evident in more than 50% of the canal surface. Remaining tubular orifices were reduced in dimensions because of partial occlusion by debris.
4. Smear layer covering more than 75% of the canal surface. Very few dentinal tubules were evident.

For the scoring procedure, two examiners had been previously trained in 20 samples, obtaining a weighted quadratic kappa of 0.71. To further reduce the subjectivity of the measurements, both examiners acted at the same time in blinded fashion respect to the groups, in order to get a single score per sample.

Contact angle evaluation

Specimen preparation

Ethical clearance was obtained from the institutional review board. Twenty extracted human anterior teeth were selected and stored in a similar manner to those samples used in smear layer evaluation. The teeth were decoronated at the cementoenamel junction and split longitudinally into 40 halves using a slow speed diamond disk (Horico, Berlin, Germany) under water cooling. Each root-half was then grounded flat and smooth on a circular grinding machine with a series of ascending grades of silicon carbide abrasive papers (800, 1000 and 1200 grit) under distilled water to remove any surface scratches and to provide a smooth surface for the analyses. Specimens were then randomly divided into four groups ($n = 10$) based on the irrigation regimen similar to smear layer evaluation.

Contact angle measurement

Following the final irrigation, each specimen was rinsed with 5 mL of deionized water to remove any precipitate of test solutions and dried were with paper points. Each specimen was then positioned on a flat glass surface in a Dynamic Contact Angle Analyzer (FTÅ 200, First Ten Angstroms, Inc., Portsmouth, VA). This equipment has a flexible video system for measuring the static and dynamic contact angles, surface tension and interfacial tension. The root canal sealer AH Plus Jet^{TM} (Dentsply Maillefer, Konstanz, Germany) was mixed according to the manufacturer's instructions and 0.2 mL of the sealer was placed over the surface of the specimen from each group. The volume of the sealer was controlled by means of a micropipette (Eppendorf AG, Hamburg, Germany). Three drops of the sealer was evaluated for each specimen and the spreading process was recorded for 60 s. Images of the droplets were then captured using the FTÅ software to determine the static contact angles made by the sealer and the mean values were calculated.

Statistical analysis

The data of the score of the smear layer were statistically analyzed by Kruskal–Wallis and Mann–Whitney *U* tests for intergroup comparisons. Friedman and Wilcoxon signed-rank tests were used to compare in each group the different canal thirds. Contact angle scores were analyzed using One way ANOVA for group comparison and Tukey HSD test for intergroup comparisons. The level of statistical significance was set at $p < .05$. All statistical analyses were performed by means of SPSS 17.0 software (SPSS Inc, Chicago, IL).

Results

Smear layer evaluation

The results of the smear layer scores in each experimental group are shown in Table 1. Analysis of various thirds of the root canal system demonstrated that, in coronal third, groups 1 (MA) and 2 (MA + CTR) showed similar efficacy and was better when compared to group 3 (MA + CTR + CHX) ($p = .023$). There were no statistical differences between the experimental groups in the middle third ($p = .093$) and apical thirds ($p = .546$). On inter-group comparison, there was no significant difference between groups 1 (MA) and 2 (MA + CTR) ($p = .393$). However, there was a statistical difference when group 3 (MA + CTR + CHX) was compared to groups 1 (MA) and 2 (MA + CTR) in which, group 1 (MA) and 2 (MA + CTR) removed smear layer more effectively than group 3 (MA + CTR + CHX) ($p = .023$). In the negative control group (distilled water), all the specimens were heavily-smeared in the coronal, middle and apical thirds of the root canal system. Figure 1 demonstrates the representative scanning electron microscopic images of root canal walls treated with the experimental solutions.

Table 1. Smear scores recorded from different experimental groups at various thirds of the root canal system.

Experimental solutions		Smear scores 1	2	3	4
Group 1	Coronal	9	1	0	0
7% MA	Middle	4	4	2	0
	Apical	0	3	4	3
Group 2	Coronal	9	1	0	0
7% MA + 0.2% CTR	Middle	5	3	2	0
	Apical	3	1	4	2
Group 3	Coronal	3	7	0	0
7% MA + 2% CHX + 0.2% CTR	Middle	1	4	4	1
	Apical	1	1	5	3
Group 4	Coronal	0	0	0	10
Distilled water	Middle	0	0	0	10
	Apical	0	0	0	10

MA: maleic acid; CTR: cetrimide; CHX: chlorhexidine gluconate.

Contact angle evaluation

The mean values of the contact angle made by AH Plus sealer on root canal dentin surface that was treated with various irrigation groups are shown in Figure 2. When the control group (distilled water) was compared with groups 1 (MA), 2 (MA + CTR) and 3 (MA + CTR + CHX), there was a highly significant difference between them in which the control group had lower contact angle ($p < .001$). When groups 1 ($p = .019$) and 2 ($p = .005$) were compared with group 3, there was a significant difference between them in which, group 1 (MA) and group 2 (MA + CTR) had a lower contact angle than group 3(MA + CTR + CHX). However, there was no significant difference between groups 1 and 2 ($p = .961$).

Discussion

The present study compared the efficacy of three different final irrigation regimens with MA in removal of canal wall smear layer and their effect on the wettability of epoxy resin-based sealer on root canal dentin surface. Even though previous have demonstrated that the addition of surfactants (CTR) and antiseptics (CHX) to MA increases its antimicrobial efficacy against *E. faecalis* biofilm [15–17], the results of the present study revealed that, MA alone or MA combined with CTR had better canal wall smear layer removal ability and also reduced the contact angle of AH Plus sealer when compared to MA + CTR + CHX combination. Hence, both the null hypothesis has to be rejected. The poor performance of CHX, when combined with MA and CTR in removal of smear layer and reduction in the contact angle of AH plus sealer may be attributed to the reduced efficacy of CHX to remove the smear layer and decalcify root canal dentin [25,26]. When the smear layer is removed the surface roughness increases due to the opening of the dentinal tubules. Wenzel [27] examined the effect of surface roughness on wetting behaviour and concluded that, the contact angle decreases with the increase of surface roughness. This may be the reason why,

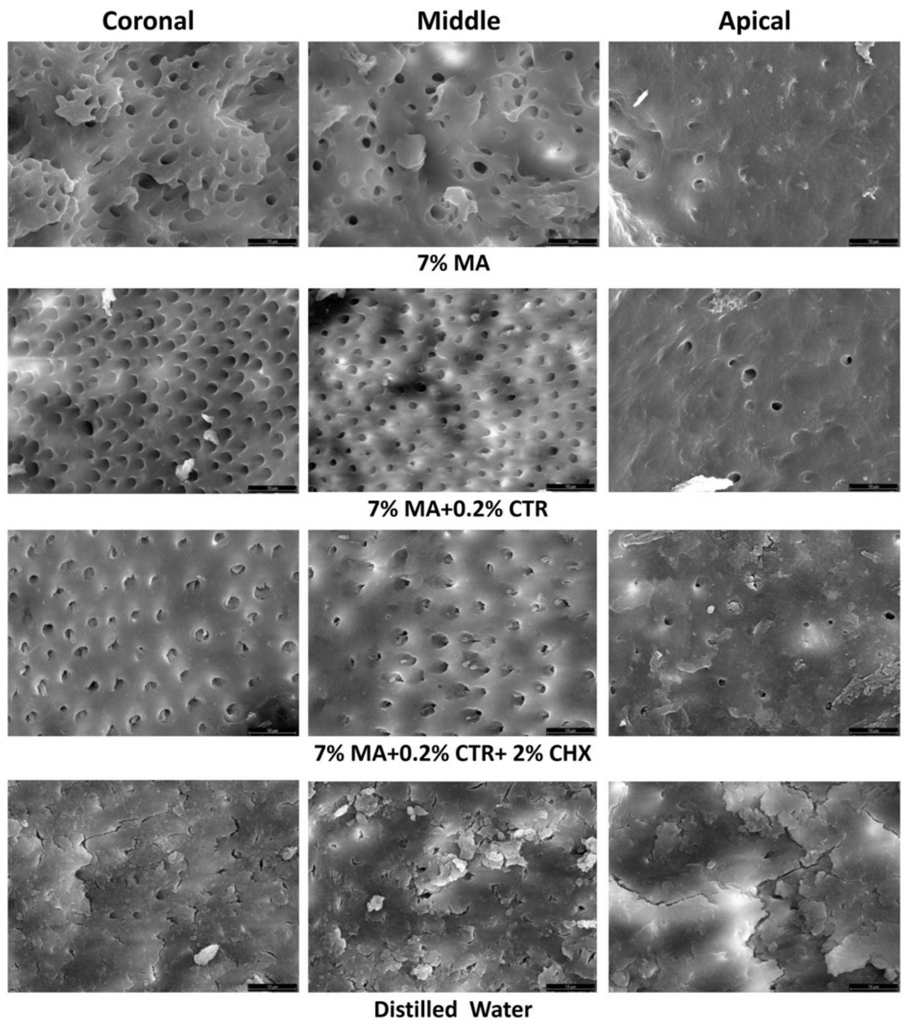

Figure 1. Representative scanning electron microscopic images coronal, middle and apical thirds of root canal walls treated with MA, MA + CTR; and it can be observed that, in the MA + CTR + CHX group, minimal dentinal tubules were open in all the thirds of the root canal system.

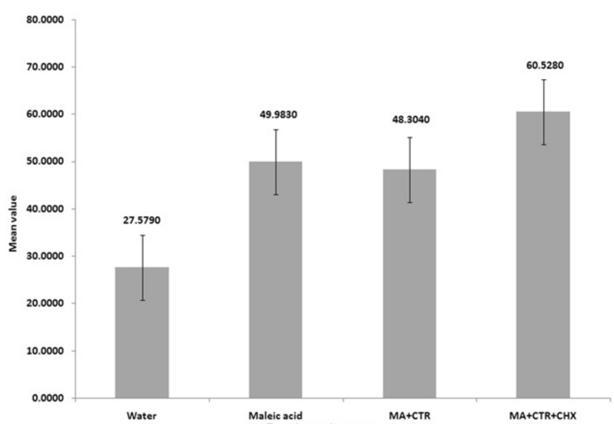

Figure 2. Mean values of the contact angles produced by AH Plus sealer to root canal dentin treated with different irrigating solutions.

MA alone or MA in combination with CTR demonstrated a better smear layer removal and decreased contact angle of AH plus sealer. These results are in accordance with the previous studies which have demonstrated that, irrigation with 7% MA removed canal wall smear layer effectively and increased the surface roughness of root canal dentin [9,28].

The poor wettability of AH plus sealer to root canal dentin treated with MA + CTR + CHX combination may also be because of root canal dentin, which consists of collagen, which has a low surface free energy, and hydroxyapatite, which has a high surface energy [29]. Due to the weak demineralising ability of CHX, the use of MA + CTR + CHX combination solution as the final rinse must have resulted in a thin layer of demineralised collagen fibrils on the root canal dentin surface which is responsible for the poor wettability of AH plus sealer [30].

Surface tension is the result of intermolecular attraction of a liquid in contact with a solid surface. When this intermolecular attraction is weakened, the surface tension decreases. Surface tension may be reduced by using a surfactant [31]. In the present study, the combination of MA with CTR showed better smear layer removal and wettability of AH plus sealer to root canal dentin. This can be attributed to the surfactant effect of CTR which reduced the surface tension of MA and increased the wettability of MA + CTR solution [32].

All static contact angle measurements were performed by using a controlled-volume (0.2 mL) of Ah plus sealer. This was done because any volumetric change could affect the value of contact angle measurement [33].

In conclusion, the present study showed that, MA alone or in combination with CTR as a final irrigation regimen removed canal wall smear layer effectively and increased the wettability of AH plus sealer to root canal dentin. However, further *in vivo* studies needs to be performed to confirm the same.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

[1] Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Sur Oral Med Oral Pathol Oral Radiol Endod. 1965;20:340–349.

[2] Siqueira JF Jr, Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008;34:1291–1301.

[3] Siqueira JF Jr, Roças IN. Optimising single-visit disinfection with supplementary approaches: a quest for predictability. Aust Endod J. 2011;37:92–98.

[4] Violich DR, Chandler NP. The smear layer in endodontics – a review. Int Endod J. 2010;43:2–15.

[5] Czonstkowsky M, Wilson EG, Holstein FA. The smear layer in endodontics. Dent Clin North Am. 1990;34:13–25.

[6] Gutmann JL. Clinical, radiographic, and histologic perspectives on success and failure in endodontics. Dent Clin North Am. 1992;36:379–392.

[7] Siqueira JF Jr. Aetiology of root canal treatment failure: why well-treated teeth can fail. Int Endod J. 2001;34:1–10.

[8] Zehnder M. Root canal irrigants. J Endod. 2006;32:389–398.

[9] Ballal NV, Kandian S, Mala K, et al. Comparison of the efficacy of maleic acid and ethylenediaminetetraacetic acid in smear layer removal from instrumented human root canal: a scanning electron microscopic study. J Endod. 2009;35:1573–1576.

[10] Ulusoy I, Gorgul G. Effects of different irrigation solutions on root dentine microhardness, smear layer removal and erosion. Aust Endod J. 2013;39:66–72.

[11] Ballal NV, Kundabala M, Bhat KS, et al. A comparative in vitro evaluation of cytotoxic effects of EDTA and maleic acid: root canal irrigants. Oral Surg Oral Med Oral Pathol Oral Radiol. 2009;108:633–636.

[12] Neelakantan P, Subbarao C, Subbarao CV, et al. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. Int Endod J. 2011;44:491–498.

[13] Grawehr M, Sener B, Waltimo T, et al. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J. 2003;36:411–415.

[14] Rasimick BJ, Nekich M, Hladek MM, et al. Interaction between chlorhexidine digluconate and EDTA. J Endod. 2008;34:1521–1523.

[15] Ferrer-Luque CM, González-Castillo S, Ruiz-Linares M, et al. Antimicrobial residual effects of irrigation regimens with maleic acid in infected root canals. J Bio Res. 2015;22:2–5.

[16] Baca P, Mendoza-Llamas ML, Arias-Moliz MT, et al. Residual effectiveness of final irrigation regimens on *Enteroccus faecalis* infected root canals. J Endod. 2011;37:1121–1123.

[17] Ferrer-Luque CM, Conde-Ortiz A, Arias-Moliz MT, et al. Residual activity of chelating agents and their combinations with citrimide on root canals infected with *Enterococcus faecalis*. J Endod. 2012;38:826–828.

[18] Pringle JH, Fletcher M. Influence of substratum wettability on attachment of freshwater bacteria to solid surfaces. Appl Environ Microbiol. 1983;45:811–817.

[19] Baier RE. Principles of adhesion. Oper Dent. 1992;(Suppl 5):1–9.

[20] Milosevic A. The influence of surface finish and in-vitro pellicle on contact-angle measurement and surface morphology of three commercially available composite restoratives. J Oral Rehabil. 1992;19:85–97.

[21] Erickson RL. Surface interactions of dentin adhesive materials. Oper Dent. 1992;5:81–94.

[22] Eick JD, Gwinnett AJ, Pashley DH, et al. Current concepts on adhesion to dentin. Crit Rev Oral Biol Med. 1997;8:306–335.

[23] Nidambur Vasudev B, Adlyn T, Khechen K, Narayan Prabhu K, et al. Wettability of root canal sealers on intraradicular dentine treated with different irrigating solutions. J Dent. 2013;41:556–560.

[24] Dai L, Khechen K, Khan S, et al. The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris. J Endod. 2011;37:80–84.

[25] Valera MC, Chung A, Menezes MM, et al. Scanning electron microscope evaluation of chlorhexidine gel and liquid associated with sodium hypochlorite cleaning on the root canal walls. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;110:e82–e87.

[26] Ferrer-Luque CM, Perez-Heredia M, Baca P, et al. Decalcifying effects of antimicrobial irrigating solutions on root canal dentin. Med Oral Patol Oral Cir Bucal. 2013;18:e158–e161.

[27] Wenzel RN. Resistance of solid surfaces to wetting by water. Ind Eng Chem. 1936;28:988–994.

[28] Ballal NV, Mala K, Bhat KS. Evaluation of the effect of maleic acid and ethylenediaminetetraacetic acid on the microhardness and surface roughness of human root canal dentin. J Endod. 2010;36:1385–1388.

[29] Akinmade AO, Nicholson JW. Glass-ionomer cements as adhesives. Part I. Fundamental aspects and their clinical relevance. J Mater Sci: Mater Med. 1993;4:95–101.

[30] Tay FR, Gutmann JL, Pashley DH. Microporous, demineralized collagen matrices in intact radicular dentin created by commonly used calcium-depleting endodontic irrigants. J Endod. 2007;33:1086–1090.

[31] Cameron JA. The effects of a fluorocarbon surfactant on the surface tension of the endodontic irrigant, sodium hypochlorite. A preliminary report. Aust Dent J. 1986;31:364–368.

[32] Giardino L, Ambu E, Becce C, et al. Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic. J Endod. 2006;32:1091–1093.

[33] Good RJ, Koo MN. The effect of drop size on contact angle. J Colloid Interface Sci. 1979;71:283–292.