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Original Article

Impact of Irrigation Solutions on the Apical Sealing Potential of Different Endodontic Sealers Used with the Continuous-wave Obturation Technique: An *In vitro* Study

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Abstract

Aim: This study is aimed to evaluate the impact of three irrigating solutions on the apical sealing ability of three endodontic sealers. **Materials and Methods:** One hundred maxillary central incisors were decoronated, and their roots were assigned into three experimental groups ($n = 30$ each) according to the nature of the irrigating solution and two control groups ($n = 5$ each). Each experimental group was divided equally into three subgroups ($n = 10$ each) according to the type of root canal sealer. All root canals were instrumented using ProTaper Universal rotary system and irrigated with 3% sodium hypochlorite (NaOCl/Group 1), 3% hydrogen peroxide (H_2O_2/Group 2), and 2% chlorhexidine gluconate (CHX/Group 3). The final irrigating solutions for all groups were 17% ethylenediaminetetraacetic acid (EDTA) and distilled water. The continuous-wave technique was used to fill the prepared canals with the following materials: MTA-Fillapex/Gutta-percha (Subgroup A), AH Plus/Gutta-percha (Subgroup B), and Realseal SE/Realseal (Subgroup C). The microleakage was assessed after 72 hours using the linear dye penetration method. The results were statistically analyzed with one-way analysis of variance. **Results:** Significant differences were found only between the experimental groups ($P < 0.05$). All root canal sealers after irrigation with 3% H_2O_2 showed the lowest amount of leakage. The highest apical leakage was observed when 3% NaOCl and 2% CHX were used. **Conclusions:** The type of irrigating solution could affect the apical sealing ability of experimental sealers used with the continuous-wave obturation technique. Root canal irrigation with 3% H_2O_2 +17% EDTA increased the apical sealing ability of all root canal sealers. Root canal irrigation with 3% NaOCl or 2% CHX +17% EDTA decreased the apical sealing ability of all root canal sealers.

Keywords: AH plus, continuous-wave obturation technique, microleakage, MTA Fillapex. Root canal irrigation, Realseal SE

Introduction

An effective root canal treatment requires appropriate cleaning, shaping, and obturation of the root canal system.[1] Root canal instrumentation is not adequate to get ride-off all microorganisms from the infected root canal system due to the enormous variations in its anatomical structure. Consequently, different types of chemical irrigants, such as sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX), and hydrogen peroxide (H_2O_2), have been proposed by different authors to reduce the bacterial load in the infected root canals.[2-5] However, these irrigating solutions were not able to remove the smear layer that is formed after the root canal instrumentation.[5] Therefore, 17% ethylenediaminetetraacetic acid (EDTA) was recommended to be used as an adjunctive solution to remove this layer and improve the quality of adhesion and sealing capacity of root canal sealers.[5]

The use of irrigating solutions may alter the chemical and physical properties of the radicular dentin, and in turn, they may reduce the adhesion or bonding capability of the

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obturation materials with adjacent dentin.[6-8] Some irrigating solutions such as NaOCl and H_2O_2 can cause significant changes in the collagen structure by dehydration and removal of fibrils required for the formation of the hybrid layer.[6] Therefore, the monomer penetration into the radicular dentin may be insufficient and consequently, the bond strength of the bondable root canal sealers becomes weak.[7] On the contrary, it has also been shown that the use of CHX could improve the integrity of the hybrid layer and resin-dentin bond stability.[8]

The root canal filling must seal the root canal space to prevent apical or coronal microleakage, and entomb irritants within the root canals.[9] Gutta-percha is currently the most common material to fill root canals when used with appropriate endodontic sealers.[10] The use of an endodontic sealer is essential to obtain a fluid-tight seal between the radicular dentinal wall and the core filling material.[11]

Epoxy resin-based sealers such as AH Plus (Dentsply Maillefer, Ballaigues, Switzerland) are widely used because of its good physical and chemical properties and excellent sealing ability.[12] MTA-Fillapex is a relatively new paste-paste MTA-based root canal sealer developed by Angelus (Londrina, Parana/Brazil) and can promote cementum regeneration and provide perfect sealing ability, according to the manufacturer.[13] Reaseal SE (SybronEndo, CA, USA) is a dual-cured self-etching methacrylate-based root canal sealer. It can bond with the Resilon and radicular dentin to form a Monoblock unit, which may inhibit leakage and increase the root strength.[14]

Warm vertical compaction is a common obturation technique that promotes the plasticization of the core filling material and improves its adaptation to the root canal walls.[15] To obtain adequate vertical compaction of gutta-percha or Resilon, Buchanan introduced the continuous-wave vertical condensation technique.[16]

Microleakage was considered the primary cause of endodontic failure.[1] In this manner, leakage tests are basic in assessing the excellence of endodontic treatment. The passive dye penetration method is the most widely used because of its sensitivity, ease of use, and convenience.[17]

The present study compared the effect of three irrigating solutions (3% NaOCl, 3% H_2O_2 and 2% CHX) on the apical seal of three endodontic sealers (MTA-Fillapex, AH Plus, and Realseal SE) when the continuous-wave filling technique was used. The null hypothesis of the current study was that the type of irrigation solution would not affect the apical sealing ability of endodontic sealers.

Materials and Methods

The present study was done after taking ethical approval (No. RD-15-02) from the institutional Research Ethics Committee.

One hundred extracted human maxillary central incisors with sound roots were collected from the dental clinics of the same institute after extensive macroscopic and microscopic examination. The teeth were randomly divided into three study groups [Figure 1] of 30 samples each, according to the nature of the irrigating solution, and two control groups of 5 teeth each. Each study group was divided into three subgroups of 10 samples, each according to the type of root canal sealer.

The crowns of selected teeth were cut so that the length of roots was standardized at 14 mm. The root canals were accessed, and the pulp tissues were removed using a small size barbed broach. The size of the initial file for selected samples should equal to or less than #25 K-file. The working length was decided by inserting a #15 K-file (Dentsply, Maillefer, Ballaigues, Switzerland) into the root canal until it was noticeable at the apical foramen. Then, 1 mm was deducted from that length. The accuracy of the working length was confirmed radiographically. The canals were prepared using ProTaper Universal NiTi rotary files (Dentsply Maillefer, Ballaigues, Switzerland) until reaching to size F4. EDTA gel (TG Chelcream, UK) was used as a lubricant during the root canal preparation procedures.

After using each file, root canals were irrigated with 5 ml of the following experimental irrigating solutions: 3% NaOCl (Group 1), 2% Chlorohexidine (Group 2), and 3% H_2O_2 (Group 3). All prepared canals were finally flushed with 5 ml of 17% EDTA solution (MD-Cleanser, META BIOMED Co. Ltd., Korea) for 1 min, followed by 5 ml distilled water. The irrigation was performed using a 27-gauge Max-i-Probe needle (Dentsply Maillefer, Ballaigues, Switzerland), which was inserted as deep as possible into the canal without binding. Apical patency was confirmed by introducing a size 10 k-file through the apical foramen before and after the root canal preparation. All prepared samples were kept moist in distilled water until the time for root canal obturation.

A size #10 finger spreader (Mani Inc., Tocigi-Ken, JA) was inserted from the external root surface through the apical foramen into the prepared root canal of each sample in the experimental and positive control groups. Except for the coronal surface and apical foramen, all external surfaces of roots were coated with three layers of nail varnish. Each layer was left to dry before applying the next layer of varnish. After

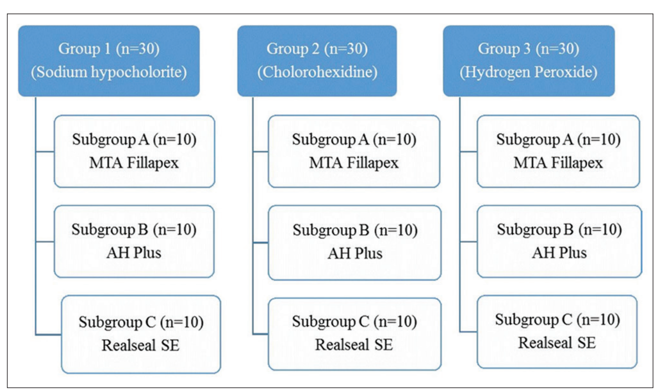

Figure 1: Schematic diagram showing the different experimental groups and subgroups

confirming the setting of varnish, the spreader was lightly removed; thus, the apical foramina were kept open. Regarding samples of the negative control group, their root surfaces were coated with nail varnish as in the experimental groups but including the apical foramen.

Continuous-wave warm vertical compaction obturation technique was used to fill the root canals with the following combination: MTA Fillapex/ProTaper Gutta-percha size F4 (Subgroup A), AH Plus/ProTaper gutta-percha size F4 (Subgroup B), and Realseal SE/ReaSeal points size 40/0.06 (Subgroup C). The master cone was accepted if a tug back sensation was felt if the cone did not show apical tug-back, it was trimmed apically with a straight scissor. Samples in the negative control group were kept empty, while in the positive control group, samples were filled with gutta-percha only using the same obturation technique but without using root canal sealer.

Elements Free Obturation system (Kerr Corporation 1717 West Collins Orange, CA, USA) was used for down filling of gutta-percha and Realseal and backfilling of gutta-percha. Obtura III device (Obtura Spartan, 175 Earth City, Missouri, US) was utilized for the backfilling of softened Realseal pellets. The canals were dried using F4 paper points (Dentsply Maillefer, Ballaigues, Switzerland). All sealers were manipulated following the directions of the manufacturer and then placed into root canals with the aid of a rotary Lentulo spiral size 30. The tip of the prefitted master cone was coated with a thin layer of experimental sealer and introduced into the canal until reaching the full working length. Elements Free Down-pack unit was programmed at 200°C for gutta-percha subgroups (Subgroups A and B), and at 150°C for Reaseal subgroup (Subgroup C).

A Buchanan heat plugger (Fine-medium size) that fixed to Elements Free Downpack unit was inserted along the master cone to 5 mm short of the working length. Heat application was then discontinued, and light apical pressure was maintained for 10 s, to overcome the shrinkage of the gutta-percha or Resilon, then the plugger was taken out after application of a short heat burst. Elements free Backfill (200°C) was used for the backfilling of gutta-percha, while Obtura III device (150°C) was used for the backfilling of Realseal Pellets using a 23G needle tip. The coronal surface of the roots obturated with Realseal was light-cured for 40 s.

The quality of obturation was evaluated radiographically, and samples with inadequate obturation were replaced with new ones. All canal orifices were sealed with light-cured glass ionomer cement and then coated with two layers of nail varnish. All samples were incubated for 1 week at 37°C with 100% humidity to allow the complete setting of sealers.

Samples were immersed in 2% methylene blue dye and stored at 37°C for 72 hours; then, they were cleaned under running tap water. The nail varnish was scraped with a scalpel blade no. 11. The apical 7 mm of each root was longitudinally cut, followed by a horizontal cut on one of the proximal surfaces using a flexible diamond disc under a water coolant. The proximal portion of each sample was then separated, and the filling material was removed using an endodontic explorer to allow a better evaluation of dye penetration.

Microleakage in each sample was evaluated under a digital stereomicroscope (Leica EZ4W, Germany) at ×12.5. Photos were captured for the apical portion of the root, and the maximum apical dye penetration was measured in millimeters using Image Tool software (ImageJ software; National Institutes of Health, MD, USA) [Figure 2]. To remove the operator variable, a single operator accomplished all the experimental procedures.

The statistical analysis was performed using IBM SPSS version 20 (IBM Corporation 1 New Orchard Road Armonk, New York, United States). Normal distribution of data and its homogeneity were assessed by Kolmogorov–Smirnov and Levene's tests, respectively. One-way analysis of variance (ANOVA) test was selected to generally compare the mean values of dye penetration in all groups and subgroups. If there were significant differences between groups and subgroups, Tukey's or Games-Howell's Post-hock test, depending on the results of the homogeneity test, was used to perform pairwise comparisons of dye penetration mean values among groups and subgroups. Moreover, two-way ANOVA test was performed to study the effect of irrigation solutions, root canal sealers, and their interactions on the apical microleakage of obturated samples. All the levels of statistical significance were established at a $P < 0.05$.

Results

The mean measurements and standard deviations of the apical dye leakage in all groups are given in Table 1 and demonstrated in Figure 3. All samples displayed variable degrees of apical dye leakage [Figure 4]. The positive control group showed the maximum amount of dye penetration, while the negative control group showed no dye penetration.

One-way ANOVA test showed significant differences ($P < 0.05$) between irrigating solutions (Groups) regarding their effect

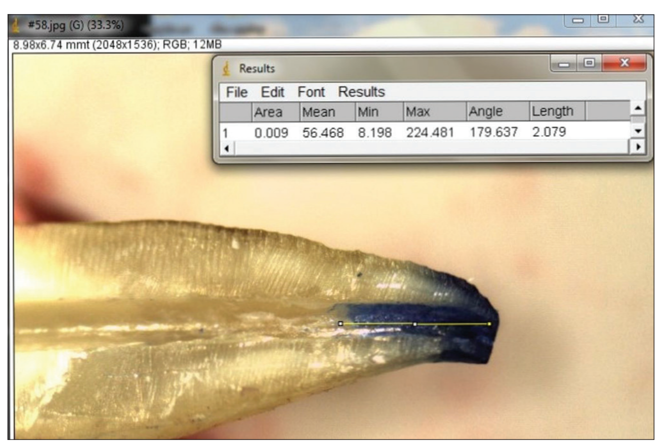

Figure 2: Measurement of linear dye leakage using ImageJ software

Table 1: Mean values of apical dye leakage (mm) in root canals filled with different obturation materials after using various irrigating solutions

Groups (irrigation solutions)	Apical linear dye penetration (mean±SDs in mm)			
	Subgroups (root canal sealers)			
	Subgroup A (MTA-Fillapex/GP)	Subgroup B (AH Plus/GP)	Subgroup C (Realseal SE/Realseal)	ANOVA (*P*)
Group 1 (Sodium hypochlorite)	2.43±0.0.84^{A*}	2.30±0.26^{A}	2.11±0.25^{A}	0.414
Group 2 (Chlorohexidine)	2.66±0.91^{A}	2.44±0.52^{A}	2.66±0.45^{A}	0.701
Group 3 (Hydrogen peroxide)	1.40±0.22^{B}	1.44±0.83^{B}	1.30±0.82^{B}	0.898
ANOVA (*P*)	0.001 (G^mes-Howell test)	0.012 (G^mes-Howell test)	0.000 (Tukey test)	

*Means with the different superscript letters in each column (Groups) or raw (Subgroups) had a significant difference ($P<0.05$). GP – Gutta-percha, SDs – Standard deviation, ANOVA – Analysis of variance.

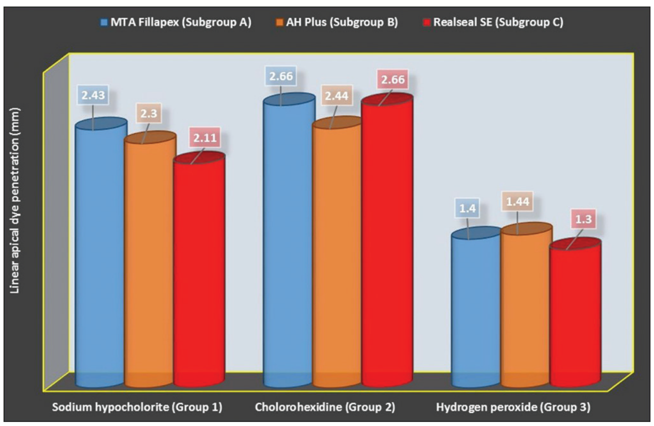

Figure 3: Effect of irrigating solutions on sealing ability of different root canal sealers when used with continuous-wave obturation technique

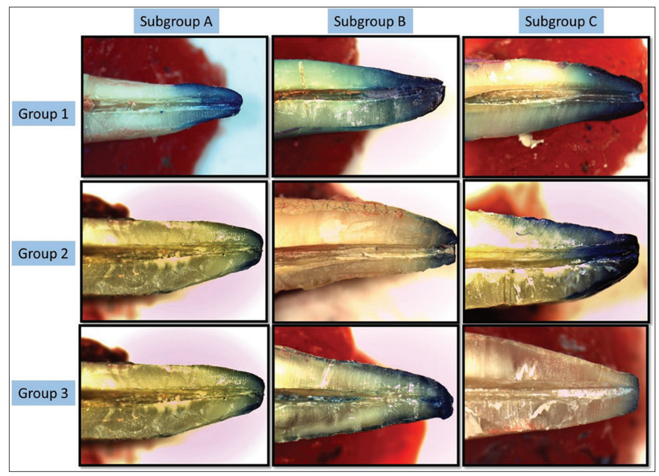

Figure 4: Apical dye leakage of selected samples filled with different obturating materials after using different irrigating solutions for all groups and subgroups (*Group 1 [Sodium hypochlorite]; Group 2 [Chlorohexidine]; Group 3 [Hydrogen peroxide]; Subgroup A [Gutta-percha/MTA Fillapex]; Subgroup B [Gutta-percha/AH Plus]; Subgroup C [Realseal/Realseal SE])

on the sealing ability of root canal sealers [Table 1]. Samples irrigated with 3% H_2O_2 +17% EDTA (Group 3) showed the lowest significant amount of leakage; while samples irrigated with 3% NaOCl or CHX +17% EDTA (Groups 1 and 2) showed the highest amount of leakage for all experimental sealers

When the microleakage mean values of root canal sealers within each group were compared, no significant differences were observed among them ($P > 0.05$). Two-way ANOVA test [Table 2] corroborated the previous results and signified that the apical leakage of all obturation materials was mainly affected by the type of irrigating solution.

Discussion

The development and maintenance of three-dimensional sealing of the root canal system is the key to successful root canal treatment. Different obturation materials and techniques were introduced and evaluated, hoping to obtain an airtight apical seal. The development of new root canal filling materials may overcome the problem of microleakage associated with the currently used materials.

The sealing ability of any obturation technique depends on the quality of the root canal sealer.[7] In the current study, three types of well-known root canal sealers were used: MTA-Fillapex, AH Plus, and Realseal SE. AH Plus is considered the gold standard to which other types of root canal sealers are compared. It has excellent physical and chemical properties as well as good sealing ability.[12] The Realseal SE is a self-etching methacrylate-based sealer that was claimed to create a Monoblock unit with radicular dentin when used with Resilon core material. It has the ability to prevent leakage and improve the root strength.[14] MTA-Fillapex is a new MTA-based sealer that has excellent biocompatibility. Moreover, it has good bioactivity, osteoconductivity, and sealing ability.[13] Root canal irrigants may alter the physical and chemical properties of radicular dentin. These changes could affect the adhesion quality of the root canal filling materials to the affected radicular dentin.[6-8] Therefore, the current study is aimed to compare the effect of 3% NaOCl, 2% CHX, and 3% H_2O_2, as irrigating solutions, on the apical sealing ability of AH Plus, MTA-Fillapex, and ReaSeal SE when used with the continuous-wave obturation technique. The using of extracted human teeth in the current study displayed variabilities, which closely mimic the clinical circumstances. Extracted maxillary central incisors with nearly similar apical diameters and root lengths were selected and instrumented to # F4 ProTaper

Table 2: Statistical results of univariate analysis of variance (two-way analysis of variance) that determine the effect of irrigating solutions and root canal sealers on the apical leakage of experimental samples

Source	Sum of Squares	df	Mean square	*F*	*P*
Type of irrigating solution (X1)	23.70	2	11.85	29.95	0.000
Type of root canal sealers (X2)	0.319	2	0.16	0.403	0.669
Irrigation type × Sealers	0.610	4	0.153	0.39	0.818
Error	32.05	81	0.396		
Total	447.63	90			

Universal nickel-titanium rotary instrument. Consequently, variables such as anatomical variation, canal size, and the width of the apical foramen, which can affect the apical leakage, were reduced.

During instrumentation, samples in each group were irrigated with one type of tested irrigation solution followed by 17% EDTA to remove smear layer.[5] Then, all samples were finally flushed with distilled water to remove any traces of previous irrigation solutions. This protocol permitted assessing the impact of one irrigant type on the sealing capacity of the experimental root canal sealers.

The dye penetration method was used in the current study to evaluate the apical microleakage because it is an inexpensive, reliable approach and easy to manipulate.[17] Besides, it has a high degree of staining ability and a molecular weight lower than that of bacterial toxins.[18] The endodontic filling materials that prevent the penetration of small molecules such as dyes can avoid leakage of larger particles such as bacteria and their byproducts.[18] A 72-hours immersion period within the dye solution was used in the present study, as the experimental protocol did not include an additional active penetration device such as a vacuum. Some authors reported that the elimination of air during the dye leakage studies might result in overestimated values of dye extensions.[13]

The continuous-wave technique was selected to fill the root canals in the present study. It can reduce the voids formation, improve the adaptation of root filling material with the radicular dentin, and allow filling of inaccessible areas.[15] The experimental design in the present study was assessed using positive and negative control groups. The positive control group indicated that leakage testing was a suitable method for maximum dye penetration without a sealer. The absence of dye penetration for samples in the negative control group stated the effectiveness of using three layers of varnish to prevent dye penetration through root surfaces and apical foramen.

The null hypothesis of this study was not approved. The results showed that the apical sealing ability of the tested sealers used with the continuous-wave obturation technique was significantly affected by the irrigating solutions.

The results of the present study are coordinating with the outcomes of past studies despite diverse obturation procedures and leakage evaluation strategies.[19-21] These studies showed no significant differences between Epiphany/Resilon and AH plus/gutta-percha in terms of the apical sealing ability when the root canals were irrigated with NaOCl.[19-21] Moreover, no significant differences were observed between the tested sealers used in the current study after using each irrigating solution. However, some authors showed that MTA-Fillapex resulted in more microleakage than AH Plus.[9,17]

In general, high leakage values were observed for samples irrigated with NaOCl and CHX solutions, while low leakage values were observed for samples irrigated with H_2O_2. Sodium hypochlorite has been appeared to harm the organic constituents of dentin, basically through collagen disintegration, and can prevent the formation of a uniform hybrid layer.[7] Furthermore, NaOCl breaks down into sodium chloride and oxygen, which can interfere with resin sealer polymerization and reduce the bond strength with radicular dentin.[7]

Some authors found that the use of CHX could improve the integrity of the hybrid layer and resin-dentin bond stability.[8] However, the results of the present study displayed that the apical sealing ability of AH plus, Epiphany SE diminished when the chlorhexidine was used as an irrigation solution, and these results are matching with the outcomes of Bodrumlu *et al*.[22] The cause of this may be attributed to the inability of CHX to dissolve organic tissues from the root canal system. Moreover, the chemical bonding of CHX with the dentin may prevent the adhesion of the endodontic sealer to the root canal walls.[23] Nikhil *et al.*[24] compared the depth of penetration of MTA-Fillapex and AH Plus sealers into the dentinal tubules with the use of a confocal laser microscope. They reported that MTA-Fillapex sealer penetrated more in-depth into the dentinal tubules. However, studies by Sönmez *et al*.[13] and Ferreira *et al*.[20] showed that MTA-Fillapex resulted in more microleakage than AH Plus when irrigating the canals with NaOCl and EDTA. These results are contrary to the current findings which showed that both materials had similar high leakage values. The temperature associated with the continuous-wave technique may increase the amount of apical leakage by accelerating the setting time of experimental sealers which my adversely affect their flow property.[25] Moreover, the heat generation during warm vertical compaction may prevent the relief of polymerization stresses of resin-based sealers and make them partially polymerized before finishing the obturation procedure.[26]

The utilizing of H_2O_2 as an irrigating solution can remove remnants of pulp tissue and dentin debris. However, its use could affect the bond strength of resin cement negatively to root canal dentin.[27] Hydrogen peroxide can break down to water and oxygen, especially when used with NaOCl. The released oxygen causes potent inhibition of the interfacial polymerization of resin-based bonding materials,[27] which may increase the setting time of the resin-based sealers and counteract the side effect of rising temperature during the continuous-wave obturation technique. Furthermore, raising the setting time could allow more sealer penetration into dentinal tubules and hence improving the sealing ability of resin-based sealer. Bojar *et al*.[28] showed that the surface treatment of bovine dentin with oxygenated materials such as ozone improved dentin shear-bond strength of two root canal sealers (AH-26 and EX Fill). Therefore, a similar effect may be expected when 3% of H_2O_2 irrigation is used.

Realseal manufacturer does not recommend the use of NaOCl and H_2O_2 to avoid damage the dentine wall adhesion. However, EDTA or CHX was recommended as a final irrigant. The final use of EDTA eliminates the smear layer that permits the collagen fibers to be exposed, hence improving the hydrophilicity of the dentine surface and supports the hybridization of the hydrophilic materials such as methacrylate-based sealers.[29] Therefore, the leakage can be minimized by increasing the micromechanical bond between the adhesive agents and the collagen matrix of dentin.[26] The results of the current study do not match the results of Vilanova *et al*.[30] who reported that a greater adhesive strength was obtained between Epiphany and the root canal walls when the root canals were irrigated with 1% NaOCl, followed by 17% EDTA. The reasons for this discrepancy may be due to the longer time of application for NaOCl (30 min) and EDTA (5 min). However, the results of Ozturk and Özer[7] may support the current results. Those authors[7] showed that the use of NaOCl reduced significantly the bond strength of dentin bonding agents significant decrease in bond strength of dentin bonding with the dentine walls.

Further laboratory and clinical studies must be conducted to evaluate the effect of 3% H_2O_2 as a single irrigating solution or in combination with other solutions on the sealing ability of currently available root canal sealers to confirm the current results.

Conclusions

Within the limitations of the present study, the subsequent conclusions can be drawn:

1. The type of irrigating solution affected significantly the apical sealing ability of experimental sealers when used with the continuous wave obturation technique.
2. The apical sealing ability of root canal sealers increased when the root canals were irrigated with 3% H_2O_2 +17% EDTA solution.
3. When the root canals were irrigated with 3% NaOCl or 2% chlorohexidine followed by 17% EDTA, the apical sealing ability of tested root canal sealers decreased.
4. The apical sealing ability of root canal sealers was statistically similar after using each irrigant solution.

Clinically significant

The apical sealing ability of different root canal sealers is an essential parameter for the success of any obturation technique. The irrigation materials could alter the physical and chemical properties of radicular dentin and hence could the sealing ability of root canal sealers. The selection of an appropriate irrigation solution that improves the sealing ability of root canal sealers is an important clinical feature. Continuous-wave obturation technique is a popular, effective technique. The effect of three common irrigation materials (3% NaOCl, 3% H_2O_2, and 2% CHX) on the apical seal of different endodontic sealers (AH Plus, MTA-Fillapex, and Realseal SE) was evaluated in the current study. Only one irrigation material was evaluated each time. None of the experimental root canal sealers could prevent apical leakage after using each irrigating solution. However, the use of H_2O_2 showed the least amount of leakage for all sealers. The sealing ability of root canal sealers decreased when NaOCl and CHX were used. One primary recommendation is to use H_2O_2 as a part of irrigation protocol.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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