

Effect of Two Irrigation Protocols and Obturation Techniques on the Apical Sealing Ability of Different Root Canal Sealers: An *In vitro* Study

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Abstract

Aim: The aim of this study was to compare the influence of two irrigation protocols and obturation techniques on the apical seal of three endodontic sealers. **Materials and Methods:** Crowns of 130 single-rooted teeth were cut and 10 roots were selected as controls, and the remaining roots were divided into four experimental groups ($n = 30$) according to the type of irrigation protocol and obturation technique. Each group was divided into three subgroups ($n = 10$) according to the type of root canal sealer. All root canals were prepared with size F4 Protaper Universal and irrigated with 3% sodium hypochlorite (NaOCl) + 3% hydrogen peroxide (H_2O_2) (Groups 1 and 3) or 3% NaOCl + 2% chlorhexidine (CHX) (Groups 2 and 4). Two gutta-percha obturation techniques were used: single-cone (Groups 1 and 2) and continuous wave (Groups 3 and 4) techniques. The following sealers were tested: MTA Fillapex (Subgroup A), AH Plus (Subgroup B), and Realseal SE (Subgroup C). The apical seal was assessed using dye penetration method, and the data were statistically analyzed. **Results:** In the first group, AH Plus showed the best apical seal (0.46 ± 0.098 mm), while RealSeal SE system showed the worst apical seal (3.47 ± 0.434 mm). In the second group, MTA Fillapex (0.58 ± 0.115 mm) and AH Plus (0.63 ± 0.109 mm) showed the best apical seal. In the third and fourth groups, all types of sealers showed similar high leakage patterns. Marked increase in the apical seal of RealSeal was observed in the third group (1.71 ± 0.453 mm). **Conclusions:** The type of irrigation protocol and obturation technique could affect the apical seal of tested sealers. When the single-cone technique was used, AH Plus with NaOCl + H_2O_2 and MTA Fillapex with NaOCl + CHX irrigation protocols showed significantly a high apical seal. RealSeal SE showed the worst apical seal when canals were irrigated with NaOCl/ H_2O_2 and filled with the single-cone technique. All sealers showed inferior results when used with the continuous wave technique.

Keywords: Apical microleakage, obturation techniques, root canal irrigation, root canal sealers

INTRODUCTION

The aim of a successful root canal treatment is to clean and shape the root canal system to receive a three-dimensional impermeable filling.^[1] Gutta-percha is the most common core material, which can be used with different types of root canal sealers and obturation techniques to fill the root canal system.^[2]

Warm vertical compaction is a commonly used obturation technique developed by Schilder in 1976. It could improve the adaptation of the obturation materials to the root canal walls and filling of lateral and accessory canals.^[3] However, this technique requires a longer time and is difficult to apply. In an attempt to simplify the warm vertical condensation

of gutta-percha, Buchanan suggested the continuous wave condensation technique using the System B heating device.^[4]

With the widespread use of rotary NiTi instruments, and the introduction of matched-taper gutta-percha or Resilon cones, the noncompaction matched taper single-cone obturation

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technique has become popular due to its simplicity and efficacy in obturating the root canals in a very short time.^[5]

The quality of any obturation technique depends mostly on the type of root canal sealer that is compatible with a certain core material.^[6] AH Plus (Dentsply Maillefer, Ballaigues, Switzerland) is the most frequently used epoxy resin-based sealer because it has appropriate physical properties, good tissue biocompatibility, and good sealing ability.^[7] RealSeal SE (SybronEndo, Orange, CA, USA) is a self-etching methacrylate sealer, which was claimed to produce monoblock unit with radicular dentin when used with Resilon and thus preventing leakage and improving the root strength.^[8] MTA Fillapex is a relatively new endodontic sealer developed by Angelus (Angelus Londrina/Parana/Brazil) that consists of MTA, salicylate resin, natural resin, bismuth oxide, and silica. This material has many advantages, such as excellent biocompatibility, bioactivity, osteoconductivity. In addition, it has appropriate physiochemical properties such as good radiopacity, flow, and alkaline pH.^[9]

Different endodontic irrigating solutions such as sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX), hydrogen peroxide (H₂O₂) and ethylenediaminetetraacetic acid (EDTA) have been recommended during root canal instrumentation to enhance cleaning and disinfection of the root canal system. However, these solutions may cause chemical and structural changes of radicular dentin that could affect the adhesion or adaptability of obturation materials to the dentin surface.^[10] Some irrigating solutions are potent oxidizing agents such as NaOCl and H₂O₂, which can inhibit resin polymerization of methacrylate-based sealers, and subsequently, decrease their bond strength to radicular dentin, increase microleakage.^[11]

Most of the endodontic failures are caused by the microleakage resulting from incomplete obturation.^[12] Therefore, leakage tests are essential in evaluating the excellence of the endodontic treatment. Several methods have been used to assess the apical sealing property of root canal filling materials.^[13] Passive dye penetration method is the most widely used because of its sensitivity, ease of use and convenience.^[13]

The purpose of this study was using the apical dye leakage technique to compare the influence of two irrigation protocols and obturation techniques (single-cone versus warm vertical obturation technique) on the apical sealing ability of three root canal sealers: MTA Fillapex, AH Plus, RealSeal SE.

The null hypothesis tested was that there were no significant differences in the maximum dye penetration among experimental root canal sealers when different irrigation protocols and obturation techniques were used.

MATERIALS AND METHODS

Samples grouping and root canal preparation

One hundred and thirty maxillary central incisors were divided randomly into four experimental groups (*n* = 30) according to the type of irrigation protocol and obturation technique,

and two control groups of five samples each [Figure 1]. Each experimental group was divided into three subgroups of ten samples each according to the type of root canal sealer [Figure 1].

The crowns were sectioned, and the length of roots was standardized at 14 ± 1 mm. The size of the initial file for each sample was 0.25 mm or less. After determination the working lengths, root canals were prepared with ProTaper Universal rotary system (Dentsply Maillefer, Ballaigues, Switzerland), until reaching to a master apical file of size F4. Between files, root canals were irrigated with 5 ml of the following irrigating protocols: 3% NaOCl + 3% H₂O₂ (Groups 1 and 3), or 3% NaOCl + 2% CHX (Groups 2 and 4). Finally, all canals were irrigated with 5 ml of 17% EDTA solution (MD-Cleanser, META BIOMED Co. Ltd, Korea) for 1 min, followed by 5 ml distilled water as a final irrigation. The irrigation was done using a 27G Max-i-Probe needle (Dentsply Maillefer, Ballaigues, Switzerland).

Root canal obturation

Before obturation, all external surfaces of each root in experimental and positive control groups were coated with three layers of nail varnish except on the coronal surface and apical foramen. In the negative control group, all root surfaces including the apical foramen were coated with nail varnish.

Samples in the negative control group did not receive root canal fillings, while in the positive control group; they were obturated with single gutta-percha cone size F4 but without sealer. Three types of root canal sealers in combination with core materials were used: MTA Fillapex/ProTaper gutta-percha size F4 (Subgroup A), AH Plus/ProTaper gutta-percha size F4 (Subgroup B) and RealSeal SE/Realseal point size 40/0.06 (Subgroup C). Samples in the first and second groups were filled with single-cone obturation technique, while samples in the third and fourth groups were filled with continuous-wave obturation technique. All canals were dried using F4 paper points (Dentsply Maillefer, Ballaigues, Switzerland). The master gutta-percha and RealSeal points were calibrated at the correct working length and placed into the root canals, followed by radiographic confirmation of their apical adaptation. Each root canal sealer was mixed according

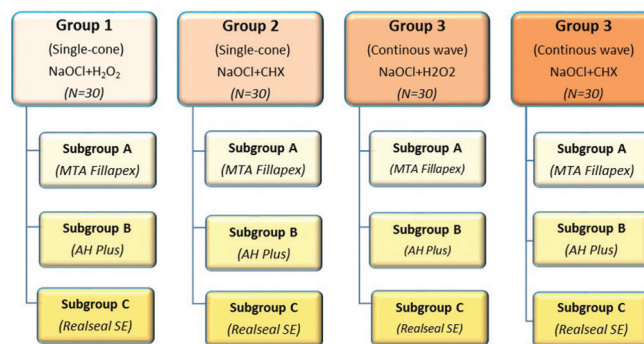


Figure 1: Schematic diagram showing different experimental groups and subgroups

to the manufacturer's (Dentsply Maillefer, Ballaigues, Switzerland) instruction and applied to root canals using a #30 Lentulo spiral (Dentsply Maillefer, Ballaigues, Switzerland) rotated at 300 rpm and 3 mm away from the apex.

Single-cone obturation technique (Groups 1 and 2)

The tip of the prefitted master cone was coated with a thin layer of its respected sealer and inserted into the canal to its full working length. The coronal excess of gutta-percha and Resilon was sheared off using a heated endodontic plugger and the excess sealer was then removed.

Continuous wave obturation technique (Groups 3 and 4)

The continuous wave obturation was performed using Elements free system (Kerr Corporation 1717 West Collins Orange, CA, USA) for down filling of gutta-percha and RealSeal (Resilon) and backfilling of gutta-percha. Obtura II (Obtura Spartan, 175 Earth City, Missouri, US) was used for backfilling of RealSeal. Buchanan heat plugger of fine-medium size was selected so that it easily penetrated into the canals up to 5 mm short of the working length.

Elements free Downpack was set at 200°C for gutta-percha vertical compaction (Subgroups A and B), and at 150°C for RealSeal points (Subgroup C). The heated activated plugger was inserted through the master cone until reaching to the previously established length. Heat activation was then stopped and light apical pressure was retained for 10 s to counteract the shrinkage of the core material. Then, the plugger was withdrawn after application of a short heat burst. Elements free Backfill with Elements gutta-percha cartridge (200°C) was used for backfilling of gutta-percha, while Obtura II device (150°C) was used for backfilling of RealSeal pellet using a 23G needle tip. The softened material was vertically compacted with a preselected Machtou plugger (Dentsply Maillefer, Ballaigues, Switzerland). The coronal surface of each sample filled with Resilon/RealSeal SE was light-cured for 40 s with an light-emitting diode curing light (Lite 696, Dentamerica, USA) to polymerize the surface of the dual-cured methacrylate sealer.

The quality of obturation was assessed radiographically, and samples with insufficient obturation were excluded from the study and replaced with new ones. After finishing obturation, the coronal surface of each root was sealed with light-cured glass ionomer cement and coated with three layers of nail varnish. All samples were incubated for 1 week at 37°C and 95% humidity to allow complete setting of sealers.

Microleakage assessment

The apical portion of each sample was fixed into a plastic tube filled with an aqueous solution of 2% methylene blue dye. All samples were incubated at 37°C for 72 h, after which they were thoroughly rinsed under running tap water and the nail varnish was removed with a scalpel blade no. 11. The apical 7 mm of each root was longitudinally sectioned in a buccolingual direction, and then a horizontal cut was made on the mesial or distal surface using a flexible diamond disc

under water coolant. The apical 7 mm of the proximal portion of each sample was cut to expose the filling material that was then removed using an endodontic explorer to allow better evaluation of dye penetration.

Microleakage in each sample was evaluated under a digital stereomicroscope (Leica EZ4W, Germany) at $\times 20$. Pictures were captured, saved, and the maximum apical dye penetration leakage was linearly measured [Figure 2] using Image J software program (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

The results were statistically analyzed by one-way ANOVA and Tukey's *post hoc* test using IBM SPSS version 20 (IBM Corporation 1 New Orchard Road Armonk, New York 10504-1722, United States). All the level of statistical significance was set at $P < 0.05$.

RESULTS

The mean values and standard deviations of the apical dye leakage are presented in Table 1 and illustrated in Figure 3. The positive-control specimens revealed maximum dye penetration, while the negative control specimens had no dye penetration into the roots. All experimental samples in each group demonstrated different degrees of apical dye leakage [Figure 4].

When experimental groups were compared, significant differences were found in dye penetration ($P < 0.05$). When MTA Fillapex was used, the second group showed the best results (0.58 ± 0.115 mm). When AH Plus/gutta-percha was used, the first group showed the best results (0.46 ± 0.098 mm), and when RealSeal SE was used the third group showed the best results (1.71 ± 0.453 mm) but without significant difference when compared with the second group (2.14 ± 0.302 mm).

When subgroups were compared within each group, significant differences were found only in the first and second groups ($P > 0.05$). In the first group, AH Plus

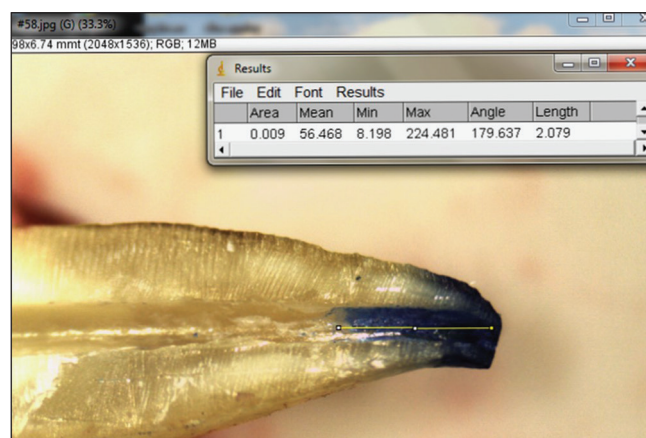


Figure 2: Measurement of linear dye leakage using ImageJ software

Table 1: Statistical comparisons between the experimental groups and subgroups

Groups	Irrigation protocol and obturation technique	Apical linear dye penetration, mean±SD (mm)			ANOVA (P)
		Subgroup A* (MTA/GP)	Subgroup B (AH Plus/GP)	Subgroup C (Realseal SE)	
Group 1 (n=30)	NaOCl+H ₂ O ₂ (single-cone)	1.11±0.368 ^{A1}	0.46±0.098 ^{A2}	3.47±0.434 ^{A3}	0.000
Group 2 (n=30)	NaOCl+CHX (single-cone)	0.58±0.115 ^{B1}	0.63±0.109 ^{B1}	2.14±0.302 ^{BC2}	0.000
Group 3 (n=30)	NaOCl+H ₂ O ₂ (continuous wave)	1.92±0.438 ^{C1}	1.87±0.468 ^{C1}	1.71±0.453 ^{B1}	0.558
Group 4 (n=30)	NaOCl+CHX (continuous wave)	2.55±0.745 ^{C1}	2.37±0.261 ^{D1}	2.39±0.212 ^{C1}	0.663
ANOVA (P)		0.000	0.000	0.000	

*Tukey HSD test – Means with the different superscript letters in each column (groups) or numbers in each row (subgroups) had a significant difference (P<0.05). GP – Gutta-percha, CHX – Chlorhexidine, SD – Standard deviation, HSD – Honestly significant difference, NaOCl – Sodium hypochlorite, H₂O₂ – Hydrogen peroxide, MTA – MTA Fillapex, SE – Self Etch

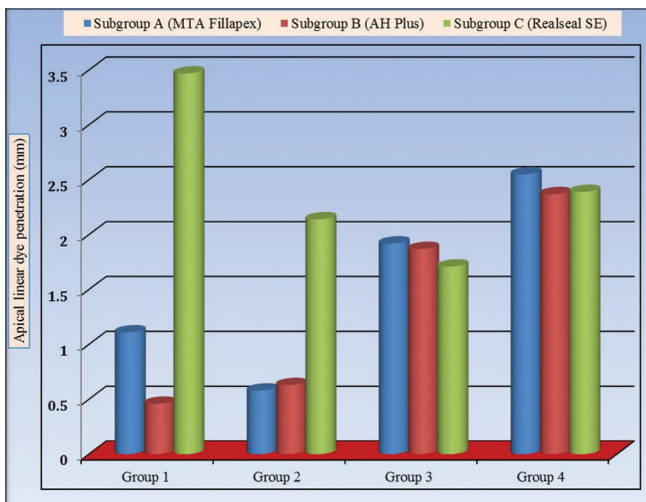


Figure 3: Graphic presentation of apical leakage mean values of experimental groups and subgroups

showed significantly the lowest apical leakage mean value (0.46 ± 0.098 mm), while RealSeal SE showed the highest apical leakage mean value (3.47 ± 0.434 mm). In the second group, MTA Fillapex (0.58 ± 0.115 mm) and AH Plus (0.63 ± 0.109 mm) showed significantly less apical leakage when compared with RealSeal SE (2.14 ± 0.302 mm). In the third and fourth groups, no significant differences were observed between the apical leakage mean values of tested root canal sealers (P < 0.05).

DISCUSSION

Root canal cleaning and shaping play a vital role in the success of endodontic treatment. However, this does not exclude the importance of the obturation quality, which mostly depends on the sealing ability offered by the endodontic sealer.^[6] The aim of the current study was to compare the effect of two irrigation protocols and two obturation techniques on the sealing ability of three root canal sealers. The irrigation protocols used were 3% NaOCl + 3% H₂O₂ (Groups 1 and 3) and 3% NaOCl + 2% CHX (Groups 2 and 4), and the obturation techniques were single-cone (Groups 1 and 2) and continuous wave (Groups 3 and 4). The root canal sealers tested in the current study were MTA Fillapex (Subgroup A), AH Plus (Subgroup B),

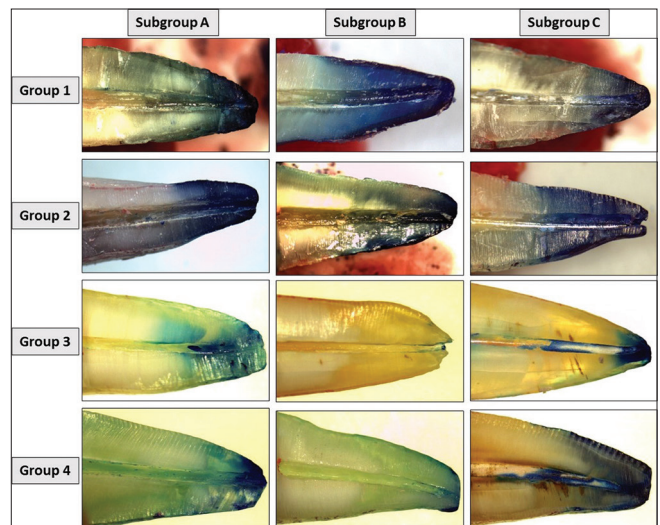


Figure 4: Apical dye leakage of selected samples after using different irrigation protocols and obturation techniques (Group 1-Single-cone + NaOCl/H₂O₂, Group 2:-Single-cone + NaOCl/CHX, Group 3- Continuous-wave + NaOCl/H₂O₂, Group 4-Continuous-wave + NaOCl/CHX), Subgroup A- MTA Fillapex, Subgroup B-AH Plus, Subgroup C- Realseal SE

and RealSeal SE (Subgroup C). These types of sealers were selected because they had excellent physical properties, tissue biocompatibility, and sealing ability.^[7,8,14]

Teeth with nearly similar apical diameters and root lengths were selected and instrumented to # F4 ProTaper Universal. Subsequently, variables such as anatomical variation, canal size, and the diameter of the apical foramen, which can influence the apical leakage, were minimized. In addition, the rotary instrumentation was found to be faster, more comfortable, and more efficient than hand instrumentation.

During instrumentation, samples in each group were irrigated only with one of the irrigation protocols, and the last irrigant was 17% EDTA to remove smear layer, which was followed by distilled water to remove any traces of previous irrigation solutions. By this way, the effect of each irrigation protocol and obturation technique on the sealing ability of each root canal sealer was evaluated.

Several methods have been used to assess the apical sealing property of root canal filling materials.^[13] Methylene blue dye penetration method was selected in the current study because it is an inexpensive, reliable method, easy to manipulate, and it has a good staining capability.^[13] Some authors reported that the dye leakage method is adequately valid if the experimental conditions such as the type of sample, type of dye, and duration of immersion into the dye have been standardized.^[15]

The experimental model in the current study was evaluated by using positive and negative control groups. The positive control group indicated that leakage testing was an appropriate method for maximum dye penetration without a sealer. The negative control group showed no dye leakage, indicating that the using of three layers of varnish is effective to prevent dye penetration through root surfaces and apical foramen.

The results of the current study showed that the sealing ability of AH Plus and MTA Fillapex was better when used with the single-cone technique than when used with the continuous wave technique. These findings were matched with the results of Yilmaz *et al.*^[16] who showed that the single-cone obturation technique showing a significantly better sealing ability than that of continuous wave obturation technique.

The results of the present study showed that the sealing ability of AH Plus was superior to the other types of sealers when the root canals were irrigated with NaOCl + H₂O₂ and obturated with single-cone technique. However, MTA Fillapex and AH Plus showed similar sealing ability when the root canals were irrigated with NaOCl + CHX. These results are matching with the results of the Ferreira *et al.*^[14] and Sönmez *et al.*^[17] who showed that AH Plus has a better sealing ability than that of MTA Fillapex when the root canals were irrigated with NaOCl and EDTA.

The sealing ability of RealSeal SE was the worst among all sealers when the root canals were irrigated with NaOCl + H₂O₂ and obturated with the single-cone technique. The cause of this may be due to; the combination of NaOCl solution with H₂O₂ may cause deep changes in the collagen structure by dehydration and/or removal of fibrils that form the hybrid layer, thus reducing the sealer penetration into dentin, and subsequently the bond strength.^[18] The release of oxygen nascent originating from NaOCl and H₂O₂ may retard interfacial polymerization.^[19] The polymerization shrinkage of methacrylate-based sealer and high C-factor may also negatively affect adhesion and sealing ability of RealSeal SE.^[11] The findings of the present study indicated that RealSeal SE had significantly higher leakage values than that of AH Plus and MTA Fillapex when the root canals were irrigated with NaOCl + CHX and obturated with single-cone technique. These results are in agreement with that of Morris *et al.*^[20] who have reported that NaOCl and CHX resulted in a reduction in the bond strength between Resilon and dentine.

The better apical sealing ability of AH Plus, especially with single-cone obturation technique may be explained by its excellent adhesiveness, ability to penetrate into

microirregularities of the dentinal wall, setting expansion, and ability to form a covalent bond with amino groups of exposed radicular dentin collagen.^[21] The effect of H₂O₂ on the apical dye leakage of AH Plus was not previously assessed. However, the existence of oxygen released from the dissociation of H₂O₂ when in contact with organic debris could improve the adhesion of AH Plus by increasing the permeability of dentin and enhance its chemical bond with amino groups of exposed dentin collagen.^[22]

In the current study, the use of NaOCl + H₂O₂ irrigation protocol decreased significantly the apical sealing ability of MTA Fillapex/single-cone gutta-percha. This may be explained by the retardation effect of the oxygen nascent to the penetration of the MTA Fillapex into the dentinal tubules during its setting, and the formation of voids at the interfacial layer between dentin and the sealer that could negatively affect the interfacial bond strength and adhesion of the sealer. However, the use of NaOCl + CHX irrigation protocol increased significantly the sealing ability of MTA Fillapex when used with single-cone technique. This may be explained by its greater penetration depth into dentinal tubules, and its setting and volumetric expansion.^[23]

All root canal sealers used with the continuous wave technique showed more leakage values than that of single-cone obturation technique. At the same time, no significant differences were observed between experimental sealers after using each irrigation protocol. This means that each irrigation protocol affected similarly the sealing ability of all tested sealers. When the effect of irrigation protocol was compared after using continuous-wave techniques, no significant difference was found between both irrigation protocols when MTA Fillapex was used. Nevertheless, low apical leakage values were observed with AH Plus and RealSeal when NaOCl + H₂O₂ irrigation protocol was used.

The temperature used with the continuous wave technique may accelerate the setting time of sealers, and consequently decreases their penetration ability into the dentinal and increase leakage.^[24] Furthermore, the setting time acceleration may prevent the relief of polymerization stresses by the slow flow of methacrylate-based sealer.^[24] DeLong *et al.*^[25] indicated that the bond strength of MTA Fillapex significantly decreased when continuous wave technique was used because of the pressure applied during obturation. The current results are matching with the results of those authors.

One of the exciting findings was improving the sealing ability of the single-cone RealSeal SE obturation system when used with NaOCl + H₂O₂ irrigation protocol. Oxygen from such chemicals causes strong inhibition of the interfacial polymerization of resin bonding material.^[19] This may increase the setting time of the sealer and counteract the effect of raising the temperature during continuous-wave obturation technique. The increasing of the setting time could allow more sealer penetration into dentinal tubules and hence improve sealing ability of methacrylate resin-based sealer.

The results obtained in the present study justify the rejection of the null hypothesis set out previously, i.e., there were no significant differences in the maximum dye penetration among experimental root canal sealers when different irrigation protocols and obturation techniques were used.

Finally, despite the single-cone technique used in the current study showed significantly better seal when used with AH Plus or MTA Fillapex, it was not able to prevent apical dye leakage totally. As such, it is not possible directly to correlate the amount of leakage to the clinical outcomes of endodontic treatments. Therefore, clinical studies are required to confirm the significance values of the current results.

CONCLUSIONS

Within the limitations of the current study, the following conclusions can be drawn:

1. None of the experimental root canal sealers could prevent totally the apical leakage
2. The apical sealing ability AH Plus was the highest when the root canals were irrigated with 3% NaOCl + 3% H₂O₂ and obturated with single-cone technique
3. The sealing ability of MTA Fillapex was significantly improved when the root canals were irrigated with 3% NaOCl + 2% CHX and obturated with the single-cone technique
4. The sealing ability of RealSeal SE was the worst among all sealers when the root canals were irrigated with 3% NaOCl + 3% H₂O₂ and obturated with the single-cone technique
5. All root canal sealers used with the continuous wave technique showed high leakage values but without significant differences. However, RealSeal SE used with the continuous wave technique showed significantly better apical seal when the root canals were irrigated with 3% NaOCl + 3% H₂O₂
6. 3% NaOCl + 2% CHX irrigation protocol increased the leakage values of all sealers that were used with the continuous wave technique.

Clinical significant

Single-cone obturation technique could be a substitute to continuous wave obturation technique when the type of the root canal sealer and irrigation protocol were properly selected. None of the experimental root canal sealers could totally prevent the apical leakage. However, the use AH Plus during single-cone gutta-percha technique may decrease the apical leakage when 3% NaOCl + 3% H₂O₂ + 17% EDTA irrigation protocol is used. In addition, the use of MTA Fillapex during single-cone gutta-percha obturation technique may decrease the apical leakage when 3% NaOCl + 2%CHX + 17%EDTA irrigation protocol is used. It is better to avoid RealSeal SE obturation system with single-cone technique and 3% NaOCl + 3% H₂O₂ + 17% EDTA irrigation protocol.

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Conflicts of interest

There are no conflicts of interest.

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