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Influence of final irrigation protocols and endodontic sealer on bond strength of root filling material with root dentin previously treated with photodynamic therapy

Matheus Albino Souza*, Marina Gabrieli Padilha Rauber, Natalia Zuchi, Laís Vargas Bonacina, Rafaela Ricci, Caroline Tumelero Dias, Karolina Frick Bischoff, Janessa Luiza Engelmann, Huriel Scartazzini Palhano

School of Dentistry, University of Passo Fundo, Passo Fundo, RS, Brazil

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ABSTRACT

Introduction: The aim of this study was to evaluate the influence of final irrigation protocols and endodontic sealer on bond strength of root filling material on root dentin previously treated with photodynamic therapy (PDT).

Methods: One hundred root canals were prepared up to #F3 file of Pro-Taper system to receive the root filling material. All samples were submitted to PDT and randomly divided into five groups ($n = 20$) according to final irrigation protocols: Group 1-distilled water + ultrasonic activation (US); Group 2–17% EDTA; Group 3-QMix; Group 4–17% EDTA + US; Group 5-QMix + US. Each group was randomly divided into two subgroups ($n = 10$), according to the endodontic sealer used for cementation of gutta-percha points: AH Plus or MTA Fillapex. The bond strength was evaluated by a push-out test. The patterns of failure were observed under optical microscopy. The bond strength was evaluated using a two-way Anova followed by the Tukey post-hoc test, and the failure mode was evaluated using the chi-square test ($\alpha = 5\%$).

Results: The use of 17% EDTA and QMix associated or not to US improved the bond strength of root filling material with either endodontic sealer ($p < 0.05$). AH Plus showed higher bond strength than MTA Fillapex ($p < 0.05$). There was a higher predominance of cohesive failure in all groups, regardless of the tested final irrigation protocols and endodontic sealer ($p < 0.05$).

Conclusions: The use of 17% EDTA and QMix, regardless of association with US, and the use of AH Plus improve the bond strength of the root filling material on root dentin previously treated with PDT.

1. Introduction

The success of endodontic therapy depends, among several factors, on proper sealing of the root canal system using root filling materials with ideal biological and physical properties [1]. The association between gutta-percha and the endodontic sealer is the most crucial for an ideal root canal filling. Several endodontic sealers including AH Plus and MTA Fillapex are being used for this purpose. The AH Plus is an epoxy resin-based endodontic sealer that is considered the gold standard for the improvement of the overall quality of the root filling. It presents with low polymerization stress, adequate flow, and high bond strength to root dentin [2]. Meanwhile, MTA Fillapex is a calcium silicate-based endodontic sealer developed to explore the balance between the biological and physical-chemical properties of MTA. It is an ideal sealer with adequate flow and ability to promote the biomineralization process [3]. In this scenario, the main goal is to prevent microbial leakage and promote adhesion to root canal dentin, creating a favorable environment for tissue regeneration in the periapical region [4].

In addition to an adequate root canal filling, the effective decontamination of the root canal system is essential for the success of endodontic therapy. However, it is known that the endodontic instrument does not touch all the root canal walls during instrumentation [5]. In addition, the use of chemical auxiliary substances associated with endodontic instrumentation are not able to promote complete neutralization of microorganisms from root canal spaces [6]. Thus, auxiliary decontamination procedures such as photodynamic therapy (PDT) are necessary to improve cleaning. The procedure of PDT

* Corresponding author at: University of Passo Fundo, BR 285/São José, Building A7, Suite 2, Zip code: 99052-900, Passo Fundo, RS, Brazil.
E-mail address: matheussouza@upf.br (M.A. Souza).

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provides effective support to conventional chemomechanical preparation. It involves a light source originating from a low-power laser and a non-toxic photosensitizer, presenting the ability to eliminate endodontic pathogens by the formation of reactive oxygen species [7].

However, this photosensitizer of PDT is a viscous substance that presents with the ability to impregnate the root canal walls and depths of dentinal tubules, forming a chemical smear layer [8]. It creates a favorable environment for microbial microleakage and inadequate adhesion of the root filling material to root canal dentin [9]. Thus, final irrigation protocols are necessary to remove the photosensitizer after PDT. Ethylenediaminetetraacetic Acid (EDTA) is the most commonly used final irrigant in endodontics with chelating action and the ability to remove the smear layer [10]. The QMix is a final irrigant solution that shows effective results in order to remove debris and smear layer [11]. In addition, ultrasonic activation (US) promotes the ability of final irrigants to remove the smear layer [12]. However, there are no studies in literature regarding the added use of US with final irrigants to remove the photosensitizer from root canal walls, evaluating its influence on the bond strength of the root filling material on root dentin.

The purpose of this study was to evaluate, *in vitro*, the influence of added use of US with EDTA 17% and QMix, on bond strength of the root filling material on root dentin previously treated with PDT. The hypotheses of the present study were: (i) Use of US improves bond strength and (ii) the type of endodontic sealer does not have an influence on the bond strength of the root filling material on root dentin.

2. Material and methods

This study was approved by the Ethics Commission of the University of Passo Fundo (Passo Fundo, RS, Brazil).

2.1. Sample collection and preparation

One hundred single-rooted extracted human teeth were used in the present study. All teeth were obtained from the Biobank of the School of Dentistry of the University of Passo Fundo (Passo Fundo, RS, Brazil). Immediately after extraction, the teeth were rinsed with saline solution and stored in labeled individual plastic vials containing 10% buffered formalin solution (Natupharma, Passo Fundo, RS, Brazil). Then, the teeth were kept under refrigeration for no longer than 1 month. Dental crowns were sectioned with a rotary diamond disc (#911H, Brasseler, Savannah, GA, United States) so that all roots retained a length of 15 mm. The root length of 15 mm was measured with a ruler, a mark was made on each root in the measurement of 15 mm and the cut was performed at this length, standardizing the length of all roots.

All roots were prepared by only one operator who is specialist on endodontics, using the same protocol for pulp tissue removal in order to standardize the canal diameter. The working length was established by introducing a K-file #10 (Dentsply-Maillefer, Ballaigues, Switzerland) into the canal until its tip was visualized at the apical foramen. From this measurement, 1 mm was subtracted to obtain the working length. Each tooth was fixed in a portable lathe machine, in order to maintain the tooth secured during the root canal preparation. The roots were enlarged to working length using the ProTaper system (Dentsply-Maillefer), following the sequence: S1 to F3. Distilled water (DW) (Natupharma, Passo Fundo, RS, Brazil) was used as irrigant solution and replenished at each instrument change. The ProTaper files (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA) were used in a 16:1 gear reduction handpiece powered by a torque-controlled electric motor (VDW Silver Reciproc Motor, VDW) at a constant rotation speed of 300 rpm in a crown-down manner according to the manufacturer's instructions, by using a gentle in-and-out digital motion.

The root canals were then filled with 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil), and all roots were put into 10 mL plastic vials containing 17% EDTA, such that there were ten samples per vial, so that the roots remained completely covered by the solution. Each plastic vial was inserted into an ultrasonic cleaning machine (Bio Free, Gnatus, Ribeirão Preto, SP, Brazil) for one minute in order to remove the smear layer formed by root canal preparation. After that, the root canals were irrigated with 5 mL of DW and dried with absorbent paper points (Tanari, Manacapuru, AM, Brazil).

The specimens were embedded in epoxy resin (Silaex, São Paulo, SP, Brazil), to avoid extravasation of tested final irrigants, and to facilitate PDT and root canal filling.

2.2. PDT protocol

After root canal preparation, all root canals were filled with 0.01% (0.1 mg/mL) methylene blue (Chimio Lux DMC, São Carlos, SP, Brazil) until extravasation to the root canal entrance. The photosensitizer was retained in the root canal for 5 min, as pre-irradiation time. After that, a low intensity laser (Therapy XT® DMC, São Carlos, SP, Brazil) was used at 100 mW power and continuous emission in the red part of the spectrum (660–690 nm wavelength), with an intra-canal optical fiber of 600 μm diameter, attached at 2 mm short of the working length. The root canals were irradiated for 90 s, with 9 J of total dose delivery and 320 J/cm^2 of energy density remaining in the intra-canal fiber in static position, as recommended by the manufacturer. Then, all roots were irrigated with 5 mL of DW, followed by the aspiration of root canals.

2.3. Final irrigation protocols

After the PDT protocol, the 100 samples were randomly divided into five groups (n = 20) according to the final irrigation protocol, i.e., DW + US (control group); 17% EDTA; QMix; 17% EDTA + US; and QMix + US.

In the groups without US, the root canals were completely filled with the tested solution until extravasation to the root canal entrance. The tested solution remained in contact with the root canal walls for a period of one minute. After that, irrigation with 5 mL of DW was performed, thereby concluding the procedure to remove the photosensitizer.

In the groups with US, the root canals were completely filled with the tested solution until extravasation to the root canal entrance. Next, US was performed using an ultrasonic device (Nac Plus Ultrasonics, Adiel, Ribeirão Preto, SP, Brazil). The stainless-steel endodontic tip, size ET40 (Satelec-Acteon, Mount Laurel, NJ, USA), was inserted 3 mm short of the working length and activated for one minute. Scale power: 3, for endodontics (75% power), was used to promote US. Finally, irrigation with 5 mL of DW was performed, thereby concluding the procedure to remove the photosensitizer.

The root canals were dried with the aspiration cannula and absorbent paper points.

2.4. Root canal filling

Each group was randomly divided into two subgroups (n = 10) according to the endodontic sealer used with the root canal filling: AH Plus epoxy resin-based sealer (Dentsply Maillefer, Ballaigues, Switzerland) or MTA Fillapex calcium silicate-based sealer (Angelus, Londrina, PR, Brazil), as described below.

In the groups filled with AH Plus, the root canals were filled by the lateral compaction technique using gutta-percha points and AH Plus endodontic sealer (Dentsply, Switzerland). After mixing, the sealer was applied along the walls of the root canals using the lentulo spiral. The gutta-percha ProTaper master cone #F3 (Dentsply, Switzerland) was lightly coated with the endodontic sealer and inserted to the working length. Then, XF gutta-percha accessory points (Dentsply Maillefer, Ballaigues, Switzerland) were introduced into the root canals with the aid of finger spreaders (Dentsply Maillefer, Ballaigues, Switzerland). The gutta-percha accessory points were used until the finger spreader did not penetrate more than five millimeters into the root canal. Then, the excess of root filling material was removed by cutting with a #2 heated plugger (SS White Duflex, Rio de Janeiro, RJ, Brazil).

In the groups filled with MTA Fillapex, the root canals were filled in the same way as described previously in the groups that were filled with AH Plus. After root canal filling, buccolingual radiographs were taken of all groups using the same exposure settings for each specimen. The radiographs were developed and fixed using the same time and temperature settings, in order to verify the quality of root canal filling.

2.5. Evaluation of bond strength

After root canal filling, all specimens were stored at 37 °C and 95% humidity for 21 days. This is time enough to complete seating of root filling material, acoording to manufacturer's instruction. Subsequently, the roots were sectioned transversely from the root canal entrance, into 1 mm-thick discs in a metallographic cutter with a diamond disk, at a speed of 350 rpm under cooling. The first disc was discarded, and the next five root discs were selected from each sample, resulting in 50 specimens per subgroup (n = 5 × 10 = 50). Each disc was subjected to the push-out test on a mechanical testing machine (Emic DL 2000, São José dos Pinhais, PR, Brazil) at a speed of 1 mm/min using a stainless steel cylindrical plunger of 0.8 mm diameter. The plunger tip was positioned so that it only contacted the root filling material (Fig. 1). The push-out force was applied in an apico-coronal direction until bond failure occurred, which was manifested by extrusion of the obturation material and a sudden drop along the load deflection. The force required to displace the material from the root canal was recorded in newtons (N) and calculated in megapascals (MPa).

The bond strength calculation and evaluation of the patterns of failure were based on a previous study by Dias et al. [13]. The bond strength (δ) in megapascals was calculated using the formula $\delta = F/A$, where F is the force (N) used by the test machine and A is the area acted upon (mm^2). To calculate the area, the following equation was applied: $A = 2\pi r \times h$, where π is a constant of value = 3.14, r is the radius of the intra-radicular space (mm), and h is the height (mm). Furthermore, the patterns of failure were observed in each disc under optical microscopy (Zeiss, São Paulo, SP, Brazil) at 50× magnification. The classification is illustrated in Fig. 2 and it was established as follows: 1: adhesive failure between the dentin and the root filling material presenting as absence of root filling material on the dentinal walls of the root canal; 2: cohesive failure of the root filling material diagnosed by the presence of root filling material on the dentinal walls of the root canal; 3: mixed failures (1 and 2) may be observed.

2.6. Statistical analysis

The bond strength was evaluated using a two-way ANOVA followed by the Tukey post-hoc test, allowing a quantitative analysis of these data. The distribution of failure mode among the groups was evaluated using the chi-square test, allowing a descriptive analysis. All tests were set at a 5% level of significance. Data were analyzed using Stat Plus AnalystSoft Inc. version 6.0 (Vancouver, BC, Canada).

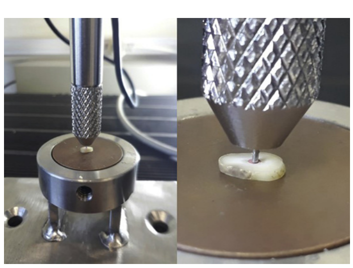

Fig. 1. A - The push-out test on a mechanical testing machine at a speed of 1 mm/min using a stainless steel cylindrical plunger of 0.8 mm diameter. B - The plunger tip positioned so that it only contacted the root filling material, applying the push-out force in an apico-coronal direction until bond failure occurred.

3. Results

The mean and standard deviation of the effects of final irrigation protocols on bond strength of the root filling material to the root canal dentin are presented in Table 1. The use of 17% EDTA and QMix, associated or not to US, improved the bond strength of the root filling material, that was statistically higher than that of the control group, regardless of the endodontic sealer used with the root canal filling ($p < 0.05$). The use of US over final irrigants did not improve the bond strength of root filling material, regardless the endodontic sealer used during root canal filling ($p > 0.05$). The groups where the root canals were filled with AH Plus showed higher bond strength of the root filling material to root dentin when compared to groups where the root canals were filled with MTA Fillapex, regardless of the final irrigation protocol ($p < 0.05$).

The percentage of pattern of failure (%) after the tested final irrigation protocols and root canal filling with both endodontic sealers are presented in Table 2. The chi-square test revealed no statistically significant differences in the pattern of failure among the groups. There was, however, a higher predominance of cohesive failure in all groups, followed by mixed and adhesive failures, in that order, regardless of the final irrigation protocol and endodontic sealer used with the root canal filling ($p < 0.05$).

4. Discussion

PDT is an effective therapeutic modality that brings about elimination of microorganisms that survived conventional chemomechanical preparation of the root canal system. Despite the fact that there are several variables associated with the parameter application, the PDT protocol in the present study was based on previous studies that used PDT as the antimicrobial strategy to promote decontamination of the root canal space [6,7]. Regarding the limitation of PDT, the photosensitizer remains over the dentin structure and obliterates the dentinal tubules, working as a chemical smear layer that can interfere in the adhesion of root filling materials [8]. According to the results of the present study, the use of an ineffective final irrigation protocol, such as DW + US, can contribute to the presence of residual photosensitizer, influencing the adhesion of root filling materials to root canal dentin. This was also demonstrated in a previous study that revealed the effects of smear layer on the sealing ability of the root canal filling [9]. It has been reported that the use of final irrigation protocols with no smear layer removal properties (control group) resulted in the lowest bond strength values. These findings point to the fact that the use of effective final irrigation protocols is necessary to remove the photosensitizer and provide ideal conditions for the adhesion of root filling materials to root canal dentin.

Excessive final irrigation using irrigants with decalcifying properties in the root canal space causes peritubular and intertubular dentinal erosion. These chemical substances bring about the removal of inorganic components from the dentinal structure, inducing damage to the mechanical properties and fracture resistance of root dentin [14]. On the other hand, a short exposure to the final irrigants results in low performance in smear layer removal [15]. It can interfere with the adhesion of the root filling material to the root canal dentin [9]. According to Çalt and Serper [14], one minute of final irrigation protocol is adequate time to promote smear layer removal with no damage to dentin structure. The final irrigation protocols remained for one minute in the root canal in the present study, in order to remove the smear layer precipitated by the use of the photosensitizer of PDT and improve the adhesion of the root filling material to root canal dentin.

The power of attraction between bodies of different chemical species is a physical principle that is known as bond strength. In dentistry,

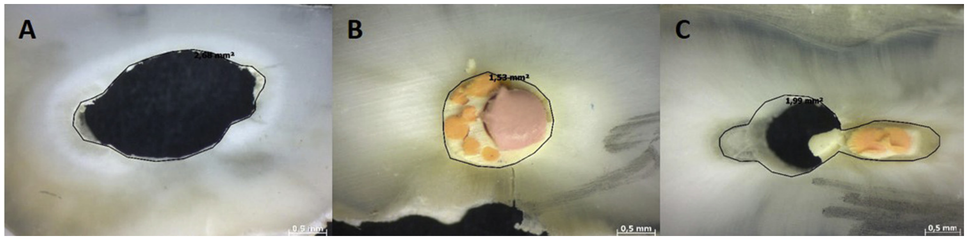

Fig. 2. Optical microscopy images illustrating the patterns of failure after push out test – A: adhesive failure; B: cohesive failure; C: mixed failure.

Table 1
Mean (standard deviation) bond strength of the root filling material to root canal dentin (MPa) of tested final irrigation protocols.

Group	N	Push-Out Bond Strength AH Plus	Push-Out Bond Strength MTA Fillapex
1. DW + US	50	3.81 (0.59) ^{A,a}	1.17 (0.24) ^{A,b}
2. EDTA	50	8.22 (1.28) ^{B,a}	2.59 (1.01) ^{B,b}
3. QMix	50	8.85 (1.21) ^{B,a}	2.61 (1.02) ^{B,b}
4. EDTA + US	50	9.61 (2.04) ^{B,a}	2.67 (0.96) ^{B,b}
5. QMix + US	50	12.84 (3.63) ^{B,a}	2.70 (0.84) ^{B,b}

* DW: distilled water; US: ultrasonic activation.
**Different superscript capital letters in each column indicate a statistically significant difference ($p < 0.05$). Different superscript lowercase letters in the row indicate a statistically significant difference ($p < 0.05$).

Table 2
Percentage of pattern of failure (%) after tested protocols and root filling.

Groups	Failure mode			Failure mode		
	AH Plus			MTA Fillapex		
	Adhesive	Mixed	Cohesive	Adhesive	Mixed	Cohesive
DW + US	10.00	28.00	62.00	8.00	24.00	68.00
EDTA	6.00	28.00	66.00	6.00	22.00	72.00
QMix	18.00	24.00	58.00	6.00	16.00	78.00
EDTA + US	14.00	24.00	62.00	4.00	22.00	74.00
QMix + US	16.00	26.00	58.00	12.00	16.00	72.00

* DW: distilled water; US: ultrasonic activation.

the bond strength between different materials and dental structures is quantified by the push-out test, which is considered a reliable method to provide this evaluation. [16]. More recently, it was consolidated as the method to evaluate the bond strength of root filling materials to root canal dentin [13]. The push-out test was adopted in the present study in order to evaluate the influence of final irrigation protocols and type of endodontic sealer on the bond strength of the root filling material to root dentin previously treated with PDT. According to literature, the push-out test is compatible with the clinical situation and produces effective power for material displacement and induces less stress at the bond interface during disc preparation [14]. The main limitation of the application of the push-out test here is that the discs were only obtained from the cervical and middle thirds in the present study. The bond strength of root filling materials was not evaluated in the apical third, where the smear removal is more critical [17].

The results of the present study revealed higher bond strength values in the groups treated with 17% EDTA and QMix alone, when compared to control groups treated with DW + US, when both tested endodontic sealers were used with the root canal filling. These results are in agreement with those of previous studies, where the use of final irrigants improved the bond strength of similar materials to the root canal dentin [18,19], despite the fact that the root dentin was not previously treated with PDT in these studies. It can be justified by the effective removal of the photosensitizer from the root canal walls promoted by the tested final irrigants. The study by Souza et al. [8] showed similar results revealing that the use of 17% EDTA and QMix provided better removal of photosensitizer from root canal walls after PDT. However, this previous study reveals only the presence of the residual photosensitizer when irrigation with DW and US is performed. This previous study does not evaluate to what extent the presence of this residual photosensitizer interferes with the adhesion of root filling material and does not evaluate the influence of endodontic sealer in this proposal, as differentially demonstrated in the present study. In addition, as much as EDTA is an effective final irrigant to remove the smear layer, it presents with some limitations. Among these are: low antimicrobial activity [20], cytotoxicity [21], and major modifications of the mechanical properties of dentinal structure [22]. For these reasons, use of alternative techniques is necessary to promote effective photosensitizer removal with no additional damaging effects to endodontic therapy.

The QMix is a final irrigant solution that produces effective results in smear layer removal due the presence of 17% EDTA and surfactant agent in its composition [11]. This ability can be the one factor that interfered with the results of the present study, as the group treated with QMix alone showed higher bond strength values when compared to the control group (DW + US), and similar bond strength values when compared to the 17% EDTA group, regardless of the type of endodontic sealer used with the root canal filling. In spite of the presence of EDTA in its composition, QMix is less aggressive to human mesenchymal stem cells [23] and induces less decalcification and erosion than 17% EDTA used alone as a final irrigant [24]. Furthermore, QMix contains chlorhexidine in its composition. It presents with a broad antimicrobial spectrum [7] and preserves dentin collagen by the inhibition of metalloproteinases [25]. Due to these properties, chlorhexidine promotes the elimination of endodontic pathogens and ensures favorable conditions for the adhesion of root filling materials. For these reasons and the results of the present study, it may be concluded that final irrigation with QMix after PDT could be an alternative to promote the removal of the photosensitizer.

According to Souza et al. [8], the use of US can aid 17% EDTA and QMix in photosensitizer removal after PDT and contribute to cleaning the root canal walls. The US induces hydrodynamic turbulence on the final irrigants within the root canal, increasing the hydrostatic pressure and temperature. The final reaction produces waves that are loaded with reactive oxygen species that collide against the root canal walls and penetrate the dentinal tubules [26]. Despite its high bond strength values, the groups treated with the combination of US and the final irrigants and groups treated with final irrigants alone showed similar results on comparison. In other words, US did not improve the bond strength of the root filling material to root dentin, rejecting the first hypothesis of the present study. This was because the discs were obtained from the cervical third and the beginning of the middle third, which were easily reached by the final irrigant solution, even without US. Greater effectiveness of US could have been observed if discs were obtained from the apical third and tested with the push-out assay.

The groups filled with AH Plus revealed higher bond strength values when compared to groups filled with MTA Fillapex, regardless of the tested final irrigation protocols. This rejects the second hypothesis of present study and was in accordance with previous researches that also observed low bond strength values for MTA Fillapex [27,28]. The epoxy-resin-based endodontic sealers such as AH Plus, have high flowability and long polymerization time. Moreover, cohesion between the AH Plus molecules increases the resistance to displacement of the root filling material from the root dentin, providing greater adhesion [28]. On the other hand, the calcium silicate-based endodontic sealers such as MTA Fillapex, display a higher ratio of salicylate resin and extended setting time. During the setting time, the presence of salicylate resin induces an initial volumetric shrinkage that increases the contraction factor. Thus, higher amounts of salicylate resin associated with extended setting time induces dimensional changes and formation of gaps between root canals and the root filling material [28]. This affects the bond strength of the calcium silicate-based endodontic sealer. For these reasons, the present study does not recommend the use of MTA Fillapex for the promotion of the root canal filling after PDT and final irrigation protocols.

The results of the present study showed high bond strength values at the root dentin in the groups that were filled with AH Plus, when compared to previous studies [9,29]. Factors such as the effective ability of tested final irrigation protocols and proper instrumentation to promote smear layer removal (composed of dentin chips, irrigant solutions, microorganisms and organic matter) can explain these findings. In these conditions, the chemical smear layer formed by the photosensitizer is more susceptible to removal. Therefore, the dentin surface is cleaned in a better way, creating favorable conditions for better adhesion of the root filling material to root dentin. In addition, the epoxy resin-based endodontic sealers have a recognized ability to penetrate and provide mechanical interlocking with the root dentin [28]. It contributes to higher bond strength of the root filling material to a cleaned root dentin and helps to explain the predominance of cohesive failure in all tested groups, demonstrating a high percentage of failure of the root filling material.

Despite the limitations of the present study, it is possible to conclude that the use of 17% EDTA and QMix, regardless of association with US, as well as the use of epoxy resin-based AH Plus endodontic sealer improve the bond strength of the root filling material to root dentin previously treated with PDT.

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Declaration of interest

The authors claim no conflicts of interest.

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