



Influence of final irrigation protocols and type of resin cement on bond strength of glass fiber posts in root dentin previously treated with photodynamic therapy

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ABSTRACT

Introduction: This study aimed to evaluate the influence of final irrigation protocols and type of resin cement on the bond strength of glass fiber posts (GFPs) in root dentin previously treated with photodynamic therapy (PDT). **Methods:** One hundred root canals were prepared up to #4 Gates Glidden drill to receive the GFPs. All samples were subjected to PDT, and randomly divided into five groups (n = 20) according to final irrigation protocol: distilled water + ultrasonic activation [US]; 17% EDTA; QMix; 17% EDTA + US; and QMix + US. Each group was randomly divided into 2 subgroups (n = 10) according to the type of resin cement used for cementation of GFPs: Rely-X ARC dual-resin; or Rely-X U200 (#M, St Paul, MN, USA) self-adhesive resin cement. Bond strength was evaluated using the push-out test. Failure patterns were observed under an optical microscope. Bond strengths were compared using two-way ANOVA, followed by the Tukey post-hoc test; failure modes were compared using the chi-squared test ($\alpha = 5\%$).

Results: The use of US over final irrigants improved the bond strength of GFPs, regardless of resin cement used ($p < 0.05$). The type of resin cement did not influence the bond strength of GFPs ($p < 0.05$). There was a higher predominance of cohesive failure in all groups, regardless of resin cement tested ($p < 0.05$).

Conclusions: The use of US on final irrigation improved the bond strength of GFPs, while the type of resin cement (dual or self-adhesive) did not influence the bond strength of GFPs in root dentin previously treated with PDT.

1. Introduction

The microorganisms represents the most prevalent cause of pulp and periapical pathologies, being necessary its elimination from root canal system in order to reach the success of endodontic therapy [1]. However, the conventional chemo-mechanical preparation by using irrigant solutions and endodontic instruments do not promote complete neutralization of microorganisms during endodontic therapy [2]. Thus, auxiliary resources of decontamination are necessary to aid in this proposal. The photodynamic therapy (PDT) involves a low-power laser and a non-toxic photosensitizer, with the ability to eliminate endodontic pathogens by the formation of reactive oxygen species, thus providing effective support to conventional chemo-mechanical preparation [2,3]. However, the photosensitizer used in PDT is a viscous substance that impregnates the root canal walls and dentinal tubules,

forming a chemical smear layer [4], which can induce microleakage and decrease the bond strength of filling materials to the root canal dentin [5]. Consequently, final irrigation protocols must be used to remove this photosensitizer after PDT.

The coronal restoration of the endodontically treated teeth is also another factor that may be essential to achieve successful endodontic therapy [6]. During this procedure, glass-fiber posts (GFPs) are widely accepted to provide adequate restoration retention in teeth with limited residual structure [6]. The GFPs presents similar elastic recovery when compared to dentin, aesthetic properties and ability to be bonded to dentin using adhesive techniques and materials [7]. In this scenario, several cements and adhesive systems have been developed to provide adhesion of GFPs to root dentin. Dual-resin cements associated with previous dentin conditioning have demonstrated high bond strengths [8]. At the same time, the use of self-adhesive resin cement has been

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recommended to promote better bond strength of GFPs to the root dentin, resulting in effective polymerization and preservation of the bonding interface [9]. However, the root canal walls must be cleaned and the dentinal tubules be opened, in order to establish an adequate adhesion of GFPs and resin cements to root canal dentin [8,9]. Then, the final irrigation protocols are also indicated in this proposal.

EDTA is the most used final irrigant used in endodontic procedures, providing chelating action and facilitating smear layer removal [10]. QMix, which consists of EDTA, chlorhexidine and a surfactant agent, has also been used as a final irrigant, demonstrating effective results in removing debris and the smear layer [11]. In addition, ultrasonic activation (US) improves the ability of the final irrigants to facilitate smear layer removal [8,12]. However, there are no studies in the literature using US over the final irrigants to remove the photosensitizer from root canal walls to evaluate its influence on the bond strength of GFPs cemented with resin cements in root dentin.

The purpose of this *in vitro* study was to evaluate the influence of added use of US with EDTA 17% and QMix, on bond strength of the GFPs cemented with dual- and self-adhesive resin cements in root dentin previously treated with PDT. We tested the null hypotheses that (i) the use of US over final irrigants does not improve the bond strength; and (ii) the type of resin cement has influence on the bond strength of GFPs cemented in the root dentin previously treated with PDT.

2. Materials and methods

This study was approved by the local Ethics in Research Committee, under protocol 735.208.

2.1. Sample collection and preparation

One hundred single-root 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 using a rotary diamond disc (#911H, Brasseler, Savannah, GA, United States); 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 #10 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) into the canal until its tip was visible at the apical foramen. From this measure, 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 the 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 renewed 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 flared at their coronal and middle thirds using Gates Glidden drills no. 2, 3 and 4 to a depth of 10 mm, to provide adequate space for cementation of GFPs. The Gates Glidden drills were used in a low speed handpiece powered by micro electric motor at a constant rotation speed of 10.000 rpm in a crown-down manner, by using a gentle in-and-out digital motion. The root canals were then

filled with 17% EDTA (Biodinâmica, Ibitiporã, PR, Brazil), and all roots were placed into 10 mL plastic vials containing 17% EDTA, with 10 samples per vial so that the roots remained completely covered by the solution. Each plastic vial was inserted into a US cleaning device (Bio Free, Gnatus, Ribeirão Preto, SP, Brazil) for 1 min to remove the smear layer left by the root canal preparation. Subsequently, 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 the tested final irrigants, and to facilitate PDT and cementation of GFPs. All procedures and parameters from this section were based in previous study of Souza et al. [4].

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 then remained in the root canal for 5 min as the pre-irradiation time. After that, a low-intensity laser (Therapy XT® DMC, São Carlos, SP, Brazil) was used at 100 mW of power, and continuous emission in the red part of the spectrum (660–690 nm wavelength), with an intra-canal optical fiber with 600 µm of diameter attached 2 mm short of working length. The root canals were irradiated for 90 s, delivering a total dose of 9 J and 320 J/cm² of energy density, maintaining the intra-canal fiber in a static position, as recommended by the manufacturer. All roots were then irrigated with 5 mL of DW, followed by aspiration. All procedures and parameters from this section were based in previous study of Souza et al. [4].

2.3. Final irrigation protocols

After the PDT protocol, the 100 samples were randomly divided into five groups (n = 20 each) according to the final irrigation protocol used: DW + US; 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 root canal walls for 1 min. Subsequently, 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. US was then performed using an US 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 1 min. Scale power 3 for endodontics (75% power) was used for US. Finally, irrigation with 5 mL of DW was performed, thereby concluding the procedure to remove the photosensitizer.

The root canals were dried using an aspiration cannula and absorbent paper points. All procedures and parameters from this section were based in previous studies of Souza et al. [4] and Çalt & Serper [16].

2.4. Cementation of GFPs

Each group was randomly divided into 2 subgroups (n = 10 each), according to the resin cement used for cementation of GFPs: Rely-X ARC dual resin cement (3M ESPE, St. Paul, MN, USA) or Rely-X U200 self-adhesive resin cement (3M ESPE), as described below.

2.4.1. Protocol for cementation for Rely-X ARC

The GFP no. 1 (White Post – FGM, Joinville, SC, Brazil) was cleaned with 35% phosphoric acid for 30 s, rinsed for 30 s and gently air-dried. The silane application (3M ESPE) was performed for 1 min, followed by Single-Bond (3M ESPE) adhesive application (3M ESPE) and light polymerization for 40 s with a halogen light source with a power of

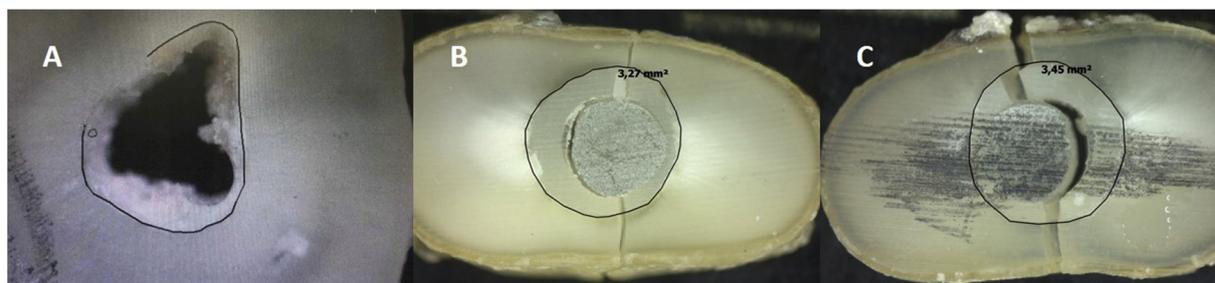


Fig. 1. Optical microscopy images illustrating the patterns of failure after push out test – A: adhesive failure; B: cohesive failure; C: mixed failure.

600 mV/cm² (Optilux, Demetron Res. Corp, Danbury CT, USA). The root canal dentin was etched using 35% phosphoric acid for 15 s, rinsed for 30 s, and gently air-dried with paper points. Single Bond was applied in dentin walls using microbrushes, air-dried for 5 s, and light polymerized for 40 s. Subsequently, Rely-X ARC dual-resin cement was mixed and injected into the root canal of the 10 samples of each group with a suitable Centryx syringe and Acudosse needle (DFL, Rio de Janeiro, RJ, Brazil). The GFP was then covered with the same cement and positioned within the root canal at a 10 mm level, and held under digital pressure for 20 s. After this period, excess cement was removed. The cement was then polymerized using a 600 mW/cm² halogen light source (Optilux) for 30 s on each face (buccal, palatal, mesial, distal and occlusal). After GFPs cementation, 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 right position of GFP and the quality of GFP cementation.

All procedures and parameters from this section were based in the manufacturer's instruction.

2.4.2. Protocol for cementation of Rely-X U200

GFP no. 1 (White Post – FGM) was etched in the same way as described above. The root canal dentin was not etched. Subsequently, Rely-X U200 self-adhesive cement was mixed and injected in the same manner as described above, repeating the same procedures for GFP positioning and stabilization, as well as polymerization. After GFPs cementation, 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 right position of GFP and the quality of GFP cementation.

All procedures and parameters from this section were based in the manufacturer's instruction.

2.5. Evaluation of bond strength

After GFP cementation, all specimens were stored at 37 °C and 95% humidity for 21 days. According to manufacturer's instruction, this is time enough to complete seating of filling material after adequate photoactivation. 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, totaling 50 specimens per subgroup ($n = 5 \times 10 = 50$). 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 filling material. The push-out force was applied in an apico-coronal direction until bond failure occurred, which was manifested by extrusion of the filling 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).

After the push-out test in the universal testing machine, each disc was immediately transported in an Eppendorf tube containing distilled water to the optical microscope site, in order to calculate the bond strength and evaluate the failure patterns. Each disc was removed from the Eppendorf tube with a tweezer, dried with absorbent paper and positioned in the center of optical microscopy (Zeiss, São Paulo, SP, Brazil), at 50× magnification. The bond strength calculation and the failure pattern evaluation were based on a previous study by Dias et al [13]. The bond strength (δ) in megapascals was calculated using the formula $\delta = F/A$, in which F is the force (N) used by the test machine and A is the area. To calculate the area, the following equation was applied: $A = 2\pi r \times h$, in which π is the constant value 3.14, r is the radius of the intra-radicular space, and h is the height (mm). The radius of the intra-radicular space of each disc was measured under optical microscopy, with the aid of a software which provided this mensuration. The height of each disc was measured by using a digital pachymeter. Furthermore, the failure patterns were observed in each disc under optical microscopy (Zeiss, São Paulo, SP, Brazil) at 50× magnification. The classification was established as follows: 1: adhesive, between the dentin and the filling material, absence of filling material on the dentine walls of the root canal; 2: cohesive, failure of the filling material (GFP/cement), presence of filling material on the dentin walls of the root canal; and 3: mixed, both failures (1 and 2) could be observed (Fig. 1). All procedures and parameters from this section were based in previous study of Dias et al. [13].

2.6. Statistical analysis

The normal distribution of results was confirmed by the Kolmogorov–Smirnov test ($p = 0.4233$). Bond strength was evaluated using a two-way analysis of variance, followed by the Tukey post-hoc test, enabling a quantitative analysis of these data. The failure mode distribution among the groups was evaluated using the chi-squared test, enabling a descriptive analysis of these data. All tests were set at a 5% level of significance. Data were analyzed using Stat Plus AnalystSoft Inc. version 6.0 (Vancouver, BC, Canada).

3. Results

The means and standard deviations of bond strength of the filling material to the root canal dentin are presented in Table 1. The use of final irrigation protocols improved the bond strength of GFP, being statistically higher when compared with the control group, regardless of the resin cement used during cementation ($p < 0.05$). The use of US over final irrigants improved the bond strength of GFP, and was statistically higher compared with all other groups, regardless the resin cement used during cementation ($p < 0.05$). The type of resin cement used during the cementation did not influence the bond strength of the GFP, which was statistically similar in all tested groups ($p < 0.05$).

The chi-squared test revealed no statistically significant differences in failure patterns among the groups, with a higher predominance of cohesive failure in all groups, followed by mixed and adhesive failures, respectively, regardless of the resin cement used during cementation.

Table 1
Bond strength of filling material to root canal dentin of the tested final irrigation protocols.

Group	n	Push-out bond strength, Mpa	
		Rely-X U200 [*]	Rely-X ARC [*]
1. DW + US	50	5.78 (2.04) ^{A,a}	4.71 (1.98) ^{A,a}
2. EDTA	50	15.91 (2.37) ^{B,a}	14.62 (2.64) ^{B,a}
3. QMix	50	18.27 (2.18) ^{B,a}	17.62 (2.09) ^{B,a}
4. EDTA + US	50	24.74 (2.46) ^{C,a}	22.39 (2.55) ^{C,a}
5. QMix + US	50	28.88 (3.50) ^{C,a}	27.54 (3.72) ^{C,a}

*Data presented as mean (SD), unless otherwise indicated. *3 M, St Paul, MN, USA.

**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 ($p < 0.05$). DW, distilled water; US, ultrasonic activation.

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

Groups	Failure mode Rely-X U200			Failure mode Rely-X ARC		
	Adhesive	Mixed	Cohesive	Adhesive	Mixed	Cohesive
DW + US	14.00	20.00	66.00	10.00	20.00	70.00
EDTA	16.00	18.00	66.00	14.00	18.00	68.00
QMix	8.00	26.00	66.00	16.00	20.00	64.00
EDTA + US	10.00	16.00	74.00	16.00	20.00	64.00
QMix + US	10.00	12.00	78.00	10.00	20.00	70.00

* DW: distilled water; US: ultrasonic activation.

($p < 0.05$) (Table 2).

4. Discussion

Bond strength is a physical principle that can be defined as the force of attraction between bodies of different chemical species [14]. In dentistry, the push-out test has been used as one of the main resources to quantify bond strength between different materials and dental structures. More recently, it has been applied to evaluate the bond strength of filling materials to root canal dentin [8,13]. According to the literature, the push-out test is compatible with clinical situations, produces effective forces for material displacement, and induces less stress at the bond interface during disc preparation [15]. In addition, discs are obtained only from the thirds filled by the GFP (cervical and medium), thus simulating clinical reality. For these reasons, this test was adopted in the present study to evaluate the influence of final irrigation protocols and resin cement on bond strength of GFPs in root dentin previously treated with PDT.

The excessive time of final irrigation with chelating properties in the root canal space causes peritubular and intertubular dentinal erosion, inducing damage to mechanical properties and fracture resistance of root dentin [16]. On the other hand, short exposure to the final irrigants results in low performance of smear layer removal [17]. According to Çalt and Serper [16], 1 min of final irrigation is sufficient to facilitate smear layer removal with no damage to dentin structure. Consequently, the final irrigation remained for 1 min in the root canal in the present study to remove the smear layer left by the use of photosensitizer in PDT.

Although PDT is an established antimicrobial method in endodontic therapy, there is no consensus in the literature regarding several parameters for its application. All PDT parameters in the present study were based in previous studies that focused on the use of PDT as a strategy to eliminate microorganisms from the root canal space [2,3].

The greatest limitation of this therapeutic modality is the chemical smear layer formed by the photosensitizer that impregnates the dentin structure [4]. The inadequate removal of this chemical smear layer can decrease the adhesion of filling materials in the root canal dentin. This was confirmed in the present study, where the use of final irrigation protocols with no smear layer removal properties (i.e., control group) resulted in the lowest bond strength values. These findings highlight the fact that simple irrigation with inert solution, even with US, is insufficient for complete removal of photosensitizer from the root canal walls. Thus, maximizing the effectiveness of final irrigation protocols is necessary to remove the photosensitizer to provide ideal conditions for adhesion of filling materials to the root canal dentin.

According to the results of present study, the groups treated with 17% EDTA and QMix alone exhibited higher bond strength values compared with the control group treated with DW + US, regardless the type of resin cement used in the GFP cementation. These findings are consistent with previous studies, in which the use of final irrigants improved the bond strength of similar materials in the root canal dentin [18,19], despite the fact that root dentin was not been previously treated with PDT. Results of present study highlight the need to facilitate effective photosensitizer removal, with no damage to the bond strength of adhesive materials to root dentin. To yield effective results, however, EDTA presents some limitations, such as low antimicrobial activity [20], cytotoxicity [21], and major modifications to the mechanical properties of dentinal structure [22]. For these reasons, alternative techniques are necessary for effective photosensitizer removal, with no additional damaging effects to endodontic therapy.

QMix is a final irrigant solution composed of different substances, and is extremely effective in facilitating smear layer removal [11]. This was confirmed in the present study as the group treated with QMix alone demonstrated higher bond strength values compared with control group (i.e., DW + US), and similar bond strength values compared with 17% EDTA alone, regardless the type of resin cement used in the GFP cementation. Despite 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 when used as the final irrigant [24]. In addition, QMix contains chlorhexidine, which exerts antimicrobial activity [3], preserves dentin collagen by inhibition of metalloproteinases [25], and is able to promote elimination of endodontic pathogens to ensure favorable conditions for adhesion of filling materials. For these reasons and, considering the results of the present study, QMix can be recommended as an effective alternative to facilitate photosensitizer removal after PDT.

The greatest effectiveness in terms of photosensitizer removal and higher bond strength values was observed in the 17% EDTA + US, and QMix + US treatments, in both tested resin cements used in GFP cementation. These results are in accordance with a previous study, in which the use of US over the tested final irrigants promoted better photosensitizer removal [4] and higher bond strength values of GFP [26]. In addition, these results reject the first null hypothesis of present study, in which US would not improve the bond strength of GFPs cemented with resin cements in root dentin. US induces hydrodynamic turbulence over any solution applied to the root canal, increasing its hydrostatic pressure and temperature. The final reaction then produces waves that are loaded with reactive oxygen species that collide with the root canal walls and penetrate the dentinal tubules [27]. In this scenario, the association of tested final irrigants and US resulted in greater photosensitizer removal and greater bond strength of GFP cemented with both tested resin cements in root canal dentin.

Dual and self-adhesive resin cements were tested in the present study to evaluate their influence on bond strength of GFPs to root dentin. Regardless the tested final irrigation protocols to promote photosensitizer removal after PDT, both tested resin cements demonstrated high values of bond strength of GFP to root dentin, with no significant differences. This rejects the second null hypothesis of the present study. The Rely-X ARC dual resin cement exhibits post-

maturation hygroscopic expansion, which offsets its initial setting shrinkage so the interface between dentin and cement remains more stable [28]. On the other hand, the acidic monomers in Rely-X U200 self-adhesive resin cement demineralize and infiltrate the tooth substrate, providing micromechanical retention. Simultaneously, the reaction between the phosphoric acid monomers of the cement and hydroxyapatite of the tooth substrate can offer chemical retention [29]. Although similar results on bond strength of GFP to root dentin, the use of Rely-X U200 self-adhesive resin cement simplifies the step of the bonding procedure and overcomes the limitations of complex, multistep applications [30]. This advantage should be taken into account when choosing the resin cement for GFP cementation to the root dentin.

The results of present study revealed high bond strength values of filling material to root dentin after photosensitizer removal by using final irrigation protocols when compared with previous studies [9,26]. It is due to the fact of effective ability of tested final irrigation protocols to facilitate smear layer removal. Furthermore, the resin cements demonstrate higher penetration and mechanical interlocking with the root dentin, providing better adhesion [8,9]. It helps to explain the higher predominance of cohesive failure in all groups. On the other hand, there was an absence of a smear layer by instrumentation consisting of dentin chips, irrigant solutions, microorganisms, and organic matter. Under the conditions of present study, the chemical smear layer formed by the photosensitizer was more amenable to removal. Therefore, the dentin surface is cleaned in a better way, creating favorable conditions for improved adhesion of the filling material to the root dentin.

During the sample preparation for push-out test, the discs were only obtained from the cervical third and the beginning of the middle third, being easily reached by the final irrigant solution and allowing a greater action of US. It happened because there was no a plunger tip with smaller diameter and ability to promote the displacement of restorative material in a higher depth of root canal, with no contact with root dentin. It can be considered some limitation of present study, being recommended additional studies to evaluate the effects of final irrigation protocols on bond strength of GFPs and resin cements in a higher depth of the root dentin previously treated with PDT. Based on the results of present study, the use of QMix + US to remove the photosensitizer from root canal walls is recommended, since this protocol is effective in this proposal [4] and the QMix induces benefits to endodontic therapy [3,23–25], as described previously. In the same way, the use of Rely-X U200 is recommended for cementation of GFPs, since this resin cement provides higher bond strength values to root canal dentin, as well as reduces the steps and clinical time for post cementation during restoration of endodontically treated teeth [9].

5. Conclusion

In conclusion, despite the limitations of the present study, the use of US over final irrigants improved the bond strength of GFP cemented with resin cements in root dentin previously treated with PDT. In addition, the type of resin cement used in GFP cementation—dual or self-adhesive—did not influence this evaluation.

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The authors claim no conflicts of interest.

The authors have no financial affiliation (e.g., employment, direct payment, stock holdings, retainers, consultancies, patent licensing arrangements or honoraria) or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest is disclosed.

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