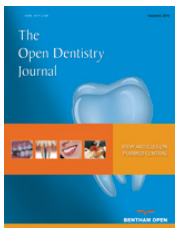

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RESEARCH ARTICLE

Effect of 0.2% Chitosan Associated with Different Final Irrigant Protocols on the Fiber Post Bond Strength to Root Canal Dentin of Bovine Teeth: An *In-vitro* Study

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Abstract:

Objective:

This in-vitro study investigated the effect of 0.2% Chitosan associated with different final irrigant protocols on the bond strength of fiber posts (FP) to root canal dentin.

Methods:

Fifty bovine incisors roots were prepared using the ProTaper Universal system, irrigated with 2.5% sodium hypochlorite, and divided into one control group (n=10) with no final irrigant protocol and four experimental groups (n=10), which were defined according to the combination of chelating solution (17% EDTA and 0.2% Chitosan) and irrigant activation/delivery method [conventional irrigation (CI), and passive ultrasonic irrigation (PUI)]. Post spaces were prepared to a depth of 12 mm using #1-5 Largo drills, and the FP were cemented using self-adhesive resin cement. Two slices of 2 mm in thickness from each third were obtained and submitted to the micropush-out test. After testing the push-out strength, the slices were analyzed under a stereomicroscope at 40× magnification for bond failure patterns determination. Statistical analysis was performed using three-way ANOVA followed by Tukey's test ($\alpha = 0.05$).

Results:

The control and 17% EDTA + CI groups exhibited significantly lower bond strength than 0.2% Chitosan + CI, 17% EDTA + PUI, and 0.2% Chitosan + PUI groups in the cervical third ($P = 0.00$). The cervical third had higher values than the middle and apical thirds in control ($P = 0.00$), 17% EDTA + PUI ($P = 0.00$), and 0.2% Chitosan + PUI groups ($P = 0.00$). Adhesive cement-dentin failure type was predominant in all groups.

Conclusion:

The use of 0.2% chitosan did not affect the bond strength of FP to root dentin. Passive ultrasonic activation of chelating solutions resulted in an improvement in bonding strength.

Keywords: Bond strength, Chelating agent, Chitosan, Fiber post, Passive ultrasonic activation, Smear layer.

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1. INTRODUCTION

Fiber Post (FP) associated with composite resin foundation materials has become the first choice to restore endodontically treated teeth presenting extensive loss of coronal structure [1, 2]. Despite the interesting biomechanical properties of FP [3 - 6] and the advances in cementation agents [7], loss of retention between FP and root dentin continues to be reported [8, 9]. Sarkis-Onofre *et al*. [9] assessed the survival and success of FP and observed an annual failure rate of 1.7% after 5 years. It has been demonstrated that the quality of adhesion at the dentin-cement-post interface can be affected by the type of adhesive

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system, cementation strategies, and the characteristics of the root canal surface [5 - 7, 10].

Root canal instrumentation produces a smear layer (SL) consisting of an accumulation of dentin, irrigant solutions, and organic tissues [7, 11]. The presence of SL decreases the dentin permeability and the adhesion of filling materials to the root canal walls [12 - 14]. Additionally, SL can block the dentinal tubules and hinder penetration and adaptation of self-adhesive resin sealers [15, 16]. Thus, removing the SL is desirable when seeking strong adhesion between FP and root canal dentin [4 - 7, 17].

Several solutions have been used for SL removal [6, 18, 19]. Ethylenediaminetetraacetic acid (EDTA) is the most widely used chelating solution [20]; however, despite it being efficient in removing SL, it has an erosive effect on dentine [11], limited antibacterial action [4], and is considered a pollutant [20]. Chitosan is a biopolymer abundant in nature and ecologically friendly [21, 22]. It has been demonstrated that chitosan is biocompatible, biodegradable, non-toxic [23], has antibacterial and antibiofilm properties [24], and despite its high chelating capacity [21], it causes little dentin erosion [12]. Different irrigant delivery devices and special techniques, such as laser technology and sonic/ultrasonic systems, have been proposed to increase the effectiveness of chemical solutions within the root canal [2, 5, 25]. Passive ultrasonic irrigation (PUI) has been used in final irrigating protocols for SL removal [19]. It is based on the principle of cavitation and acoustic streaming [26]. Although PUI is more effective in SL removal than conventional needle irrigation, its use may result in dentin erosion, which could interfere with the bond strength of FP [7, 19].

Previous studies showed the harmful effects of chemical agents used during endodontic treatment on the retention of FPs [10, 17, 27]. They justified the bond strength reduction to the capacity of these agents to induce compositional and structural modifications on the root dentin surface [25, 28]. Chelating solutions may also lead to changes in the microstructure of the dentin [11] due to changes in the calcium to phosphate ratio and consequently in the proportion of the inorganic and organic components [29]. Changes in biomechanical properties of the dentin may have critical clinical repercussions, mainly for the endodontically treated tooth restored with FP [7].

To date, few studies have evaluated the effect of different protocols for the removal of SL on the push-out bond strength of FP to root dentin [4, 7, 8, 17, 30]. In addition, information on the possible effects of chitosan on the bond strength of FP cemented with self-adhesive resin cement is scarce. Therefore, the present study aimed to examine the effect of 0.2% chitosan, with or without PUI, compared to 17% EDTA, on the bond strength of FP to root canal dentin. The null hypotheses tested were that there would be no differences in bond strength of FP to the root dentin regardless of the (i) chelating solution, (ii) irrigant activation/delivery method, and (iii) level of the root canal.

2. MATERIALS AND METHODS

The sample size was calculated using G* Power 3.1.2 software (Universitat, Düsseldorf, Germany), considering an alpha error probability of 0.05 and power of 80% (effect size = 0.50). The software recommended 10 samples per group as the sample size.

Fifty bovine incisors with roots anatomically similar in size and shape, root canals less than 1 mm in cervical diameter, as measured with a digital caliper (Mitutoyo, Tokyo, Japan), and with mature apices [3, 10, 27] were selected for this study. They were stored in 0.2% thymol solution (Pharm, Phloraceae, Cuiabá, MT, Brazil) at room temperature until use.

The teeth were decoronated below the cementoenamel junction using a double-faced diamond disc (KG Sorensen, São Paulo, SP, Brazil) operated perpendicularly to their longitudinal axis to produce standardized roots of 17 mm in length from the apical end. Initially, a #10 K-File (Dentsply Maillefer, Rio de Janeiro, RJ, Brazil) was used to verify the patency of the canals. Next, the anatomic diameter of all roots was standardized using a #20 K-File (Dentsply Maillefer). Finally, to simulate clinical conditions, root apices were sealed with flowable composite (Top dam; FGM Produtos Odontológicos, Joinville, SC, Brazil).

The Working Length (WL) was set at 16 mm and Root Canal Preparation (RCP) was performed by using the ProTaper Universal rotary nickel-titanium system (Dentsply Maillefer, Ballaigues, Switzerland) until the F5 (50/.05) instrument. Each instrument was used for preparing only five root canals. During RCP, the canals were irrigated with 3 mL of 2.5% sodium hypochlorite (NaOCl; Pharm, Phloraceae, Cuiabá, MT, Brazil) between every instrument change. After RCP, the specimens were randomly divided into one control group (n=10), which received no final irrigant protocol, and four experimental groups (n=10), which were defined according to the combination of the following factors: chelating solution (17% EDTA and 0.2% Chitosan) and final irrigant activation/delivery method [conventional irrigation (CI) and passive ultrasonic irrigation (PUI)] (Table **1**).

The chelating agents used were prepared from analytical reagent grade materials (Pharm, Phloraceae) using purified water by reverse osmosis system with ultraviolet light (Quimis, Diadema, SP, Brazil) and electrical conductivity of <1 μS mm-2. The pH of the solutions was determined using a digital pH meter (Analion, Ribeirão Preto, SP, Brazil). The 0.2% chitosan solution was prepared with 0.2 g of chitosan (ACROS Organics Gell, Belgium; degree of deacetylation > 90%) in 100 mL of 1% acetic acid. The mixture was agitated using a magnetic agitator for 2 h [12, 21].

Concerning the final irrigant activation/delivery method, in groups 2 and 3, 5 mL of non-activated chelating solutions were delivered into root canals using a 5 mL disposable syringe (Ultradent Products, South Jordan, UT, USA) and a 29-gauge needle (NaviTip; Ultradent Products) that was inserted 1 mm short of WL without binding to the walls of the canal, and left for 3 min. In groups 4 and 5, 5 mL of the chelating solutions were passively activated for 60 s using an EMS PM 200 ultrasonic unit (EMS – Electro Medical Systems, Nyon, Switzerland) and an E1 – Irrisonic tip (HELSE, Santa Rosa do Viterbo, SP, Brazil) positioned 1 mm short of the WL, without

touching the root canal walls, so that it could vibrate freely. The ultrasonic unit was set to 10% power [19].

Finally, the specimens were irrigated with 2 mL of distilled water, dried with paper points (Dentsply Maillefer), and filled with gutta-percha points (Dentsply Maillefer) and an epoxy-resin-based sealer (AH Plus; Dentsply Maillefer). According to the manufacturer's instructions, the sealer was mixed, and the root filling used Tagger's Hybrid technique. The excess gutta-percha and sealer was removed, and the canal access was sealed with a micro-hybrid composite resin (TPH Spectrum, Dentsply Latin America, Petrópolis, RJ, Brazil). All samples were stored at 100% humidity for 24 h at room temperature.

Post spaces were prepared to a depth of 12 mm using #1-5 Largo drills (Dentsply Maillefer), which corresponded to the 1.5 parallel-sided, serrated fiber posts (Reforpost #3; Angelus, Londrina, PR, Brazil). The root canals were irrigated with 2.5% NaOCl (Pharm, Phloraceae) after each bur change. After the post-space preparation, each root was rinsed with 2 mL of saline solution and dried with absorbent paper points (Dentsply Maillefer). All roots were covered externally with wax to avoid lateral polymerization [31]. The posts were cleaned with a solution of 70% ethanol, and a silane agent (Silano, Angelus) was applied with a micro brush (KG Sorensen, Barueri, SP, Brazil) for 1 min. Self-adhesive resin cement (RelyX U200; 3M-ESPE, St. Paul, MN, USA) was manipulated according to the manufacturer's instructions and introduced into the root canal with a low-speed lentulo spiral instrument (Dentsply Maillefer) and applied to the post. The post was seated to its full depth with digital pressure. The excess cement was removed with a clean micro brush (KG Sorensen) after 1 min. Three min later, the self-adhesive resin cement was light-cured using a 1,200 mW/cm-2 source (Radii-Cal; SDI, Bayswater, Australia) for 40 s each on the cervical face of the specimen, and oblique to the buccal and lingual surfaces, for a total of 120 s. The samples were then stored in 100% humidity at room temperature for 24 h before the push-out test [3]. A single operator, an endodontist with more than ten years of experience, performed all endodontic and post-placement procedures.

The specimens were sectioned transversely to their long axis with a double-faced diamond disc (4" diameter × 0.012" thickness × 1/2"; Arbor, Extec, Enfield, CT, USA) and a precision saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) at low speed with water cooling to produce six slices. Accordingly, two 2 mm thick discs were obtained from cervical, middle, and apical thirds.

The root slices were then submitted to a micropush-out test in a universal testing machine (Instron 5960 Dual Column Tabletop Testing Systems, Instron, Barueri, SP, Brazil). A compressive load was applied at 0.5 mm/min-1 in the apical-coronal direction until failure by displacement of the FP occurred. The bond strength in MPa was calculated by dividing the load at failure (N) by the area of the bonded interface. The area of the bonded interface was calculated as follows: $A = 2\pi \times r \times h$, where A is the area of the bonded interface, $\pi = 3.14$, r is the radius of the post segment (mm), and h is the thickness of the post segment (mm) [28, 31, 32]. The thickness of each slice was measured using a digital caliper (Mitutoyo, Tokyo, Japan).

The failure pattern was determined after all specimens were air-dried. Both sides of the slices were analyzed using a stereomicroscope at 40× magnification (Leica DM 500B; Leica Microsystems, Heerbruigg, Switzerland). The failure pattern was classified into 6 types as follows: (i) adhesive between the post and resin cement; (ii) adhesive between the resin cement and root dentin; (iii) cohesive in cement; (iv) cohesive in dentin; (v) cohesive in post; and (vi) mixed, between post, resin cement, and root dentin [33]. The slices were evaluated by a trained examiner, blinded to the applications of chelating solutions, activation/delivery method, and root canal level.

2.1. Statistical Analysis

The 3.3.2 R software (R Core Team, Vienna, Austria) was used for statistical analysis. The Shapiro-Wilk test was used to test normality. Push-out bond strength data were analyzed using three-way ANOVA to examine the influence of chelating solution, final irrigant activation/delivery method, and root canal level. The Tukey test was used for post hoc multiple comparisons ($\alpha = 0.05$). The percentage of each type of failure within each group was calculated.

3. RESULTS

The mean bond strength values (MPa), the standard deviations, and the differences within the groups after micropush-out test are shown in Table **2**. The control and 17% EDTA + CI groups exhibited significantly lower bond strength than 0.2% Chitosan + CI, 17% EDTA + PUI, and 0.2% Chitosan + PUI groups in the cervical third ($P = 0.00$). Conversely, the cervical third had higher values than the middle and apical thirds in control ($P = 0.00$), 17% EDTA + PUI ($P = 0.00$), and 0.2% Chitosan + PUI groups ($P = 0.00$). Failure patterns are shown in Table **3**. Adhesive cement-dentin failure type was predominant in all groups (Fig. **1**).

Table 1. Control and experimental groups and final irrigant protocols.

Groups (n=10)	Chelating Solution	Irrigant Activation/Delivery Method
Group 1 (Control)	Distilled water	Conventional irrigation
Group 2	17% EDTA	Conventional irrigation
Group 3	0.2% Chitosan	Conventional irrigation
Group 4	17% EDTA	Passive ultrasonic irrigation
Group 5	0.2% Chitosan	Passive ultrasonic irrigation

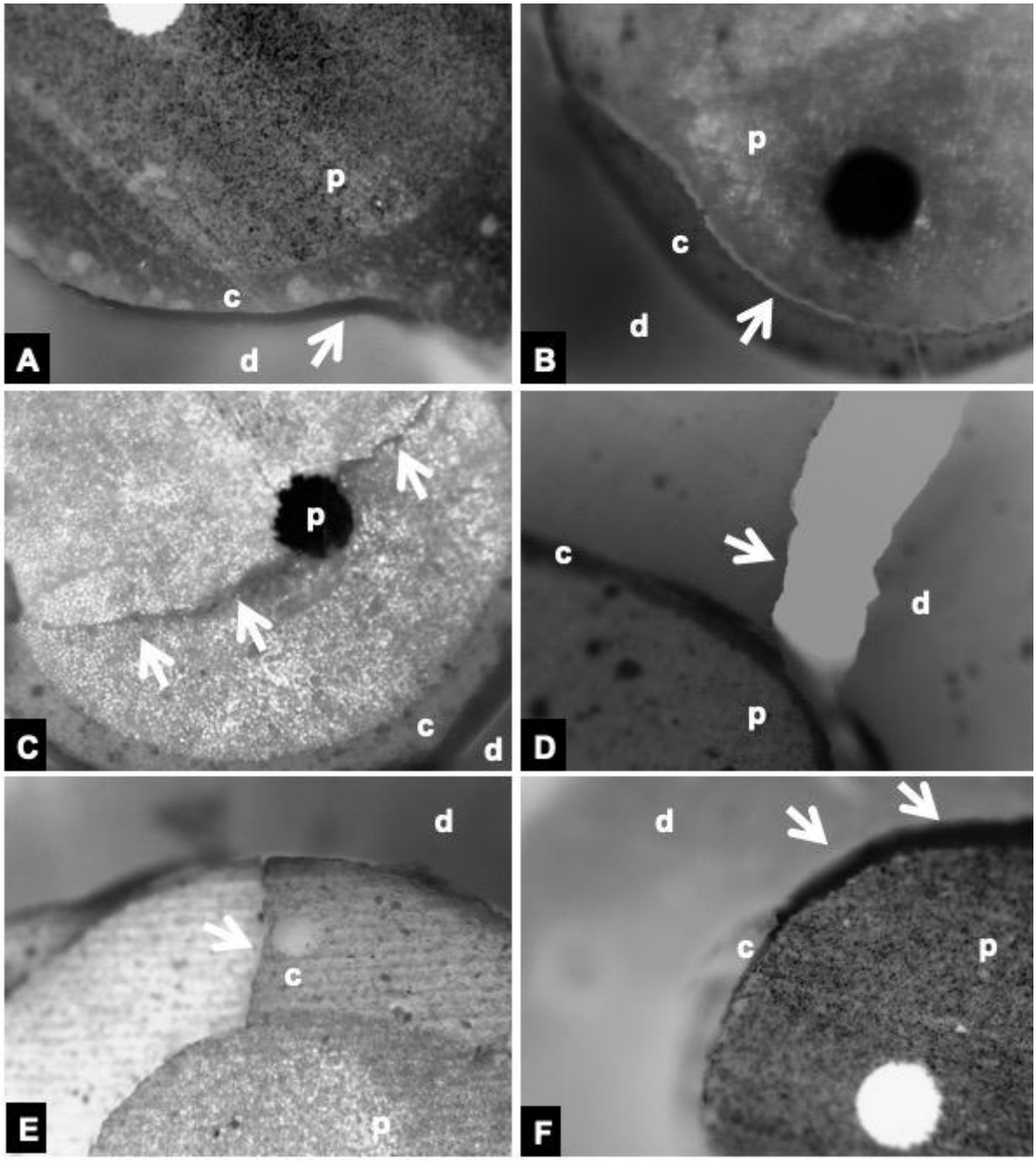

Fig. (1). Light microscopy of failure mode: (**a**) adhesive between resin cement and root dentin, (**b**) adhesive between resin cement and post, (**c**) cohesive in post, (**d**) cohesive in dentin, (**e**) cohesive in cement, (**f**) mixed, between post, resin cement and root dentin. White arrows point the failure area. d = dentin, c = resin cement, p = fiber glass post.

Table 2. Mean bond strength values in MPa and statistical categories according to the Tukey test (n = 10).

Root Third	Final Irrigant Protocols					-
	Group 1	Group 2	Group 3	Group 4	Group 5	P-value
Cervical	10.86 ± 4.64^{Ba}	7.06 ± 4.32^{Ba}	12.80 ± 6.43^{Aa}	13.31 ± 3.08^{Aa}	14.33 ± 5.37^{Aa}	0.00
Middle	8.83 ± 5.21^{Ab}	6.51 ± 4.57^{Aa}	8.96 ± 4.93^{Ab}	8.45 ± 3.08^{Ab}	8.14 ± 3.19^{Ab}	0.38
Apical	6.95 ± 4.67^{Ab}	5.59 ± 3.03^{Aa}	8.14 ± 3.39^{Ab}	7.32 ± 2.67^{Ab}	7.85 ± 4.12^{Ab}	0.17
P value	0.00	0.51	0.01	0.00	0.00	-

Group 1: Distilled water (control); Group 2: 17% EDTA + CI; Group 3: 0.2% Chitosan + CI; Group 4: 17% EDTA + PUI; Group 5: 0.2% Chitosan + PUI.
Capital letters compare groups in horizontal lines and lower-case letters in vertical lines. Different letters are statistically different from each other (P<0.05).>

Table 3. Failure patterns for experimental groups (%).

Failure Patterns	Smear Layer Removal Protocol				
	G1	**G2**	**G3**	**G4**	**G5**
(i)	8.33	5	1.67	10	5
(ii)	68.33	85	78.34	61.67	78.33
(ii)	5	5	1.66	-	-
(iv)	16.67	5	8.33	26.66	15
(v)	1.67	-	3.33	1.67	1.67
(vi)	-	-	6.67	-	-

Group 1: Distilled water (control); Group 2: 17% EDTA + CI; Group 3: 0.2% Chitosan + CI; Group 4: 17% EDTA + PUI; Group 5: 0.2% Chitosan + PUI. (i) adhesive between the post and resin cement; (ii) adhesive between the resin cement and root dentin; (iii) cohesive in cement; (iv) cohesive in dentin; (v) cohesive in post; and (vi) mixed, between post, resin cement and root dentin.

4. DISCUSSION

The null hypotheses tested in this study were rejected. Instead, the results demonstrated that the type of chelating solution, irrigant activation/delivery method, and the thirds of the root canal significantly affected the bond strength of FP to root canal dentin.

It has been demonstrated that the microtensile strength is not an appropriate method for evaluating of bond strength of intracanal materials since it promotes a high number of premature failures and high variation test results [14, 28]. On the other hand, the push-out test has demonstrated a more homogenous stress distribution, lower data variability, and no occurrence of premature failures [4, 7, 32]. Additionally, this method allows the fabrication of several specimens out of one root and testing for regional differences between root sections [28], with results comparable with the clinical conditions [3]. However, it is important to note that the specimen's geometric parameters and the elastic moduli of dentin and intracanal materials may interfere with the bond strength measurement [34]. In this way, comparing the results obtained from studies with different experimental setups should be made with caution [27].

The present study used bovine teeth, as sound human teeth are difficult to collect for dental research [3, 31, 35]. In addition, ethical issues make the use of human teeth even more difficult in scientific work [3, 31]. Bovine teeth are easier to obtain, enable a better age and canal space standardization, and reduce the risk of transmitting infectious, contagious diseases [3, 10, 27]. Despite some micro and macrostructural differences between human and bovine teeth [35, 36], several studies consider bovine teeth good substitutes for human teeth in dentin or enamel bond strength tests [10, 27, 31].

A large variety of products are commercially available for cementation of FP [2, 28]. This study selected a self-adhesive resin cement (RelyX U200), as it presents high adhesion, long-term stability, and simplicity of use when compared to conventional cement [2, 7, 37]. Self-adhesive resin cement was idealized to adhere to the tooth structure without previous acid etching [8]. Its adhesions occur through two distinct mechanisms: (i) the acidic monomers hybridize with the dentin, and (ii) the resin chemically interacts with the hydroxyapatite [17, 31]. In Bitter *et al*.'s study [33], the chemical interaction between resin-based cement and hydroxyapatite was more relevant for root dentin bonding than the material's capability to promote the hybridization of dentin. Therefore, it is expected that when applied directly to the SL covering the dentin, with no pre-treatment, the acidic monomers within self-adhesive cement demineralize the dentin and infiltrate through the mineralized tissue [37]. However, it has been demonstrated that resin cement has a low demineralization effect, especially if the dentin is covered with thick SL [38] and that this limited etching potential may cause poor adhesion [15]. In this sense, when self-adhesive resin cement is used, a chelating agent plays an important function since it has to remove the SL [8] and cannot remove excessively calcium hydroxyapatite presented in root dentin [7].

Chelators are chemicals composed of macromolecules that link to a metal ion, forming a stable, ring-shaped unit called chelato [39]. After sequestering the ion, the demineralizing solution forms a heterocyclic structure through a process called chelation [39], resulting in the breakage of the ion and facilitating its removal [40]. In the present study, two different chelating solutions (17% EDTA and 0.2% Chitosan) were examined to investigate the adhesion capability of FP to root dentin.

EDTA is the most used chelating solution in endodontics. Its reaction with the calcium ions in dentin results in calcium chelation [18], promoting decalcification of the dental structure at a depth of approximately 20-30 μm [21]. Spano *et al*. [20] evaluated the concentration of calcium ions and SL removal using 15% EDTA, 10% citric acid, 10% sodium citrate, apple cider vinegar, 5% acetic acid, 5% malic acid, and 1% sodium hypochlorite. The authors concluded that 15% EDTA resulted in the highest concentration of calcium ions, followed by 10% citric acid and that 15% EDTA and 10% citric acid were the most efficient solutions for removing SL. Despite the proven effectiveness of EDTA, increasing concern about its use has been observed. The widespread use of this solution by industry has resulted in a significant increase of this compound in rivers and lakes [12, 21]. Because EDTA has not been found naturally in nature, it has been considered a pollutant [20].

Chitosan is an oligosaccharide that has multiple functional properties [23]. This substance has a high chelating capacity for different metallic ions [22], being extensively explored by the industry [12]. Adsorption, ionic exchange, and chelation are probably responsible for forming complexes between chitosan and metal ions [21]. Silva *et al*. [21] evaluated the

efficacy of SL removal using chitosan compared with different chelating agents (15% EDTA, 10% citric acid, and 1% acetic acid). The results showed that EDTA, chitosan, and citric acid efficiently removed the SL and that EDTA and chitosan promoted the highest demineralizing effect. Two models are reported in the literature as possible action mechanisms of Chitosan [41, 42]. The first, the bridge model, is based on the theory that two or more amino groups of a Chitosan chain link to the same metal ion [41]. The second defends the idea that only one amino group of the structure of the substance is involved in the bond, the ion being "anchored" to the amino group [42].

The present study results indicate that chelating agents and final irrigation protocols have no harmful influence on the bond strength between FP and root canal dentin. On the contrary, the use of chitosan, regardless of the final irrigant protocol, resulted in higher bond strength values (Table **2**). Unfortunately, no previous published study on the effect of chitosan on the bond strength of FP to root dentin has been found, making it difficult to explain and compare the results. However, Xu *et al*. [43] demonstrated that the covalent immobilization of chitosan could significantly induce the deposition of calcium phosphate minerals on the surface of the partially demineralized dentin. Furthermore, Shrestha *et al*. [44] demonstrated that chitosan treatment improves the resistance of the dentin surface to enzymatic degradation, stable ultrastructure, and increased tensile strength. These results could help understand the high bond strength values obtained in 0.2% chitosan + CI and 0.2% chitosan + PUI protocols. Further studies based on confocal laser scanning microscopy (CLSM) are needed to better understand the repercussions of the use of chitosan in the formation of a hybrid layer [45].

On the other hand, the 17% EDTA + CI group exhibited the lowest bond strength values, with no significant difference for the control group, which received no final irrigant protocol (Table **2**). Likely, the low bond strength values observed for the 17% EDTA + CI protocol are associated with its limited ability to remove SL and thereby clean the root canal walls [8]. The presence of SL along the root canal walls hinders penetration and adaptation of self-adhesive resin sealers [15, 16], promoting weak areas in the bonding interface, which will reduce the bond strength [5]. PUI of EDTA resulted in an improvement in bonding strength values, which could be explained by the cleaning effect of PUI [19]. However, Barreto *et al*. [7] observed that EDTA use associated with ultrasonic activation might lead to dentin erosion, leading to a bond strength reduction.

In the present study, all groups' mean bond strength values were higher in the cervical and lowered in the apical thirds, as reported in previous investigations [3, 10, 27]. The significantly lower values from the apical third observed may be explained by a large amount of gutta-percha and sealer remaining in this region [17]. The presence of a large amount of filling material in the apical third and the absence of a homogenous bond interface [3] could, therefore, reduce the contact area between dentin and cementing agent, thus reducing polymerization of the resin cement [3]. Also, limitations in the flow of the viscous cement, reduced accessibility to the apical area, the factor cavity configuration (C-factor), and differences in the anatomical and histological characteristics of different regions of the root canal [2, 17, 28] may contribute to these results.

In this study, most adhesive failures occurred at the interface between root canal dentin and resin cement (Table **3**). This result conforms with the results of previous studies demonstrating that FP cemented with self-adhesive resin is weakest at the resin cement-root dentin interface [6, 7, 27, 45] and could be attributed to the presence of residues of filling materials on the root canal walls and inside dentinal tubules, chemical and structural alteration of root dentin, the type of adhesive system and cementation strategies [5, 7].

Some limitations in the present study should be considered: (i) samples were not submitted to thermal and mechanical influences, which may simulate oral cavity conditions and provide more realistic results [46, 47]; (ii) a control group with PUI was not performed. As the isolated action of PUI could not be stated, (iii) the teeth were decoronated, which may eliminate any coronal reservoir of the chelating solution. Therefore, when activating the solution using PUI, a considerable amount of the solution may be lost coronally.

Further clinical studies are needed to confirm the present study's results and evaluate the effect of new chemical and mechanical protocols of the SL removal on the long-term stability of composite resin build-up using FP with self-adhesive resin cement.

CONCLUSION

Within the limitations of this study, it may be concluded that:

1. The use of chitosan in different final irrigant protocols did not influence the bond strength of fiber posts cemented with self-adhesive resin cement to root dentin.

2. PUI of chelating solutions resulted in an improvement in bonding strength.

LIST OF ABBREVIATIONS

CI	=	Conventional Irrigation
RCP	=	Root Canal Preparation
SC	=	Smear Layer
WL	=	Working Length

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the Northern University of Paraná (approval number 046-15).

HUMAN AND ANIMAL RIGHTS

No human/animal were used in this research. All research procedures were in accordance with the ethical standards of the institutional and/or national research committee.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available in the (University of North Paraná) at (https://repositorio.pgsskroton.com/bitstream/123456789/3008/1/Tese%20Maura%20Cristiane%20G.%20O.%20Dorilêo.pdf).

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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