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ORIGINAL ARTICLE

Effect of a root dentin deproteinization protocol on self-adhesive cementation of fiber posts

ABSTRACT

Aim: *This study evaluated the effect of a root dentin deproteinization protocol on the bond strength of fiber post cemented with self-adhesive resin cement.*
Materials and Methods: *Twenty-four single-rooted teeth were randomly divided into two groups (n=12), as follows: G1 - cementation with self-adhesive resin cement RelyX U200 (3M/ESPE) according to manufacturer's instructions; G2 - treatment of root dentin with phosphoric acid at 37% for 15 s followed by sodium hypochlorite (NaOCl) at 5% for 5 minutes, and post cementation according to manufacturer's instructions. A light-emitting diode (LED) unit was used for photoactivation and after 24h at 37 °C in absolute humidity, teeth were sectioned into slices of 1 mm divided by different root thirds (cervical, middle and apical) and submitted to the push-out bond strength test. Two-criteria ANOVA was used to determine the statistical significance between groups ($p<0.05$).*
Results: *No statistical difference in bond strength between group cemented as manufacturer's instructions and group submitted to deproteinization was detected ($p>0.05$). As well as there was no statistical difference between the different root thirds evaluated ($p>0.05$).*
Conclusions: *The deproteinization protocol proposed was not able to improve the bond strength of self-adhesive cement to root dentin.*

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Introduction

Endodontically treated teeth often have little remaining of coronal structure and require intraradicular retainers prior to restoration (1, 2). Nowadays, there is a variety of intraradicular posts and cements for the rehabilitation of these teeth (3). Fiber posts have been widely used to restore endodontically treated teeth when the dental remnant cannot provide adequate support and retention for restoration, and are presented as an alternative to cast metal post and core (3), since their elasticity modulus are similar to dentine, as well as they have good aesthetic properties and absence of corrosion (4, 5). However, the lack of intimate adaptation between fiber post and the inner walls of the root canal requires an adhesive cementation for greater retention (6). Conventional or self-adhesive resin cements are indicated as the material of choice for performing post cementation, particularly because they provide good aesthetics and good mechanical strength, besides low solubility and good bonding to the root dentin (7).

Self-adhesive cements were introduced in order to simplify the adhesive technique, so they are used in a single application and theoretically do not require pretreatment of dental surfaces, as there is a chemical reaction between acidic phosphate methacrylates and dental hydroxyapatite (8, 9). However, they have limited infiltration in dental tissues and some studies have shown low bond strength to dentin (10, 11). Thus, it is a subject that still generates some discussions, mainly in relation to the adhesion between fiber post and root dentin, mainly because of the difficulty in removing dentin smear layer, drying the excess moisture from root canal dentin, in addition to the complete removal of gutta-percha and endodontic cement of the root canal walls after endodontic treatment (12).

The mechanical preparation of the root canal generates a smear layer that is deposited on the dentin walls and which consists mainly of mineral and organic residues. The presence of this residual layer impairs the adequate contact between root dentin and adhesive/cement during the cementation process, thus interfering in bond strength to dentin. In this way, partial or total removal of the smear layer prior to cementation may improve post retention (4). Studies have proposed root dentin pre-treatment with sodium hypochlorite (NaOCl) to remove the smear layer and improve the bond strength between resin cement and dentin (13-15). However, it is unclear whether this beneficial effect would occur for self-adhesive resin cements (4). NaOCl is a common antibacterial irrigant used in chemical-mechanical preparation of the root canals, on the other hand it is a potent antioxidant agent that can create an oxygen-rich layer on the dentin wall inhibiting the resin-based cement polymerization, thus reducing the bond strength between cement and root dentin (16). However, according to Sung et al. (17) independent of the irrigating agent used, the bond strength of a composite to dentin is much more dependent on the bonding agent used. In addition, a systematic review showed that the type of cement used strongly influences the retention of the fiber posts in the root canals, however there are other variables that also influence, such as cement application protocol, irrigation product and protocol, sample storage conditions, among others (18). Some studies have evaluated deproteinization protocols with different irrigants and bonding agents in an attempt to improve the bond strength between fiber posts and root dentin (5, 6, 19, 20), however the results were quite variable and dependent on the methodologies used in each study.

Thus, the aim of this study was to evaluate the effect of a root dentin deproteinization protocol in the bond strength of a fiber post cemented with a self-adhesive resin cement. In addition, the influence of the root thirds (cervical, middle and apical) in bond strength and the pattern of fracture of the specimens were assessed. The null hypothesis tested was that the deproteinization protocol is not capable of producing a higher bond strength when compared to conventional cementation protocol. As well as there is no difference between the bond strength obtained in the different root thirds evaluated.

Materials and Methods

This study was approved by the Institutional Research Ethical Committee, protocol number (PT/0439/11). Twenty-four single-rooted human teeth extracted for periodontal or orthodontic therapeutic reasons were selected. For inclusion, teeth should be free of coronal or root caries, absence of endodontic treatment, root length of at least 14 mm and straight single root with a single canal.

Preparation of the roots

The teeth were cleaned of soft tissue and calculus and sectioned below the enamel-cement junction perpendicular to the long axis with the aid of a diamond blade under water cooling. After, the teeth were stored in 0.1% thymol solution at 4 °C for no longer than two months. The coronal access was made with a K-file #15 (Dentsply Maillefer, Ballaigues, Switzerland) placed into the canal until it was visible at the apical foramen. Then, the working length was set 1 mm shorter than this length. K-files #15 to 40 were used for the preparation of 1 mm from the apical foramen opening. The canals were irrigated between instrumentation with 2 mL of NaOCl at 5% and 2 mL of EDTA at 17% and finally irrigated with 5 mL of distilled water and dried with paper points. Finally, in the obturation process, the single cone technique was used (Odous De Deus, Belo Horizonte, Brazil) together with the AH Plus™ endodontic cement (Dentsply DeTrey GmbH, Konstanz, Germany). The gutta-percha was cut with heated Paiva plugger and vertical condensation technique was performed at the entrance of the channel. After seven days, the root canal enlargement was performed with a Largo drill system (1, 2 and 3 respectively) at a distance of 4 mm from the apical foramen. After, the low speed drill provided by the manufacturer was used, under constant irrigation with distilled water. Then, the root canals were again gently dried with paper points and randomly divided into two groups.

Cementation protocols

Twelve specimens were assigned to group 1 (G1), where fiber posts were cemented according to the manufacturer's instructions, which consists of abundant irrigation of the root canal with distilled water and drying with absorbent paper cones. In group 2 (G2; n=12), fiber posts were cemented after deproteinization of the root dentin. Phosphoric acid at 35% was applied inside the root canal for 15 s and washed thoroughly with distilled water for 30 s. The root canal was dried with absorbent paper cones and flooded with NaOCl at 5% for 5 minutes. After, the root canal was again irrigated with 5 mL of distilled water, and again dried with absorbent paper cones.

Before the cementation protocol of the two groups each post was cleaned with alcohol, dried and covered with a silane layer (RelyX Ceramic Primer, 3M ESPE Dental Products, St Paul, MN, USA). The self-adhesive resin cement RelyX U200 (3M ESPE, St Paul, MN, USA) was taken to the interior of the root canal through the applicator and then inserted the fiber post. The excess of self-adhesive resin cement was removed and then light-activated using a light-emitting diode (LED) unit (irradiance 900 mW/cm^2 [Radii-cal LED; SDI, São Paulo, SP, Brazil]) through the post for 40s. The irradiance was measured with a digital power meter (Ophir Optronics, Danvers, MA, USA). The roots were stored at 37 ºC and 100% of humidity for 24h until the push-out bond strength test. All roots were prepared and restored by a single trained operator and following the manufacturers recommendations.

Push-out bond strength analysis

After 24h the roots were positioned and fixed in a block of acrylic resin and sectioned transversely. Twelve slices were made on each root generating 6 specimens of 1 mm per root with a diamond steel disc in a cutting machine (Isomet 2000, Bue-

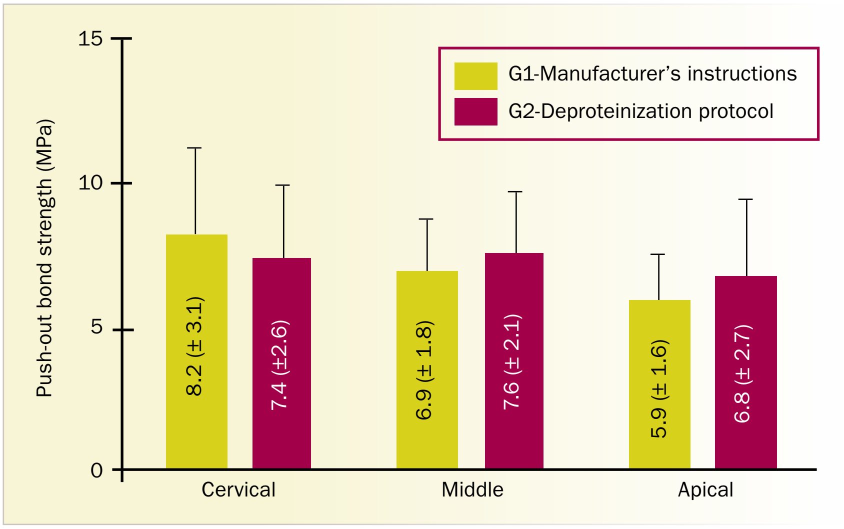

Figure 1.
Push-out bond strength (MPa) of the different groups tested (with or without root dentin deproteinization protocol prior to the application of self-adhesive resin cement RelyX U200) and the different root thirds evaluated. There was no statistical difference between the treatments proposed, as well as there was no difference between the root thirds evaluated.

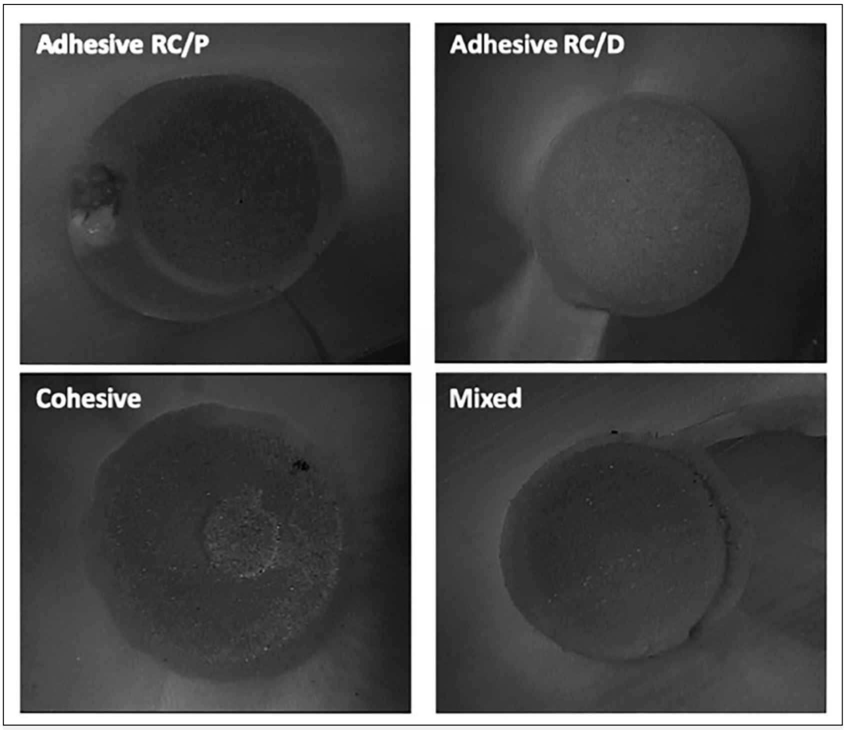

Figure 2.
Representative images of the different types of failures assessed under light optical microscope (40x).

hler, Lake Bluff, IL, USA) under water-cooling at 200 rpm. The first cut was made at 1 mm from the enamel-cement junction obtaining 6 slices per teeth respectively, and two specimens for root region: cervical, middle and apical. Each sample was placed in a metallic matrix with a central opening larger than the diameter of the post, with the coronary portion down, which was applied a cylindrical tip 0.6 mm in diameter connected to a universal testing machine (EMIC, São José dos Pinhais, PR, Brazil) at 1.0 mm/min. A digital caliper was used to measure the thickness of the slices (h), the largest (R) and smallest radius (r) of the dentin/post interface on each specimen. Then, the bonding surface was calculated using the formula of a frustoconical:

$$A = \pi (R+r) \sqrt{(R - r)^2 + h^2}$$

The bond strength (σ) was finally obtained by the formula σ=C/A, where C=load for rupture of the specimen (kgf) and A=interfacial area (mm^2).

Analysis of the failure mode
All debonded specimens were evaluated with a light optical microscope at a magnification of 40x. Two independent operators evaluated and classified the failure modes into four categories as follows: A) adhesive failure between fiber post and resin cement; B) adhesive failure between resin cement and root dentin, C) cohesive fracture of the fiber post and D) mixed failure (when more than one classification occurred in the same specimen).

Statistical analysis
The data of push-out bond strength (MPa) were analyzed using the GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The statistical analysis was performed by two-way ANOVA (cementation protocol and root region). The level of significance was set at $p<0.05$. Data regarding failure mode was presented descriptively, with frequency distribution.

Results

Push-out bond strength test
The results indicated that the differences in values of push-out bond strength between G1 (RelyX U200 applied according to the manufacturer's instructions) and G2

Table 1
Distribution of type of failure occurred in each group in percentage

Type of failure	G1	G2
Adhesive RC/P*	5.6	0
Adhesive RC/D**	61.1	52.8
Cohesive***	0	2.8
Mixed****	33.3	44.4

**RC/P Adhesive failure between resin cement and fiber post.*
***RC/D Adhesive failure between resin cement and root dentin.*
****Cohesive failure of the post or root dentin.*
*****Mixed failure when more than one classification appeared in the same specimen.*

(deproteinization protocol) were not statistically significant. Also, no significant statistical difference was detected between push-out bond strength values for the different root thirds evaluated (Figure 1).

Failure pattern

Thirty-six specimens were evaluated for each group in relation to failure pattern, and a similar behavior was observed for both groups (Table 1). All debonded specimens were evaluated using a light optical microscope at 40x magnification. The adhesive failure pattern between cement and dentin was predominant in the two groups tested, followed by mixed fractures. Only two specimens presented adhesive fracture between cement and post and one specimen presented cohesive fracture in the post (Figure 2).

Discussion

In the present study, the application of phosphoric acid at 35% followed by NaOCl at 5% was used for remove the smear layer and to demineralize the root dentin, followed by the deproteinization of the collagen, respectively. Theoretically the deproteinization would promote a greater contact between the resin cement and the inorganic portion of the dentin, which could improve adhesion. However, the results demonstrated that dentin deproteinization did not produce any benefit to the bond strength. Similarly in a study using RelyX U100 self-adhesive resin cement, dentin deproteinization did not improve adhesion performance either (6).
Studies show that the bond strength values of self-adhesive cements are similar to the bond strength values of conventional cements (21, 22), however, for this to occur, the root canal must be previously irrigated and cleaned to remove the smear layer and debris after removing gutta percha and endodontic cement (23). However, some studies have shown that the different irrigation systems, whether activated or not, are not able to completely remove the root canal sealer remnants (24, 25), and interfere with the bond strength (26).
Some studies have demonstrated the limited ability of self-adhesive resin cements to demineralize and dissolve the smear layer to reach the underlying dentin (9, 27, 28). The high viscosity of this type of cement limits its capacity of demineralization (29). Since the adhesion of the self-adhesive cement occurs through the intimate contact between cement and dentin by the micromechanical retention and chemical bond, the dentin smear layer, together with the other debris, must be removed. And for this to occur satisfactorily, the irrigation protocol is essential (20). Several studies have already tested different irrigants and their properties in relation to adhesion with the dental structure, such as EDTA and citric acid (30), 2% chlorhexidine (20, 31), sodium hypochlorite and calcium hypochlorite (20), however the most widely used irrigants, which are EDTA and NaOCl, showed unsatisfactory results and may negatively affect the results of the endodontic treatments (30). Thus, there is a need for the development of new irrigators with bacterial action and that do not interfere in the bond strength. On the other hand, NaOCl is still the most tested irrigator as a deproteinizing agent, as it promotes changes in the structure of dentin, mainly in the protein content of dentin (32).
In one study, the authors concluded that the use of NaOCl as a pretreatment for root dentin also does not interfere in the immediate adhesion of fiber posts. However, the evaluation after 12 months showed that the bond strength values obtained with the NaOCl treatment were higher than those obtained with the conventional

technique. On the other hand, the effect of coronal dentin deproteinization on the bond strength has also been studied and the results showed a reduction, or no beneficial effect in adhesion, independently of the adhesive system used (33-35). Perdigao et al. (36) demonstrated with transmission electron microscopy (TEM) an incomplete removal of the collagen fibers along the resin-dentin interface and in the walls of the dentinal tubules after treatment with NaOCl at 10% for 60s. Other authors, who also used TEM, reported that the use of NaOCl at 5% for 120s did not completely remove the collagen fibrils from dentin (37). Sauro et al. (38) reported that the application of NaOCl at 12% even for 10 minutes was not able to completely remove the collagen from the porous surface of the intra and intertubular dentin walls. Deproteinization with NaOCl is a recognized treatment for removing the organic matrix, leaving a clean and mineralized surface (5, 38). However, in some studies, NaOCl has been shown to be harmful when used as an irrigant (39), or as deproteinizing agent (6), this can occur because this effect on dentin is not always homogeneous. Thus, the concentrations and time of application play an important role in the process (38). In addition, NaOCl dissociates in sodium and oxygen and this can interfere with the polymerization of the cement, leading to reduced adhesion (40). However, a long-term *in vitro* study concluded that the use of NaOCl as a deproteinizer minimized the degradation of the conventional and self-adhesive cement interface (32). Nevertheless, Seballos et al. (20) concluded that NaOCl and CaOCl should not be used as deproteinizing agent, as they negatively affected the bond strength between fiber posts cemented with self-adhesive resin cement and root dentin. Thus, our findings could be better interpreted with the aid of complementary techniques to morphologically evaluate the deproteinization of the root dentin.

Regarding the adhesion at the different root thirds, no significant differences were found between the thirds in the two groups evaluated in this study. Other research has shown that the RelyX U100 self-adhesive cement showed the same performance in the three different root thirds evaluated (apical, middle and cervical), producing an uniform bond strength throughout the root (6). One hypotheses has been proposed to explain this, some authors suggest that this may occur due to the good adaptation promoted between the fiber post and the root canal after the introduction of the customized drilling systems by the manufacturers (41). This factor would cause a decrease in the thickness of the resin cement, which could consequently reduce the polymerization shrinkage as well as the polymerization stress (42).

Many methods have been used to measure the bond strength between endodontic posts and tooth structure (43). In the present study, the push-out test was used because it has the benefit of simulating clinical conditions as closely as possible. In our study, a predominance of adhesive failure between cement and dentin was observed, followed by the mixed failure type. No difference was observed between the fracture pattern of the groups cemented with or without deproteinization. Similar studies also demonstrate that the most prevalent failure type was adhesive between cement and dentine, followed by mixed failure (5, 44). As this is an *in vitro* study, the present study has the characteristic limitations of these methodologies. The attempt to increase the contact between the cement and the mineralized dentin tissue, by the total or partial removal of the collagen, was not able to produce better results of bond strength. Another limitation of the present study is that the surface of the root dentin has not been morphologically evaluated to observe the effects caused by NaOCl.

Conclusions

There was no difference in the performance of the self-adhesive resin cement tested when applied with or without root dentin deproteinization protocol proposed. Thus, despite not impairing adhesion, the proposed deproteinization protocol would only represent the addition of a clinical cementation step, which may increase technique sensitivity and chair time.

Clinical Relevance

Deproteinization protocol would only represent the addition of a clinical cementation step, which may increase technique sensitivity and chair time

Conflict of Interest

None.

Acknowledgements

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