Bond Strength between Fiber Posts and Root Dentin Treated with Natural Cross-linkers

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Abstract

Introduction: The aim of this study was to investigate the effects of a proanthocyanidin-rich extract (grape seed extract [GSE]) on the bond strength and stability of the adhesion of fiber posts to the root dentine using 2 adhesive systems: a total-etch and a self-etch adhesive system. **Methods:** Single-rooted human teeth were randomly divided into 6 groups: G1 (control), untreated + total-etch adhesive Adper Scotchbond Multi-Purpose (3M ESPE, St Paul, MN); G2, 6.5% GSE for 5 minutes + SB; G3, 10% GSE for 5 minutes + SB; and G4 to G6 groups were similar to previous ones; however, the self-etch adhesive system Clearfil SE Bond (Kuraray, Kurashiki, Japan) was used. Fiber posts were cemented with RelyX ARC (3M ESPE), and the specimens were immediately tested for push out or stored for 12 months. The bond strength means were analyzed by analysis of variance and Games-Howell post hoc tests ($\alpha = 0.05$). Additionally, matrix metalloproteinase inhibition by 0.65%, 0.065%, and 0.0065% GSE was examined with gelatin zymography. **Results:** The use of GSE did not affect immediate bond strength to dentin and contributed to preserve the bond strength after 12 months ($P < .05$) for both adhesives. The bond strength of SB was significantly higher than Clearfil SE Bond ($P < .05$). Gelatin zymography showed reduced matrix metalloproteinase activity when recombinant enzymes were incubated with both 0.65% and 0.065% GSE, with complete inhibition at the highest concentration. **Conclusions:** The pretreatment with GSE can be used as a natural alternative to improve bond strength stability of dentin–adhesive interfaces in root canals. *(J Endod 2015;41:1667–1671)*

Key Words

Bond strength, durability, fiber post, grape seed extracts, root canal

Glass fiber posts have been indicated for the restoration of endodontically treated teeth mainly because of their similar elastic modulus to human radicular dentin. They uniformly distribute stresses along the post-cement-dentin interface and to the remaining tooth structure, thus avoiding stress concentration and minimizing the risk of vertical root fractures (1). Resin luting cements are used to bond the posts to root canal dentin in association with a total-etching or self-etching primer (2). Nevertheless, both *in vivo* and *in vitro* studies have shown that loss of retention is the predominant failure mode in fiber post restorations (2, 3).

Resin-dentin adhesion is a complex procedure, and a lack of bond strength stability has been observed (2, 4, 5). The loss of integrity of resin-dentin bonds over time is likely because of the combined effect of hydrolytic deterioration of resinous components (6) and the host-derived enzymatic degradation of collagen fibrils (7). The latter is attributed to an endogenous proteolytic mechanism involving the activity of matrix metalloproteinases (MMPs) (7) found in the coronal (8) and radicular dentin (9). Furthermore, cysteine cathepsin (CT) activity has also been recently detected in dentin and correlated with MMP activity (10).

Studies have shown that chlorhexidine (CHX) digluconate has beneficial effects on the preservation of resin-dentin bonds by inhibiting both MMPs (2, 7) and CTs (11). However, a potential disadvantage is that CHX may leach out of hybrid layers within 18 to 24 months (12). Another option for improving the stability of dentin collagen is the use of natural cross-linking agents. Some proanthocyanidin (PAC)-rich plant extracts have been shown to stimulate interfibrillar, intrafibrillar, and intermicrofibrillar cross-links in the collagen matrix (13–17). PAC-rich extract (grape seed extract [GSE]) increases the biomechanical properties and biostability of demineralized dentin matrix (14, 15) and the immediate resin–dentin bond strength (13). Furthermore, Epasinghe et al (17) showed that PACs inhibit recombinant MMPs and CTs and also reduce dentin matrix degradation more efficiently than CHX. However, most of the studies that evaluate the effects of enzyme inhibition and collagen cross-linking on the durability of adhesive restorations and stability of dentin matrix were performed using coronal dentin; therefore, the literature does not provide data about the effect of PAC on the bond strength and adhesive durability of posts to root dentin.

Therefore, the aim of this study was to investigate the effect of different concentrations of a PAC-rich extract (GSE) on the long-term bond strength of fiber posts to the root dentin using 2 different adhesive strategies: a total-etch and a self-etch adhesive system. Moreover, the inhibition of purified MMPs by different concentrations of GSE was evaluated. The tested null hypothesis was that, irrespective of the concentration,

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dentin pretreatment with GSE could not prevent the reduction of bond stability of fiber posts to root dentin after 12 months of water storage.

Materials and Methods

Specimen Preparation

Seventy-two single-rooted human teeth with anatomically similar root segments and fully developed apices were selected. Each tooth was decoronated below the cementoenamel junction perpendicular to the longitudinal axis. The roots were cut to a uniform length of 14 mm from the apical end.

To remove pulp tissue and predentin, the root canals were enlarged using files #15, #20, #25, and #30 (Dentsply Maillefer, Ballaigues, Switzerland) and #1, #2, and #3 Largo burs (Dentsply Maillefer). The apical end (1 mm) was left unprepared to prevent the apical extrusion of solutions and luting cement. Roots were rinsed with 5 mL 0.9% sodium chloride (NaCl) solution to remove the remaining debris and were divided as follows:

1. *Control group:* No treatment
2. *Experimental groups:* 6.5% or 10% GSE (*Vitis vinifera*, Mega-Natural gold grape seed extract; Polyphenolics Madera, CA) for 5 minutes

The Scotchbond Multi-Purpose (SBMP) total-etching adhesive system was used in half of the samples of each group, and the Clearfil SE Bond (CB) self-etching adhesive system was used in the other half. GSE was applied after 37% phosphoric acid etching and before primer application when the SBMP was used. In the samples in which CB was used, GSE was applied before primer application. Both adhesive systems were light cured for 40 seconds using a halogen light-curing unit operated at 600 mW/cm^2 (Optilux; Demetron Res Corp, Danbury, CT).

The intracanal restoration was performed using fiber glass posts no. 3 (Angelus, Londrina, PR, Brazil). SBMP or CB adhesives were applied to the post surface and immediately polymerized for 20 seconds on each side. The dual-polymerizing resin luting material RelyX ARC (3M ESPE, St Paul, MN) was mixed and injected into the prepared root canal with a Centrix syringe (Centrix Dental Inc, Shelton, CT) using an appropriate needle (20-G). Subsequently, the fiber post was covered with cement, seated inside the root canal, and kept under finger pressure for 20 seconds with the excess cement removed. The cement was light polymerized for 30 seconds on each surface (buccal, palatal, mesial, and distal), resulting in a 2-minute light polymerization cycle. Specimens of each group were randomly divided into 2 subgroups according to their storage: 24 hours and 12 months of storage in water at 37°C.

Push-out Test: Specimen Preparation, Post Dislodgment, and Failure Pattern Analysis

Each root was cut horizontally with a slow-speed, water-cooled diamond saw (Isomet 2000; Buehler Ltd, Lake Bluff, IL) to produce 7 slices approximately 1-mm thick. The first slice from the top was excluded. Thus, 6 slices were considered from each root canal (n = 36).

The push-out test was performed by applying a load at 0.5 mm/min to the apex in the direction of the crown until the fiber post relined segment was dislodged from the root slice. Care was also taken to ensure that the contact between the punch tip and the fiber post section occurred over the most extended area as possible to avoid a notching effect of the punch tip on the fiber post's surface. Furthermore, the punch tip was centralized in the root canal and positioned to contact only the post/cement without stressing the surrounding root canal walls. The push-out bond strength was measured with a universal testing machine (EMIC DL 2000; São José dos Pinhais, PR, Brazil). To express the bond strength in megapascals, the load at failure recorded in newtons was divided by the area (mm^2) of the post-dentin interface. To calculate the bonding area, we used the formula $\pi(R + r)\ [(h)^2 + (R - r)^2]^{0.5}$, where R represents the coronal root canal radius, r the apical root canal radius, and h the thickness of the slice (2). The thickness of each slice was measured using a digital caliper.

The bond strength values were expressed in megapascals. The debonded specimens were measured under 20× magnification with a stereoscope to classify the failure pattern into 5 types (2):

1. Adhesive between the fiber post and resin cement (no cement visible around the post)
2. Mixed, with resin cement covering 0%–50% of the post's circumference
3. Mixed, with resin cement covering 50%–100% of the post's surface
4. Adhesive between resin cement and root canal (post enveloped by resin cement)
5. Cohesive in dentin

Bond strength values were statistically analyzed by 3-way analysis of variance (3 dentin pretreatment × 2 adhesive systems × 2 storage conditions) followed by Games-Howell post hoc tests. The significance level was set at α <0.05.

Gelatin Zymography

MMP inhibition by GSE was assessed by gelatin zymography as previously described (18). GSE extract was prepared at different concentrations in distilled water, and the pH was adjusted to 7.4. Recombinant MMP-2 and -9 (AnaSpec, Inc, Fremont, CA) were preincubated with 0.65%, 0.065%, and 0.0065% GSE for 15 minutes at 37°C and subjected to electrophoresis under nonreducing conditions on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels copolymerized with 0.1% gelatin from porcine skin (Sigma-Aldrich, St Louis, MO). Gels were washed twice in 2.5% Triton-X 100 (Sigma-Aldrich) for 30 minutes under agitation and then incubated for 24 hours at 37°C in an incubation buffer (50 mmol/L Tris-HCl, 5 mmol/L $CaCl_2$, and 0.1 mmol/L $ZnCl_2$, pH = 7.5). Negative control gels were incubated in the same buffer containing 2 mmol/L 1,10-phenanthroline. After incubation, gels were stained in 0.2% Coomassie Brilliant Blue R-250 (Bio-Rad

TABLE 1. Bond Strength Results (mean [standard deviation]) Obtained in Each Experimental Condition (N = 36)

Groups	Scotchbond Multi-Purpose		Clearfil SE Bond	
	Immediate	12 months of storage	Immediate	12 months of storage
Control (NaCl)	6.36 (1.70)^{a,b}	4.22 (1.75)^{c,e}	3.34 (1.31)^{e}	1.86 (0.89)^{f}
GSE 6.5%	7.55 (2.25)^{a}	5.84 (2.31)^{a,b,c}	3.70 (1.10)^{e}	3.72 (1.04)^{e}
GSE 10%	7.15 (1.54)^{a}	5.34 (1.92)^{b,c,d}	4.29 (1.41)^{c,e}	3.98 (1.11)^{d,e}

GSE, grape seed extract; NaCl, sodium chloride.

Means followed by different letters are significantly different.