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Effect of natural collagen cross-linker concentration and application time on collagen biomodification and bond strengths of fiber posts to root dentin

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ABSTRACT

Proanthocyanidin-rich plant-derived agents have been shown to increase dentin biomechanical properties and resistance of dentinal collagen to degradation. This study investigated the effects of proanthocyanidin-rich extract (grape seed extract, GSE; *Vitis vinifera*) on the mechanical properties of demineralized dentin matrix, and the bond strength and stability of the adhesion of fiber posts to the root dentin. Demineralized dentin beams ($n = 15$) were incubated with 6.5% or 10% GSE for 30, 60, or 120 s. The modulus of elasticity (E) and the fold increase in E were determined by comparing specimens at baseline and after treatment. The data were analyzed by two-way ANOVA and Tukey post hoc tests ($\alpha = 0.05$). For the bond strength test, single-rooted human teeth were randomly divided into seven groups: control group, no treatment; and experimental groups as described for the modulus of elasticity. Fiber posts were cemented with RelyX U200. Each group was randomly divided into two subgroups: 24 hours of water storage and 12 months of water storage. All roots were sectioned transversely in the coronal, middle, and apical regions, producing 1-mm thick slices, and the push-out test was performed. The failure modes were observed, and the bond strength means were analyzed by ANOVA ($\alpha = 0.05$). For E test, no significant difference was observed among the incubation times ($P > 0.05$); however, high concentration of GSE (10%) resulted in a statistically higher fold increase in E compared to 6.5% ($P < 0.05$). For the push out test, the immediate and stored for 12 months groups showed similar bond strength values with or without pre-treatment with GSE ($P > 0.05$), irrespective of the GSE concentration, time of treatment, and root regions ($P > 0.05$); however, the use of GSE contributed to a significant reduction in adhesive failure mode. The results indicate that 10% GSE exhibits a high cross-linking potential for dentin extracellular matrix. Moreover, the bond strength of fiber posts luted to root dentin with RelyX U200 remained stable after 12 months of water storage, with or without pre-treatment with GSE.

1. Introduction

In endodontically treated teeth that have missing a large quantity of coronary structure, the use of intraradicular posts is indicated to supply the retention of the restoration [1]. Glass fiber-reinforced posts have acquired popularity in oral rehabilitation procedures due to their elastic modulus similar to the root dentin, in objection to the elevated modulus of elasticity of cast metal post and core systems, which increases the chance of irrecoverable cracking of the remaining tooth structure [2–4]. However, adhesion between resin and dentin is considered to be a fragile factor for luting the fiber post [5,6]. Poor bonding of fiber posts may result from mechanical damage induced by occlusal forces [7] or degradation of the cement–dentin interface [6,8,9].

The degradation of resin-dentin bonds overtime is due to the agreed effect of hydrolytic deterioration of resinous components [8] and the activity of matrix metalloproteinases (MMPs) [10] and cysteine cathepsins (CTs) [11,12]. Although chlorhexidine (CHX) has been proposed as an inhibitor of hybrid layer collagen-degrading enzymes (MMPs and CTs) [8,10,12] and is known to improve the durability of bond strength [8,10], a disadvantage is that CHX may leach out of the hybrid layers

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within 18 to 24 months [13]. Proanthocyanidin (PAC)-rich plant extracts such as grape seed extract (GSE) have shown broad clinical applicability in dentistry for increasing the biomechanical properties and biostability of demineralized dentin matrix [14–16]. PACs are known to have therapeutic potential and act as immuno-modulators, antioxidants, antimutagens, and antibacterials [17]. Furthermore, Epasinghe et al. [18] showed that PACs inhibit MMPs and CTs and also minimize the degradation of the dentin matrix more efficiently than CHX.

A successful bonding of resin materials to root dentin is crucial for marginal adaptation of fiber posts. Self-adhesive cements have been used for luting fiber post due to their ease of use and lower sensibility to moisture [19]. However, no studies evaluated the adhesive durability with the use of self-resin cements for dentin treated with GSE. Therefore, the aim of this study was to investigate the effects of GSE on the modulus of elasticity of demineralized dentin matrix and the bond strength and stability of the adhesion of fiber posts to root dentin. The null hypotheses tested were the following: 1) the modulus of elasticity of demineralized dentin matrix does not increase with the use of GSE; and 2) dentin pretreatment with GSE could harm the bond strength of fiber posts to root dentin.

2. Materials and methods

2.1. Modulus of elasticity: sample preparation and biomechanical analysis

Thirty intact human molars were chosen following a protocol sanctioned by the Institutional Review Board of the University of Passo Fundo (#1.760.094). The specimens were obtained from mid-coronal dentin, as reported previously [16]. The dentin beams with 1.7 mm width × 6.0 mm length × 0.5 mm thickness were demineralized in 10% phosphoric acid (Ricca Chemical Company, Arlington, TX, USA) for 5 h. Demineralized dentin beams were randomly divided into six groups (n = 10) and treated with 6.5% or 10% GSE (*Vitis vinifera*, Mega-Natural gold grape seed extract, Polyphenolics Madera, CA, USA) pH 7.2 diluted in distilled water for 30, 60, or 120 s.

The modulus of elasticity of the demineralized dentin beams was assessed by a three-point bending testing using a 1 N load cell mounted on a universal testing machine (EZ Graph, Shimadzu, Kyoto, Japan) at a crosshead speed of 0.5 mm min^{-1}. Load displacement curves were converted to stress-strain curves and the apparent modulus of elasticity (E) was calculated at 3% strain [16]. Displacement (D) during compression was expressed in millimeters and calculated at the maximum strain of 3% using the following formula:

$$D = \frac{\varepsilon L^2}{6T}$$

where $\mathcal{E}$ is strain, L is support span, and T is thickness of the specimen. The E was determined at baseline and after exposure to different GSE concentrations and incubation times using the following formula:

$$E = \frac{PL^3}{4DbT^3}$$

where P is the maximum load, L is the support span, D is the displacement, b is the width of the specimen, and T is the thickness of the specimen. The fold increase in E before and after treatment was also calculated.

Normal distribution was checked using Kolmogorov-Smirnov test, and since the normality was satisfied, data were analyzed by two-way analysis of variance (ANOVA) and Games–Howell post hoc tests (α = 0.05). Data were analyzed using Stat Plus Analyst Soft Inc. version 6.0 (Vancouver, BC, Canada).

2.2. Push-out test: specimen preparation, post dislodgment, and failure pattern analysis

Eighty-four single-rooted human teeth with anatomically similar root segments were carefully chosen following a protocol approved by the Institutional Review Board of the local University (#631.273). Each tooth was decoronated below the cementoenamel junction, perpendicular to the longitudinal axis. The roots were sectioned to equal a length of 14 mm from the apex.

The root canals were shaped using hand files #15, 20, 25, and 30 (Maillefer, Ballaigues, VD, Switzerland) and #1, 2, and 3 Largo burs (Maillefer). Roots were rinsed with 5 mL of 0.9% sodium chloride solution (NaCl) to remove the debris and were divided as follows: control group, (no treatment) and experimental groups, complete filling of root canals with 6.5% or 10% GSE for 30, 60, or 120 s.

Intracanal restoration was done using fiberglass posts no. 3 (Angelus, Londrina, PR, Brazil). The dual-polymerizing resin luting material RelyX U200 (3 M ESPE, St. Paul, MN, USA) was mixed and inserted into the root canal with a syringe (Centrix Dental Incorporated, Shelton, CT, USA) using a proper needle (20 gauge). After, the fiber post was covered with cement and settled inside the root canal, kept under finger compression for 20 s, and the surplus of cement was removed. The cement was light-polymerized for 30 s on each surface (buccal, palatal, mesial, and distal), resulting in a 2-min light polymerization cycle. Specimens of each group were randomly divided into two subgroups according to the storage time: 24 h and 12 months storage in water at 37 °C. The water was replaced after every 15 days.

Each root was horizontally sectioned with a slow-speed, water-cooled diamond saw (Isomet 2000, Buehler) into two slices (each, 1-mm thick) for each root zone (apical, middle, and coronal). Seven slices were created from each root canal. The first slice from the apical and cervical portion was eliminated. Thus, the first two slices were used for the coronal third, the next two slices for the middle third, and the last two slices for the apical third. Thus, 12 slices originated from six teeth were taken for each group. The push-out test was carried out by applying a load at 0.5 mm/min to the apex side in the direction of the crown side until the fiber post segment was dislodged from the root slice segment. Additional care was also taken to ensure that the contact between the punch tip and the fiber post section occurred over the most extended area to prevent 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 dentin walls. The push-out bond strength was measured with a universal testing machine. To express the bond strength in megapascals (MPa), the load at failure recorded in Newtons (N) was divided by the bonded area (mm^2) of the adhesion. To calculate the bonding area, was used the formula π (R + r) $[(h)^2 + (R-r)^2]^{0.5}$, where R expresses the coronal root canal radius, r the apical root canal radius, and h the thickness of the slice [8, 9]. The thickness of each slice was measured using a digital caliper.

The debonded specimens were visualized under 20× magnification with a stereoscope to classify the failure pattern into five types: [1] adhesive between the fiber post and resin cement (no cement visible around the post); [2] mixed, with resin cement covering 0% to 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); and [5] cohesive in dentin [8, 9].

Normal distribution of the results was confirmed by Kolmogorov–Smirnov test ($P > 0.31323$). Bond strength values were statistically analyzed by three-way ANOVA. The significance level was set at $\alpha = 0.05$. The distribution of failure patterns was evaluated by Chi-square test ($P < 0.0001$). Data were analyzed using the same software as that used in the earlier test.