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Effect of dentin moisture on the adhesive properties of luting fiber posts using adhesive strategies

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Abstract: The purpose of this study was to evaluate the effect of dentin moisture (moist and dry) on the bonding of fiber posts to root dentin with different adhesive strategies (etch-and-rinse, self-etch, and self-adhesive). Seventy-two extracted single-rooted human teeth were endodontically treated and divided into six groups (n = 12) according to the moisture of dentin surface and adhesive systems as follows: a) etch-and-rinse/moist, b) etch-and-rinse/dry, c) self-etch/moist, d) self-etch/dry, e) self-adhesive/moist, and 6) self-adhesive/dry. The specimens were sectioned into six slices for push-out bond strength (BS), nanoleakage (NL) by SEM, and Vickers microhardness (VHN) of the resin cement. A universal testing machine (AG-I, Shimadzu Autograph) was used at a crosshead speed of 0.5 mm/min until post extrusion, with a load cell of 50 kg for evaluation of the push-out strength. Data on BS, NL, and VHN were evaluated by two-way ANOVA and Tukey's test ($\alpha = 0.05$). Dentin moisture as the main factor was not significantly different for the push-out test. However, higher BS values can be observed for the etch-and-rinse group. A lower percentage of NL was found in the dry dentin groups. The moisture pattern was not significant in the hardness values for the pre-etching groups. Additional moisture did not increase the evaluated properties.

Keywords: Dental Bonding; Dentin; Post and Core Technique; Resin Cements.

Introduction

Dentin has an intrinsically moist structure, composed of 50 vol% mineral in the form of a carbonate-rich apatite; 30 vol% organic matter, which is largely type I collagen; and about 20 vol% water.[1] In this way, the control of intrinsic dentin moisture added to extrinsic moisture must be considered for optimal adhesion, especially with adhesive systems that require moisture control.[2] If, on the one hand, excessive drying leads to the collapse of demineralized collagen on the surface,[1] on the other hand, the presence of excessive water negatively affects adhesion of the material.[3]

In addition to the structural characteristics of dentin that vary by region,[1] other factors are also relevant in the retention of glass fiber posts luted into the root canal, such as endodontic treatment, method of cement application, and post pretreatment.[4] Thus, in order to simplify the adhesive cementation, self-adhesive resin cements have been developed, not requiring any pre-treatment of the tooth, such as etching, priming, or bonding. According to previous systematic reviews, these cementing agents were considered a less technique-sensitive option as compared with regular resin cements.[4,5]

As moisture content after rinsing the root canal is difficult to control, self-adhesive resin cements have performed well in the cementation of glass fiber posts,[6,7] given that another characteristic pointed out by the manufacturers of self-adhesive resin cements is their greater moisture tolerance[6] because of water formation during the neutralization reaction of phosphoric acid methacrylate, basic fillers, and hydroxyapatite (data provided by the manufacturer).[7] Water plays an important role in the bonding system that involves ionization of the acidic functional monomer to demineralize, penetrate, and establish a chemical bond with calcium ions from dentin apatite, simultaneously allowing for a double (*i.e.*, micromechanical and chemical) bonding system.[8] Thus, the water present on the dentin surface together with the amount generated during cementation can influence the connection mechanism.

Literature is scarce on dentin moisture conditions for the performance of resin cements in the cementation of fiberglass posts. Thus, the aim of this study was to evaluate the effect of dentin moisture (moist and dry) of the post space on the bonding of different adhesive resin cement systems (etch-and-rinse, self-etch and self-adhesive). The null hypotheses proposed stated that the different dentin moisture would not affect a) the push-out bond strength; b) nanoleakage at the adhesive interface; and c) microhardness of resin cements.

Methodology

This study was submitted to and approved by the local Research Ethics Committee. Seventy-two extracted single-rooted human teeth with a root length greater than 15 mm were selected.

Sample calculation was performed for the push-out test (primary outcome). We based our calculations on the results of a pilot study (mean of 13.20 MPa with standard deviation of 2.2 MPa) and using the following equation:

$$n = Z^2 . s^2 / e^2$$

where Z = 1.96, the acceptable error (e) = 10% of the mean, and s = 2.2 (standard deviation). Thus, the sample size would be at least 6.28 and, therefore, eight teeth were randomly selected. For the secondary outcomes (nanoleakage analysis and microhardness test), a formal sample size calculation was not performed, but the literature was used as a reference.[9-14]

Sample preparation

The crowns were sectioned at the cementoenamel junction using a low-speed diamond saw (Isomet, Buehler, Lake Buff, USA) with copious water cooling. Canal patency was established with a 15-K file (Dentsply Maillefer, Ballaigues, Switzerland) at a 1-mm working length from the apex. The root canals were instrumented using ProTaper rotary nickel-titanium instruments (Dentsply Maillefer) with a size range of S1 to F3. Irrigation was performed with 1% sodium hypochlorite (NaOCl) (Asfer Chemical Industry, São Caetano do Sul, Brazil) after each instrument change. At the end of instrumentation, 5 mL of 17% ethylenediaminetetraacetic acid solution (EDTA) (Biodinâmica, Ibiporã, Brazil) was used to irrigate the canals for 3 min, followed by saline solution. Roots were dried with paper points (Dentsply Maillefer), and the apical 4 mm was filled with gutta-percha (Tanari, Manacapuru, Brazil) and calcium hydroxide-based canal sealer (Sealer 26, Dentsply Maillefer) using the vertical condensation technique.[15] The specimens were stored at 100% humidity at 37 ± 1°C for 7 days.

Thereafter, the post spaces were prepared with the corresponding post drill of the Exacto # 2 fiber post (Angelus, Londrina, Brazil) at a 10-mm depth. The specimens were then randomly divided by restricted randomization with two random block sizes (www.sealedenvelope.com) into six groups (n = 12) according to the moisture of dentin surface (two levels – slightly dry and moist) and adhesive systems (three levels

– etch-and-rinse, self-etch, and self-adhesive). The resulting groups of the present study are: a) etch-and-rinse/moist, b) etch-and-rinse/dry, c) self-etch/moist, d) self-etch/dry, e) self-adhesive/moist, and f) self-adhesive/dry.

Prior to sample preparation, 25 teeth were used for calibrating the operator, as well as for checking whether differences in moisture levels were statistically significant. The roots were instrumented and prepared as described earlier. The mass of each tooth was then measured under moist and dry conditions on an analytical balance.

For the moist dentin surface, post space was thoroughly washed with water and then dried with compressed air for 5 s at 2 cm followed by two #40 paper points (Figure 1A). It could be seen from the last paper point that the root canal was still moist, especially from the middle third to the apical third, while for dry dentin, the root canals were dried with compressed air for 10 s followed by three #40 paper points (Figure 1B) and the last paper point was practically dry. This method of moisture management was previously validated.[10,11]

Despite not being part of the methodology, the canal was irrigated with a plaque disclosing solution and water as a dye (Figure 2). In this way, it was possible to visualize the differences between the last paper point of moist and dry dentin; however, it is important to note that distilled water alone was used for sample preparation.

For the cementation procedures, the length of the glass fiber posts was standardized at 13 mm. While 10 mm corresponded to the previously prepared post space, the other 3 mm was used as a guide to standardize the distance of the light-curing device from the coronal root area. The surface of the posts was cleaned with gauze pads moistened with 70% alcohol for 5 s prior to cementation.

RelyX™ Ultimate resin cement (3M ESPE; St. Paul, USA), associated with the Single Bond Universal adhesive system (3M ESPE), was used for post luting in the etch-and-rinse and self-etch groups and, RelyX™ U200 was used in the self-adhesive groups. In the etch-and-rinse groups, the root canals were etched with 35% phosphoric acid (Ultra-etch™, Ultradent, South Jordan, USA) for 15 s and then washed abundantly with water for 1 min and then dried according to the moisture protocol for the corresponding group. The universal adhesive system was applied manually and actively for 20 s in the etch-and-rinse and self-etch groups. For the self-adhesive groups, the post spaces were cleaned with distilled water during 1 min before cementation.

All resin cements were handled according to the manufacturers' instructions, introduced into the root canal using the Centrix syringe (Maquira, Maringá, Brazil) and the glass fiber posts were positioned with finger pressure. The resin cement was photoactivated for 40 s using the LED

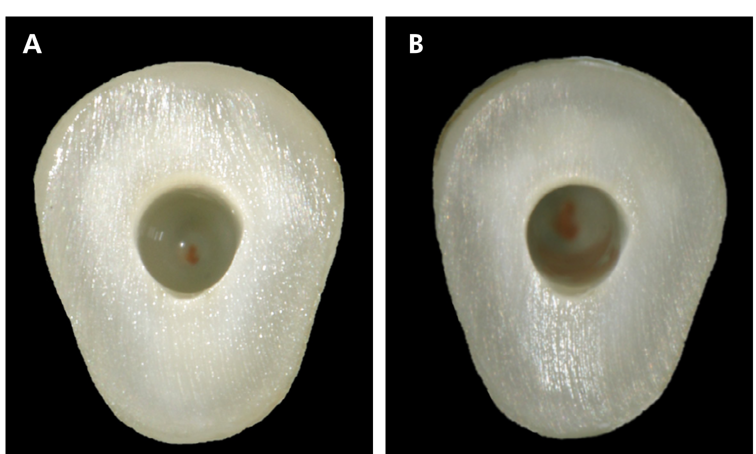


Figure 1. (A) Moist dentin, (B) Dry dentin.

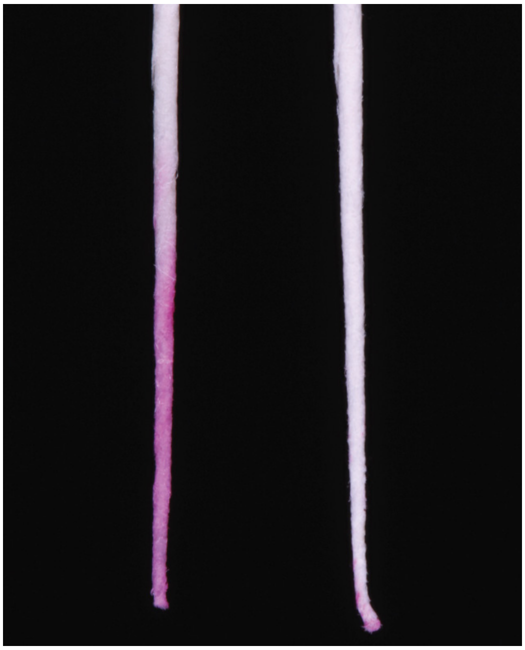


Figure 2. Last paper point used in moist (left) and dry (right) dentin.

light-curing unit with a light intensity of 1,200 mW/cm² (Radii Cal, SDI, Melbourne, Australia) measured with a radiometer (LM-1, Woodpecker, Guilin, China) on every two teeth, maintaining the light guide tip of the light-curing unit perpendicular to the post.

After the cementation procedures, all roots were stored in 100% relative humidity at 37 ± 1 °C for 24 h. The roots were transversally sectioned into seven slices with approximately 1 mm in thickness using a low-speed diamond saw under water cooling. The first coronal slice was discarded due to the presence of excess cement, resulting in two slices for each root canal third: two for apical, two for middle, and two for coronal. The coronal side of each slice was identified and its thickness measured using a digital caliper with an accuracy of 0.01 mm (Mitutoyo Digimatic Caliper, Tokyo, Japan).

Eight roots per group were randomly selected for push-out bond strength testing and four roots were selected for nanoleakage evaluation within the hybrid layer and Vickers microhardness of the resin cement.

Push-out bond strength test

Before testing, all slices were photographed on both sides under an optical microscope (magnification 40x, Olympus BX 51 model, Olympus, Tokyo, Japan) in order to calculate the coronal and apical post radii using the Image J software (National Institutes of Health, Bethesda, USA). To determine the adhesive area, the tapered design of the glass fiber post was considered and the formula of a lateral surface of a truncated cone was used.[16]

The push-out test was performed on a universal testing machine (AG-I, Shimadzu Autograph, Tokyo, Japan) at a crosshead speed of 0.5 mm/min. Each slice was positioned with the most coronal portion side down on a metallic device with a central opening. A cylindrical metallic tip, with the extremity compatible with the post diameter at each root canal third, applied a compressive force (50-kg load cell) on the post in an apical to coronal direction until debonding. The load value for post displacement was recorded in newtons (N) and the bond strength values (MPa) were obtained by dividing the load by the bonding area in mm².

The failure mode of the dentin slices was analyzed under a stereomicroscope (Olympus BX 51 model, Olympus) at 40x magnification and classified as 1) adhesive fracture between resin cement and root dentin or between resin cement and fiber post; 2) cohesive fracture (within the resin cement); and 3) mixed fracture (resin cement and dentin).[7,17] The percentages of each failure were calculated and the data plotted on a graph for qualitative analysis.

Nanoleakage analysis

As previously reported, four teeth of each group were randomly selected for this test. Three random slices per tooth (coronal, middle, and apical) were used among those selected. The specimens were immersed in a dark and small container with a 50wt% ammoniacal silver nitrate solution for 24 h at 37°C and then photodeveloped (Carestream, São José dos Campos, Brazil) for 8 h under indirect fluorescent light. The specimens were washed with running tap water, placed on metallic stubs, and polished down under wet condition using 600-, 1200-, 1500-, 2000-, 2500-, and 3000-grit silicon carbide papers (3M ESPE) for 30 s each. Finally, the samples were maintained in colloidal silica at 37 °C for 48 h. Before scanning electron microscopy (SEM) evaluation (SSX 550, Shimadzu), operating in back-scattering electron mode with an accelerating voltage of 15 kV, the specimens were gold-sputtered (Balzers SCD 050 Sputter Coater, Bal-Tec, Balzers, Liechtenstein). From each slice, one image with a 60x magnification was obtained and analyzed by Image J software (National Institutes of Health). Relative nanoleakage infiltration was calculated by the ratio between the sum of the lengths of cement-dentin adhesive interface infiltrated with silver nitrate and the total perimeter of this interface.

Microhardness analysis

The other slice of the four teeth of each group not used in the nanoleakage evaluation was used to evaluate the microhardness of the resin cement close to the root dentin. These sections were embedded in acrylic resin with the test surface (the one in the most coronal area) face up and polished down under wet condition using 600-, 1200-, 1500-, 2000-, 2500-grit silicon carbide papers (3M ESPE) and washed with tap water. The samples were stored at 37°C for 24 h.

For microhardness analysis, a 100-g load was applied for 15 s with a Vickers microhardness tester (Shimadzu HMV2, Newage Testing Instruments Inc., Southampton, USA). Four indentations were performed on the self-adhesive resin cement near the dentin, in a clockwise direction (at 3, 6, 9, and 12 h). A minimum distance corresponding to one indentation diameter was maintained from the dentin. The diagonals of each indentation were measured employing an optical microscope at 400x magnification and the VHN was calculated using the formula: $VHN = 1.8544\ F/d^2$, where 1.8544 is a constant; F represents the force in kgf (0.1 kgf) used in the test; and d represents the average of the diagonals of the indentation (mm).

Statistical analysis

First, the normality hypothesis was evaluated using the Shapiro–Wilk test ($p > 0.05$). The data obtained from the validation of the moisture conditions method were then subjected to the paired t-test using Sigma Plot 12.0 software (Systat Software, San Jose, USA). The data obtained by the push-out bond strength, nanoleakage, and microhardness tests were subjected to two-way ANOVA (adhesive strategy vs. dentin moisture) and post-hoc comparisons were performed using Tukey's test ($\alpha = 0.05$) using Dell™ Statistica™ 13.2 software (Dell Inc, Round Rock, USA). The mean difference and the 95% confidence interval for the two levels of dentin moisture for all outcomes was also calculated. Data on the fracture pattern were assessed qualitatively only.

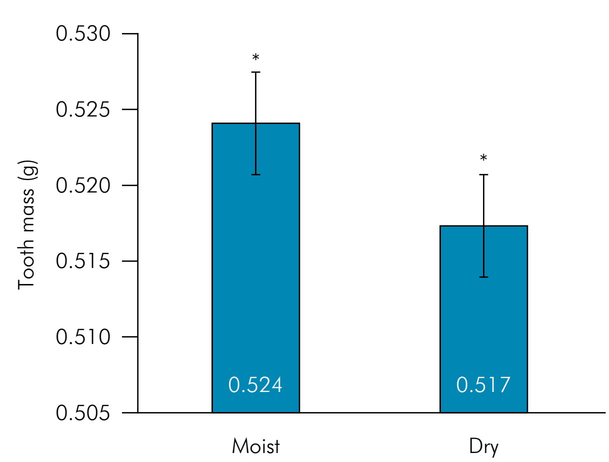
Results

Dentin moisture levels

The amount of water within the root canal between the two simulated moisture conditions (moist and dry dentin) (Figure 3) was significantly different ($p < 0.001$).

Push-out bond strength

Results for the push-out bond strength test are shown in Table 1. Two-way ANOVA demonstrated that the cross-product interaction (adhesive strategy vs. dentin moisture) was not significant ($p = 0.65$). Dentin moisture as the main factor was not



*Indicate statistically significant differences.

Figure 3. Mean and standard error of mass variation of moisture standards.

significantly different. The low mean difference between the two levels of dentin moisture also highlights this lack of statistical significance. However, when evaluating the bonding agents, higher bond strength values can be observed for the RelyX Ultimate group applied with the etch-and-rinse technique.

Qualitative assessment of the fracture pattern indicated a higher percentage of mixed fractures, and groups with the same adhesive system were similar, regardless of root moisture. The percentages obtained are shown on the graph below (Figure 4).

Interfacial nanoleakage analysis

Two-way ANOVA demonstrated that the cross-product interaction (adhesive strategy vs dentin moisture) was significant ($p < 0.05$). Mean, standard deviation, and mean difference are shown in Table 2. The groups with the lowest percentage of nanoleakage were cemented onto dry dentin. For RelyX Ultimate resin cement, there was no statistically significant difference for moist dentin between the etch-and-rinse and self-etch techniques. The RelyX U200 self-adhesive cement showed the lowest rate for the dry substrate among the materials tested.

Microhardness analysis

The data obtained by the Vickers microhardness test (mean, standard deviation, and mean difference) are shown in Table 3. The two-way ANOVA (adhesive strategy vs. dentin moisture) was significant ($p < 0.05$). The RelyX U200 group achieved statistically higher hardness values for dry dentin, while the additional moisture on RelyX Ultimate cement did

Table 1. Means, standard deviations, and mean differences for push-out bond strength (MPa)

Adhesive strategy	Bond strength (MPa)		Main factor for adhesive strategy	Mean difference (95%CI)
	Moist	Dry		
RelyX Ultimate (etch-and-rinse)	14.0 ± 2.8	14.0 ± 2.2	14.0 ± 2.5^A	0 (-2.70 to 2.70)
RelyX Ultimate (self-etch)	12.6 ± 2.4	11.6 ± 2.9	12.1 ± 2.9^B	-1 (-3.85 to 1.85)
RelyX U200 (self-adhesive)	10.8 ± 2.4	10.1 ± 2.4	10.5 ± 2.4^C	-0.7 (-3.50 to 2.10)
Main factor for dentin moisture	12.5 ± 2.8^a	11.9 ± 2.9^a		

CI: confidence interval. Values with the same superscript (capital letter: column; lowercase letter: row) are not significantly different ($p = 0.65$, Tukey's test).

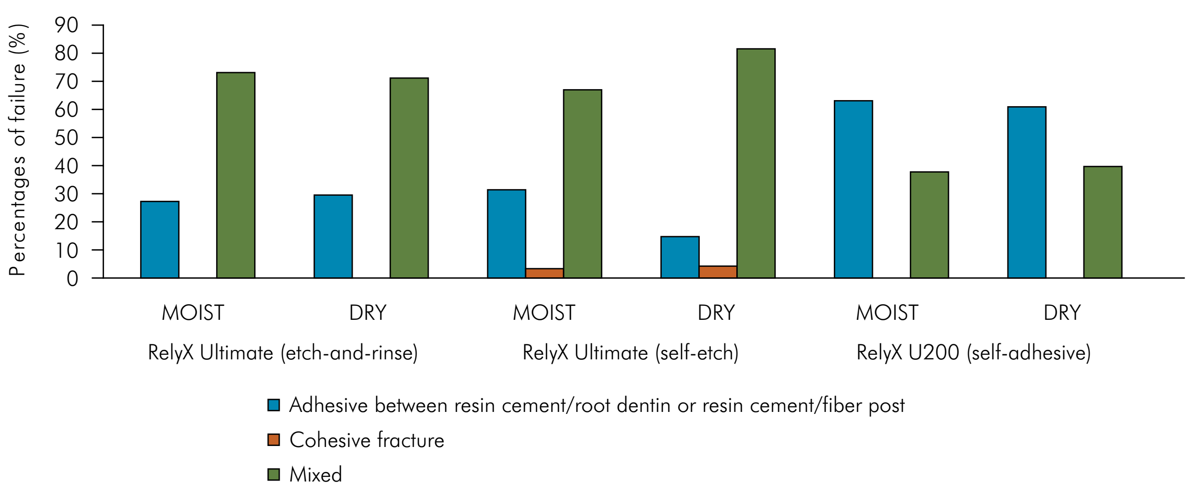


Figure 4. Failure mode distribution.

Table 2. Means, standard deviations, and mean differences for nanoleakage.

Adhesive strategy	Nanoleakage (%)		Mean difference 95% CI
	Moist	Dry	
RelyX Ultimate (etch-and-rinse)	52.8 ± 1.9^{D}	22.8 ± 4.1^{B}	-30 (-35.53 to -24.47)
RelyX Ultimate (self-etch)	47.6 ± 3.9^{D}	36.5 ± 1.9^{C}	-11.1 (-16.41 to -5.79)
RelyX U200 (self-adhesive)	60.8 ± 3.7^{E}	12.0 ± 2.0^{A}	-48.8 (-53.95 to -43.65)

CI: confidence interval. Different superscript letters indicate statistically significant differences ($p < 0.05$, Tukey's test).

Table 3. Means, standard deviations, and mean differences for microhardness.

Adhesive strategy	Microhardness (VHN)		Mean difference (95% CI)
	Moist	Dry	
RelyX Ultimate (etch-and-rinse)	70.5 ± 2.9^{C}	62.4 ± 4.1^{C}	-8.1 (-14.24 to -1.96)
RelyX Ultimate (self-etch)	66.2 ± 6.6^{C}	70.9 ± 6.3^{C}	4.7 (-6.46 to 15.86)
RelyX U200 (self-adhesive)	90.3 ± 5.3^{B}	106.2 ± 4.1^{A}	15.9 (7.70 to 24.10)

CI: confidence interval. Different superscript letters indicate statistically significant differences ($p < 0.05$, Tukey's test).

not interfere with the values and with the adhesive strategy, as there was no statistical difference between the groups.

Discussion

According to the results described in this investigation, the additional moisture applied to the post space did not affect fiber post adhesion to root dentin; therefore, the first null hypothesis was accepted, given that the moisture did not present a statistically significant difference. Similar results were also described in previous reports, in which additional moisture on the dentin surface did not increase the bond strength.[7,18,19] Excess water could affect the integrity of the resin cement and interfacial seal, creating defects within the matrix, and would predispose to bond failure.[18] In contrast, there are other reports that verified an increase in the bond strength with additional moisture,[11,20] but an ideal moisture standard has not been defined, which may contribute to the differences found.

There are still few studies on the role of moisture in post cementation; however, even though moisture in coronal dentin has been advocated for years,[21] the current literature has shown that there are no clinical advantages.[22-24] In addition, active application stands out, allowing better penetration of the monomers as a more relevant factor than the substrate moisture.[10]

When evaluating the main factor for adhesive strategy, the etch-and-rinse group was statistically superior. In the literature, it is known that etch-and-rinse adhesives associated with dual-cure resin cements ensure reliable results for fiber post cementation.[25,26] However, the technique is more sensitive due to the greater number of steps; in this way, self-adhesive resin cements become more attractive to clinicians.

The fracture pattern distribution corroborates the results obtained in the push-out test, as the graphical representations did not show discrepancies in the type of failure between moist and dry substrate for the same cementing agent. Also, there was a greater amount of mixed fracture pattern in the groups with application of the universal adhesive system, which had superior performance in the push-out test. The same relationship can be observed for the self-adhesive resin cement, in which the performance in the analysis of bond strength was lower, and there was a predominance of adhesive failure.

Regarding nanoleakage, the second null hypothesis was rejected, as dry dentin presented statistically significant and lower percentages of silver infiltration at the adhesive interface, which was also found in another report,[27] but for coronal dentin, given that the reports are still scarce in the literature on fiber post cementation.

In previous studies, an inverse correlation was observed between the degree of conversion and the permeability of the adhesive layer for simplified adhesives[28] and with the silver nitrate uptake at the bond interface.[29] The moisture level established in this study may have compromised polymerization at the adhesive interface, given that the degree of conversion of adhesives is substantially reduced in the presence of water.[30-32]

As for the microhardness evaluation, the values obtained presented statistically significant differences and, therefore, the third null hypothesis was rejected. This method was performed because it has been shown to be a simple and reliable measure to indirectly predict the relative degree of conversion.[33] Nonetheless, the data are comparable only within the same resin system because they are not linearly correlated with degree of cure if compared across different materials.[28,33]

From these results, it can be assumed that dentin moisture did not influence the quality of the polymerization degree of the conventional resin cement in the etch-and-rinse and self-etch techniques, as there were no statistically significant differences. On the other hand, the presence of water can affect the degree of conversion of adhesive systems[18,29] and, therefore, the moisture recommended in this methodology may have compromised the properties in the cementation line, which could explain the greater amount of silver infiltration.

It is known that self-adhesive resin cements require the presence of water for acidic monomers to ionize and to be able to promote acid etching and interact with dentin.[34] However, the amount needed is minimal, as excess water will dilute the concentration of acidic monomers and hinder the infiltration of the hydrophobic component of self-adhesive cements.[35,36]

Another factor that must be considered is the variation in the composition of resin cements, as the results are material-dependent.[8,16,19] Kim et al.[37] evaluated surface energy parameters, as well as the microtensile strength in coronal dentin with self-adhesive cements, and observed that in addition to substrate moisture, the surface energy of cementing agents also interferes with the behavior of adhesion. Therefore, knowing the composition of the material plays an important role in optimizing the quality of adhesion.

A systematic review with meta-analysis[5] was carried out in order to determine the influence of cementation strategies among *in vitro* studies on the retention of fiber posts in intraradicular dentin and indicated high heterogeneity among the selected studies, as well as a high risk of bias.[5] This variability in methodology among the studies makes the discussion more complex, as there is no consensus.

In vitro tests have limited results due to the difficulty in simulating all the challenging oral environment conditions because moisture control of the root dentin becomes even more challenging in clinical practice.

Conclusion

As discussed earlier, this study suggests slightly dry canal when cementing glass fiber posts with the materials tested; however, randomized clinical trials are needed to establish methods for the intraoral environment.

Luting glass fiber posts in the etch-and-rinse, self-etch, and self-adhesive strategies onto moist dentin showed similar or lower values for the properties tested. When taking into account the difficulty in controlling moisture within the post space, it is suggested that future randomized clinical trials evaluate the performance of luting posts cemented in dry root canals.

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