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ORIGINAL ARTICLE

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Confocal laser scanning microscopy investigation of bond interfaces involved in fiber glass post cementation

Avaliação de dentes restaurados com pinos de fibra de vidro e agentes cimentantes, empregando-se microscopia confocal

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ABSTRACT

Objective: This confocal microscopy study evaluated the cement/dentin and cement/post interfaces along the root canal walls when fiber glass posts were bonded to dentin using different types of cements. **Material & Methods:** Thirty endodontically treated premolars were divided into 3 groups according to the adhesive materials used in the bonding procedure: Prime & Bond 2.1/Self Cure + Enforce, RelyX Unicem and RelyX Luting. Rhodamine B dye was incorporated in the luting materials for the cementation of the fiber glass posts (Exacto, Angelus) to dentin. Three transversal slices (apical, middle and coronal) were examined under confocal laser scanning microscopy. Statistical analysis was performed using the Kappa, Kruskal-Wallis and Dunnet tests, in a significance level of 5%. **Results:** The Prime & Bond 2.1/Self Cure + Enforce presented a uniform formation of tags in the dentin but gaps in the cement/dentin interface. The RelyX Unicem and RelyX Luting presented an adhesive interface with a fewer amount of gaps, but showed shorter tag formation than the Enforce system. All cements presented the same pattern of bubbles inside the cements. The RelyX Luting presented a greater amount of cracks inside the cement in comparison with the other cements in the coronal third, while no difference was observed between RelyX Unicem and Enforce. The RelyX Luting showed the lowest quantity of cement penetration into the post. **Conclusion:** In general, the quality of bonding interfaces of fiber posts luted to root canals was affected by both location and type of cement.

KEYWORDS

Canals; Bonding techniques; Dentin; Cements.

RESUMO

Objetivo: O objetivo deste trabalho foi avaliar as interfaces cimento/dentina e cimento/pino quando diferentes agentes cimentantes foram utilizados na cimentação de pinos de fibra de vidro. **Material e Métodos:** Trinta pré-molares humanos foram tratados endodonticamente e divididos em 3 grupos de acordo com o procedimento adesivo: Prime & Bond 2.1/Self Cure + Enforce, RelyX Unicem e RelyX Luting. Corante fluorescente Rodamina B foi incorporado aos agentes cimentantes para a cimentação dos pinos de fibra (Exacto, Angelus). Os dentes foram seccionados em três terços (apical, médio e coronário) e examinados ao microscópio confocal. Os dados foram submetidos aos testes Kappa, Kruskal-Wallis e Dunnet, a um nível de significância de 5%. **Resultados:** O grupo Prime & Bond 2.1 / Self Cure + Enforce apresentou uma formação uniforme de tags na dentina, porém fendas na interface cimento/dentina. Os cimentos RelyX Unicem e RelyX Luting apresentaram uma interface adesiva com pequena quantidade de fendas, mas mostrou pequena formação de tags se comparados ao sistema Enforce. Todos os cimentos apresentaram o mesmo padrão de bolhas em seu interior. O cimento RelyX Luting apresentou grande quantidade de trincas em seu interior se comparado com os outros cimentos no terço coronário, enquanto nenhuma diferença foi observada entre os cimentos RelyX Unicem e Enforce. O cimento RelyX Luting a menor quantidade de penetração de cimento dentro do pino. **Conclusão:** Em geral, a qualidade das interfaces adesivas dos cimentos foi afetada pela localização e tipo de cimento.

PALAVRAS-CHAVE

Canal; Técnicas adesivas; Dentina; Cimentos.

INTRODUCTION

When a significant horizontal loss of dental tissue occurs at the coronal level and a small ferrule can be created in the residual tooth structure, a post-and-core build up is needed with the purpose of achieving the most reliable retention for the prosthetic crown [1]. Fiber posts are well accepted for the restoration of endodontically treated teeth, since the post in combination with adhesive materials (luting cement and restorative material) can form a structurally and mechanically homogeneous complex with the dentin [2].

Adhesion of the cement to the canal walls and post surface is a decisive factor for post retention; however, there is no consensus in the literature about the superiority of one cement compared with the others, since the outcomes of bond strength studies are conflicting [3]. This controversy occurs due to unfavorable conditions that are inherent within the root canals, such as: different characteristics of the endodontically-treated dentin, technical demand caused by restricted access to the bonding substrate and unfavorable cavity geometry to dissipate polymerization shrinkage stress [4,5].

Studies investigating different combinations of adhesive systems and luting agents for improving bond strength have been published [2]. Resin-based cements have demonstrated better initial bond strengths than water-based cements, they have been more recommended for luting fiber posts to dentin [6]. However, water-based cements, such as resin-modified glass-ionomer cements, adhere to dentin by micromechanical and chemical bonding mechanisms [7]. Although they also shrink during setting, their viscoelastic properties render them more favorable to the preservation of bond integrity than the stiffer resin-based cements [8]. Moreover, post-maturation hygroscopic expansion of resin-modified glass-ionomer cements may compensate their initial setting shrinkage resulting in more intimate cement-substrates adaptation [9]. Self-adhesive cements were introduced as a promising subgroup of resin cements (e.g. RelyX Unicem, 3M ESPE, St. Paul, MN, USA). These self-adhesive resin cements are easy to handle and provide a time-saving procedure since no etching and bonding steps are required [10].

The objective of this study was to perform a morphological evaluation of interfaces involved in the fiber post luting procedure. The null hypothesis was that morphological characteristics are not affected by the type of cement when fiber posts are bonded into the root canals.

MATERIAL & METHODS

Endodontic Procedures

Thirty single-rooted premolars were selected and stored in 0.1% thymol solution no longer than 1 month following extraction. Premolars that were selected for this study presented straight root canals and a round cross-section. The tooth crown was removed and the working length was established at 1 mm from the root apex. A crown down technique was used for instrumentation with Gates Glidden drills #4 to #1 (Dentsply Maillefer, Ballaigues, Switzerland). Each canal was also instrumented with #50 K files (Dentsply Maillefer, Tulsa, OK, USA). The teeth were irrigated with 1% sodium hypochlorite solution and 17% ethylenediamine tetra-acetic acid. Following the final irrigation, the canal spaces were dried with absorbent paper points. The lateral condensation technique was accomplished with gutta-percha and AH26 Sealer (Dentsply, Petrópolis, RJ, Brasil). The teeth were stored in 100% humidity in deionized water for 7 days at 37º C. A 9-mm depth post space was prepared with a low-speed drill from the Exact Post system (Angelus, Londrina, PR, Brasil). Each post was cleaned with ethanol and air-dried.

Bonding of Fiber Posts

Each root was randomly assigned according to the cements (n = 10). Classification, modes of application and composition of cements are described in Table 1. Luting agents were mixed and labeled with a 0.16% fluorescent dye Rhodamine B (Synth, Labsynth, Diadema, SP, Brazil) [11].

Table 1 - Description of manufacturers, modes of application, composition and batch numbers of the cements

Materials (Manufacturer)	Mode/Steps of application*	Composition	Batch #
Enforce (Enforce; Dentsply, Petropolis, RJ, Brazil)	1) Apply 35% phosphoric acid for 15 s, using a light scrubbing motion 2) Rinse thoroughly and gently dry with with air and paper points 3) Apply adhesive (Prime & Bond 2.1 + Self Cure), then dried with air and polymerize for 40 s by placing the light source on the top of post-space 4) Apply the cement onto the posts' surfaces. 5) Insert and light cure for 40 s	BisGMA, EBPADM, TEGDMA, butylhydoxitoluol, benzoyl peroxide, barium glass, silica	Catalyst 305390 Base paste 305390
RelyX Luting (3M ESPE, St. Paul, MN, USA).	1) Etch the post space with polyacrylic acid for 15 s 2) Rinse with water for 10 s and dry with air and paper points 3) Apply the cement onto the posts' surfaces and insert the post.	Powder: fluoroaluminosilicate glass, potassium persulfate, ascorbic acid catalyst system, opacifying agent Liquid: water, polycarboxylic acid, metha-crylate groups, HEMA , tartaric acid	5CX 5MP
RelyX Unicem (3M ESPE, Seefeld, Germany)	1) Rinse the post space with water for 10s 2) Dry with air and paper points 3) Activate the capsule and mix the cement into a mixer for 15 s 4) Apply the cement onto the posts' surfaces. 5) Insert and light cure for 60 s	Phosphoric acid methacrylates, dimethacrylates, inorganic fillers (72wt.%), fumed silica, initiators	232722

* According to manufacturers information.

The posts were then covered with the cements and inserted into the canal where they were chemically and/or light cured by a high-power halogen curing unit with 1200 mW/cm^2 (Astralis, Ivoclar/Vivadent, Lichtenstein). All luting agents presented both chemical and light curing, as well as the dual adhesive system (Prime & Bond 2.1 + Self Cure, Dentsply, Petrópolis, RJ, Brazil).

Post-core buildup restorations were performed with an adhesive system (37% phosphoric acid + Prime & Bond 2.1, Dentsply, Petrópolis, RJ, Brazil) and composite resin (TPH, A3, Dentsply, Petrópolis, RJ, Brazil). The specimens were stored in 100% humidity in deionized water for 1 month at 37 ºC.

Confocal microscopic analyses

The specimens were transversely sectioned with a microtome saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) in three 1 mm thick sections (cervical, medium and apical thirds).

Confocal Laser Scanning Microscopy (CSLM) was performed with the "Leica TCS SP2" (Leica, Mannheim, Germany). A HeNe gas laser was used as the light source. The excitation light had a maximum wavelength at 543 nm. The intensity of the excitation light as well as the amplification of the photomultiplier was kept constant during the investigation period. CSLM images were recorded in fluorescent mode.

Morphological characteristics were observed in each section of the root. One representative area of each section was scanned 22 μm below the sample surface and divided into 11 sections of 2 μm each. An oil immersion objective (40x, numerical aperture 1.25) was used. The analyzed image was obtained from a mean of all sections. Two evaluators received a prior calibration by an experienced researcher, and then they assigned scores to the confocal images using a double-blind method. In cases where the two examiners disagreed, both re-examined the image and reached a joint final decision.

The evaluated criteria and scores were:

I) Quality of the interfaces between the dentin and the cements (Figure 1):

0- absence of gaps with a defined and organized interface;

1- partial presence of gaps and partial organized interface, present in approximately less than 50% of the interface;

2- presence of gaps and disorganized interface, present in approximately more than 50% of the interface;.

II) Tags formation into dentin (Figure 2):

0- not detectable;

1- few tags were visible;

2- uniform tag formation was seen but with a few lateral branches

3- long resin tags with lateral branches were uniformly evident.

III, IV, V) Penetration of luting agents into posts, presence of bubbles and cracks in the cements (Figures 3-5):

0- none;

1- few, present approximately in less than 30%;

2- medium, present in approximately 30 to 50% of the interface;

3- many, present in approximately more than 50% of the interface;.

Statistical analysis

The Kappa test was carried out to verify the intra- and inter-examiners reproducibility. Data were recorded in tables, and the Kruskal-Wallis and Dunnet tests were used for statistical analysis ($p < 0.05$).

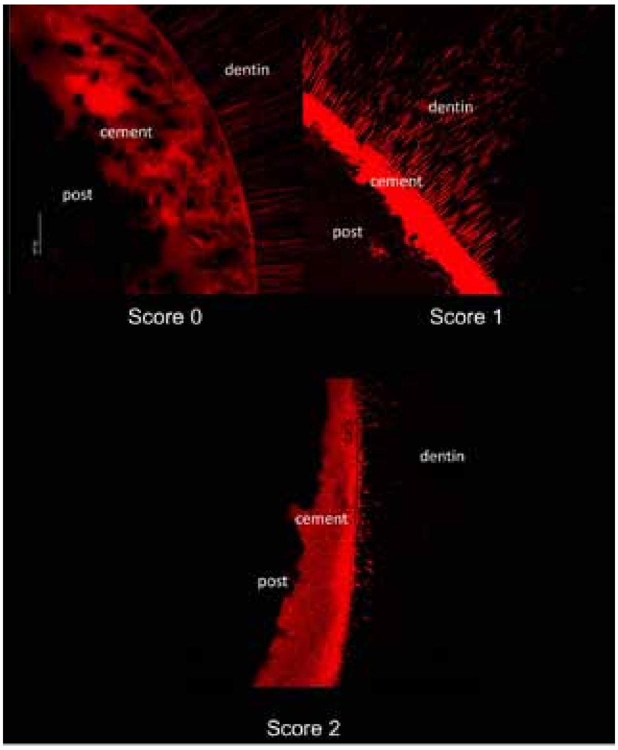

Figure 1 - Scores for the quality of the interfaces between the dentin and the cements.

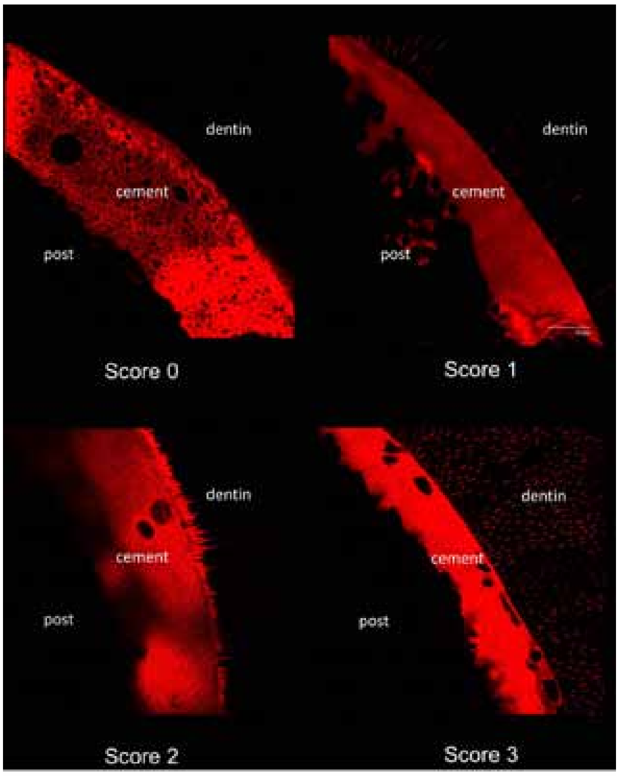

Figure 2 - Scores for the tags formation into dentin.

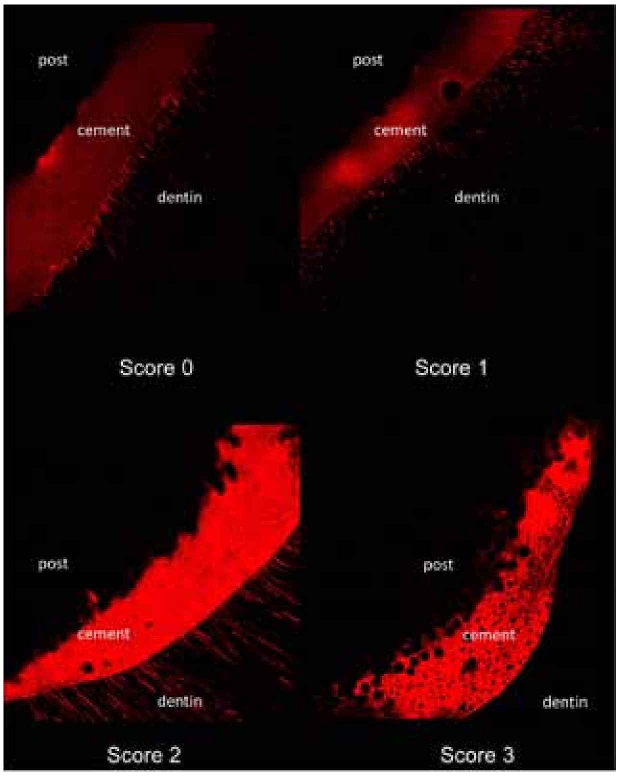

Figure 3 - Scores for the penetration of luting agents into posts.

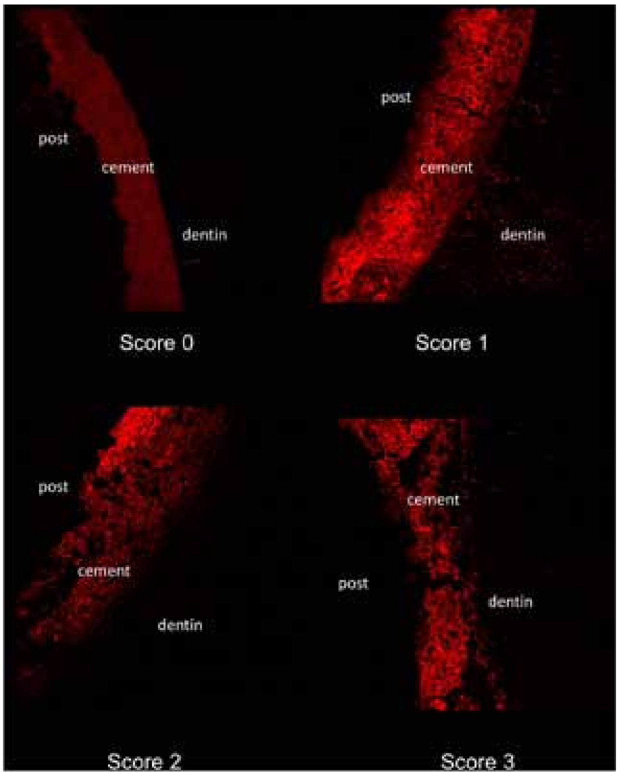

Figure 5 - Scores for presence of cracks in the cements.

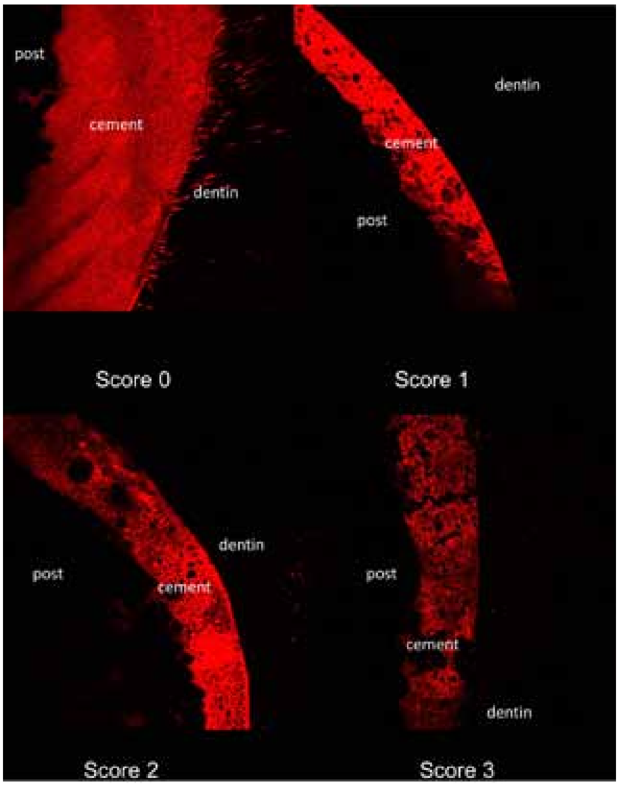

Figure 4 - Scores for the presence of bubbles and in the cements.

RESULTS

The Kappa value was 0.89 and 0.79 for the intra-examiner evaluations, and 0.77 for the inter-examiners. Results are shown in Table 2.

The Prime & Bond 2.1/Self Cure + Enforce showed a more disorganized interface with dentin, presenting gaps in the medium third when compared with the RelyX Unicem and RelyX Luting ($p = 0.0022$; Figure 6A).

The resin cement system (Prime & Bond 2.1/Self Cure + Enforce) showed the presence of tags into dentin statistically higher than the other cements studied (apical, $p = 0.003$; medium, $p < 0.001$ and cervical, $p < 0.001$; Figure 6A). However, no statistically significant difference between the RelyX Unicem and RelyX Luting was found (Figures 6B, 6C and 6D).

The RelyX Luting showed the lowest quantity of penetration of cements into the post (apical, $p = 0.015$; medium, $p < 0.021$ and cervical $p < 0.015$; Figures C and D). However,

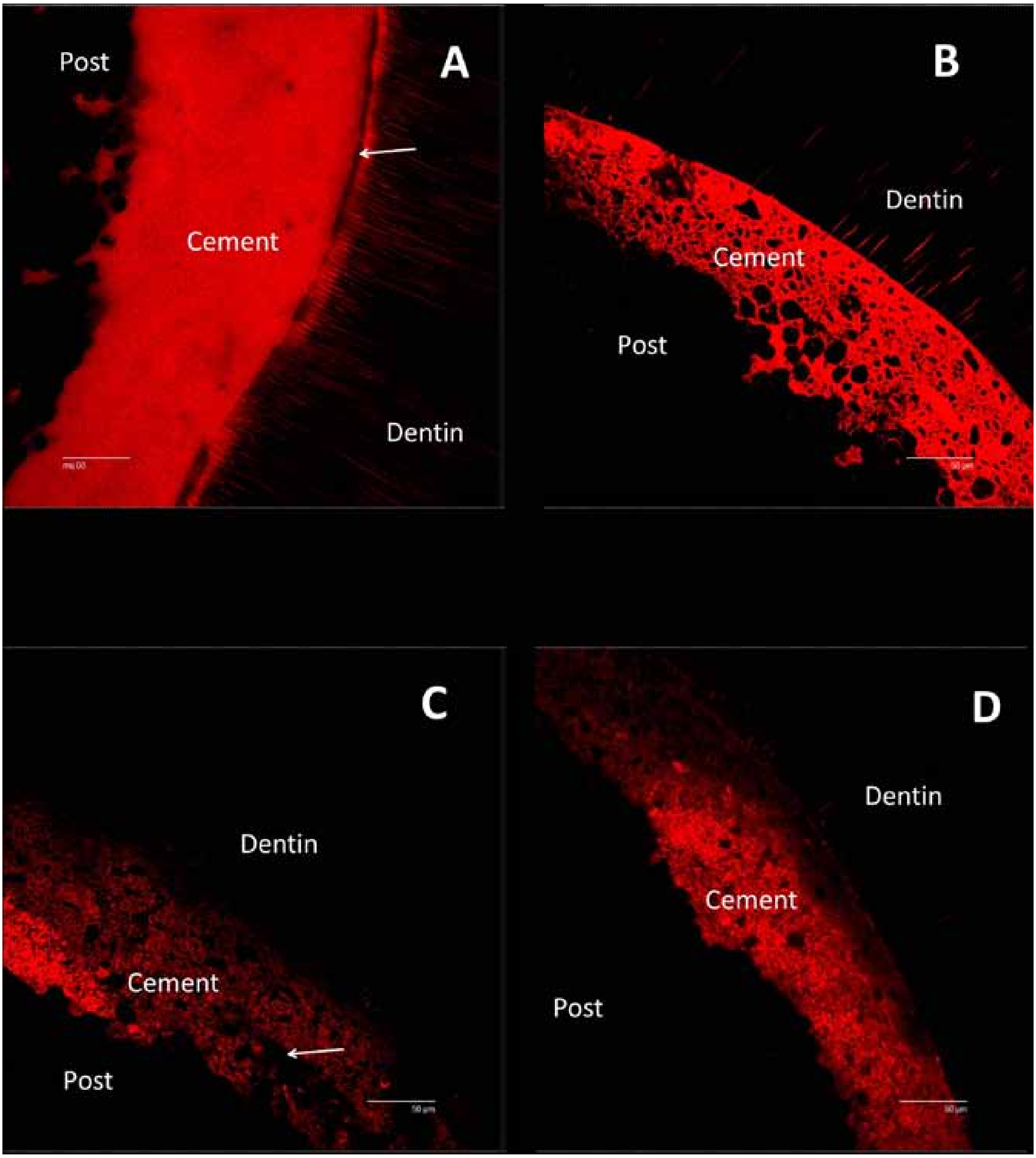

Figure 6 - CLSM representative images for each group studied. Pointer= Presence of gaps in the cement; arrow= presence of bubbles in the cement. A- Enforce system presenting gaps in the interface with dentin (score 3, arrow), although long resin tags with lateral branches were uniformly evident (score 4). A greater quantity of cement can be observed penetrating into the post (score 3). No bubbles and gaps can be observed in the cement (scores 0). B- RelyX Unicem presenting absence of gaps with a defined and organized adhesive interface (score 0) with few visible tags into dentin (score 1). A greater quantity of cement could be observed penetrating into the post (score 3). Few bubbles and no gaps can be observed in the cement (scores 1 and 0, respectively). C and D- RelyX Luting presenting partial presence of gaps and partial organized interface (score 1), with few visible tags into dentin (score 1). No penetration of the cement into the post can be observed (score 3). A medium number of bubbles (scores 2) and many cracks can be observed in the cement (score 3, arrow).

no statistically significant difference between the RelyX Unicem and Enforce was observed (Figures 6A and 6B).

Concerning the bubbles inside the cements, there were no statistically significant difference among the cements studied (p =0.415; Figures 6A-D).

The RelyX Luting presented a greater amount of cracks inside the cement in comparison with the other cements in the cervical third (P < 0.001; Figure 6C). However, no difference was observed between the RelyX Unicem and Enforce (Figures 6A and 6B).

DISCUSSION

CLSM has been used with the purpose to visualize and analyze the quality of teeth restored with adhesive materials, including the bonding process of glass fiber posts and resin cements to dentin [12,13]. However, different methodologies have been used according to the objectives of the study. The confocal microscopy analysis was chosen in this study because it has advantages over conventional light microscopy and scanning electron microscopy (SEM). In the conventional light microscopy, it can be difficult to distinguish the precise location of the interfaces [14]. With SEM, the sputter coating and vacuum chambers can cause dehydration artifacts, which in turn can rupture the interfaces [14]. The CLSM is able to focus just below the surface, and with the aid of the fluorescent dye, can clearly distinguish the interfaces [14]. The CLSM method provided more detailed information regarding the penetration and distribution of the resin cement and adhesive than the SEM imaging.

Table 2 - Quality of interfaces involved in post cementation, bubbles and cracks into cements were evaluated using the classification system with scores.

		Enforce			Unicem			RelyX Luting		
		C	M	A	C	M	A	C	M	A
Interface dentin	0	2	-*	3	4	3	3	3	2	1
	1	1	3*	3	5	5	4	1	6	6
	2	7	7*	4	1	2	3	6	2	3
Tags into dentin	0	-*	-*	-*	3	4	3	3	3	5
	1	-*	2*	4*	5	4	6	4	6	4
	2	2*	-*	1*	2	2	1	2	-	1
	3	8*	8*	5*	-	-	-	1	1	-
Penetration into post	0	4	3	3	1	-	1	3*	3*	4*
	1	2	2	-	-	2	1	4*	6*	5*
	2	1	2	3	2	6	3	3*	1*	1*
	3	3	3	4	7	2	5	-*	-*	-*
Bubbles into cement	0	1	4	3	2	3	1	2	4	1
	1	7	3	3	6	3	4	1	2	3
	2	-	-	3	-	3	4	4	-	3
	3	2	3	1	2	1	1	3	4	3
Cracks into cement	0	10	8	8	8	8	3	2*	7	4
	1	-	1	2	2	1	4	4*	1	4
	2	-	-	-	-	-	1	2*	1	1
	3	-	1	-	-	1	2	2*	1	1

Groups with asterisks statistically differ from the others.
C - cervical, M- medium and A- apical

The null hypothesis of this study was partially rejected since except for the presence of bubbles, no differences were found among the cements. Regarding the interface with dentin, the Prime & Bond 2.1/Self Cure + Enforce presented a uniform formation of tags with gaps (Figure 6A). The confocal microscopy analysis also observed that the use of etch & rinse adhesive systems produced good results in terms of density and the quality of resin tags [13,15]. Polymerization shrinkage stresses that were generated because of the highly unfavorable cavity configuration factor of the post spaces may affect the interfacial strength of resin-based material, causing gaps in the adhesive interface (Figure 6A) [4,16]. The RelyX Luting and RelyX Unicem presented an interface with absence or fewer amount of gaps, but showed shorter tag formation (Figure 6B-D). The use of slow-setting, self-adhesive/curing luting cements provides a more favorable condition for the relief of these stresses along the bonding interface. Shorter tag formation observed for the RelyX Unicem and RelyX Luting may have occurred because the methacrylate phosphoric esters, and polyalcenoic acid, responsible for substrate conditioning, are not as effective as phosphoric acid, which dissolve the smear layer created on the root canal walls during the post space preparation (Figures 6B-D) [17,18]. Corroborating the present study, the tag formation of the RelyX Unicem with dentin was quite irregular and superficial in accordance with observations of other studies [18,19].

Furthermore, retention of posts cemented with resin-modified glass-ionomer may be related to the frictional retention provided by hygroscopic expansion occurring after cement maturation, which also aids the self-sealing at the dentin-cement interface [9,20]. The similarity between the RelyX Luting and RelyX Unicem regarding the quality of the interface may be justified by the fact that this self-adhesive resin cement can also bond chemically to dentin, thus promoting a higher tolerance to moisture because water is formed during the neutralization reaction of the phosphoric acid methacrylate, basic fillers and hydroxyapatite [20].

It was observed in bond strength studies that the RelyX Luting and RelyX Unicem cements displayed predominance of cohesive fractures, whereas Enforce exhibited 70% to 80% of adhesive failures at the dentin/cement interface [6,21]. In a recent study, the self-adhesive materials and the glass ionomer cements had the highest push-out bond strength values when compared with the dual-curing resin cement [22].

Some studies indicate that bond strength values depend on the etching capacity and creation of sufficiently hybridized dentin of the adhesive systems used [17]. On the other hand, other studies demonstrated that the bond strength of fiber posts in root canals rather depend on frictional resistance instead of adhesion [9,23]. Bitter 2009 et al. [12] observed that chemical interactions between the adhesive cement and hydroxyapatite may be more crucial for root dentin bonding than the ability of the same material to hybridize dentin. Based on the cements studied in the present work, long tag formation may not be enough to guarantee adhesion if polymerization shrinkage stresses are generated, thus promoting gaps in the interface.

Regarding the micromechanical interactions of the cements with posts, when silanization of the post was performed, the self-etching RelyX Unicem system revealed low percentages of covered surfaces probably because this system might create a layer of acidic monomers on the post surface that reduced the micromechanical interlocking [24]. However, Mazzitelli et al. [25] observed no increase in the push-out bond strength values for the RelyX Unicem, independently from the post surface treatment performed. A greater quantity of interlocking into the post was observed for the RelyX Unicem (Figure 6B) such as what occurred with the Enforce in the present study (Figure 6A). Probably due its viscosity, the RelyX Luting presented few interlocking into the post. A recent study concluded that the silane application may be necessary to improve the adhesion of fiber posts luted with the self-adhesive resin cement [26]. The possibility of combining chemical and micromechanical retention on the post surface provides the most promising adhesion mechanism [2].

No differences about the presence of bubbles may be attributed to the manner in which the cements were inserted. Applying the cements onto the posts' surfaces may have proportioned the same facility for bubbles formation, independent of the materials used.

Presence of cracks into the resin-modified glass ionomer cements is a characteristic inherent to these materials, since they are sensitive to dehydration associated with the early stage of the acid–base setting reaction (Figure 6C) [27]. However, resin-modified glass-ionomer cements appeared to have a greater capacity for closure of established cracks upon rehydration [28].

Because of the limitation of the morphological microscope evaluations, it was not possible to correlate the results to the laboratory or clinical outcomes [29]. In conclusion, our study describes the morphological features of the interfaces involved in post luting performed by three different cements through CLSM images. The Prime & Bond 2.1/Self Cure + Enforce presented a uniform formation of tags and higher number of gaps in the interface with dentin. In contrast, the RelyX Unicem and RelyX Luting presented an interface with absence or fewer amount of gaps, but exhibited a shallow interaction with the dental substrate. Cements with chemical bond present a shorter clinical protocol, which can be useful during clinical practice. Further studies are still required to evaluate the laboratory and clinical performance of techniques and cements to indicate the optimal choice for clinical practice.

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