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Effect of Long-term Simulated Pulpal Pressure on the Bond Strength and Nanoleakage of Resin-luting Agents With Different Bonding Strategies

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Clinical Relevance

Simulated pulpal pressure presented different effects on the long-term adhesive performance of the resin cements and should be considered when choosing a resin-based luting cement.

SUMMARY

This study evaluated the effects of simulated hydrostatic pulpal pressure (SPP) on the microtensile bond strength (μTBS) to dentin and nanoleakage patterns produced by self-adhesive luting agents after 12 months. Three self-adhesive luting agents (RelyX Unicem [UN], RelyX U100 [UC], and Clearfil SA Luting [SA]) and three conventional luting agents (Rely X ARC [RX], Panavia F [PF], and a two-step self-etching adhesive system [Clearfil SE Bond] associated with Panavia F [PS]) were evaluated. One hundred twenty-three human molars were abraded to expose occlusal surfaces. Resin cements were used to lute cylindrical composite blocks to the teeth either subjected or not to SPP. Sixty specimens were subjected to 15 cm H_2O of SPP for 24 hours before and 24 hours or 12 months after cementation procedures. Afterward, restored teeth were serially sectioned into beams with a cross-sectional area of 1 mm^2 at the bonded interface and were tested in tension (cross-head speed of 1 mm/min). Failure mode was determined using scanning electron microscopy (SEM). Data were statistically analyzed by three-way analysis of variance and post hoc Tukey test (*p*=0.05). Two additional teeth in each group

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were serially sectioned into 0.9-mm-thick slabs, which were submitted to a nanoleakage protocol with $AgNO_3$ and analyzed with scanning and transmission electron microscopes. The μTBS values of the etch-and-rinse group (RX) were negatively influenced by SPP and long-term water storage with SPP. After 12 months, UC and SA presented premature failures in all specimens when submitted to SPP. SPP increased silver deposition in most groups in both evaluation times. The hydrostatic pulpal pressure effect was material dependent. The storage time without SPP did not affect bond strength. However, long-term SPP influenced the performance of the etch-and-rinse and self-adhesive cements regarding μTBS and nanoleakage pattern, except to UN.

INTRODUCTION

Resin-based luting agents were introduced to overcome the inherent problems of zinc phosphate cements and provide better handling and esthetic properties.[1] To promote adhesion to tooth structures, conventional resin cements use etch-and-rinse or self-etching adhesive systems.[2] Dentin is the main substrate available for adhesion in prosthetic procedures, especially in vital teeth. Dentin is a hydrated hard tissue in the vital state, when there is an outward flow of dentinal fluid through the dentinal tubules with a positive pulpal pressure, estimated to be approximately 15 cm H_2O.[3] Water presents deleterious effects for adhesive procedures, such as the plasticization of the polymer chains, leading to compromised mechanical properties and hydrolytic degradation of resin and collagen fibrils.[4-8]

The etch-and-rinse multistep adhesive technique has been considered to be complex and sensitive.[9] This technique produces a complete smear layer and smear plug removal, increasing outward flow of dentinal fluid.[10] In addition, an incompletely infiltrated hybrid layer has been reported.[11,12] Some adhesive systems behave as semipermeable membranes[13] and can allow outward fluid flow through the dentin-adhesive interface, even after polymerization.[14-16] These disadvantages probably account for a higher incidence of postoperative sensitivity after bonding procedures,[17] pulpal damage,[18] and premature degradation of the resin-dentin interface.[7,8,19]

Self-etching adhesive systems were developed in an attempt to reduce the technique sensitivity of etch-and-rinse systems. Self-etching monomers simultaneously etch and infiltrate dentin, providing micromechanical retention after polymerization.[20] The maintenance of smear plugs when self-etching adhesive systems are used minimizes moisture contamination by dentinal fluid transudation when compared with the use of etch-and-rinse adhesives.[21] Simplified single-step all-in-one self-etching adhesives have been reported to allow water diffusion through the adhesive interface even after polymerization.[13,14,16,22]

A new type of luting material that does not require any pretreatment of the tooth surface with adhesive systems has been developed, the so-called self-adhesive cement.[23-25] This material aims to combine the favorable properties of conventional (zinc phosphate, glass ionomer, and polycarboxylate cements) and resin-luting agents.[25] After the first self-adhesive cement was developed (RelyX Unicem; 3M ESPE, St Paul, MN), it rapidly gained popularity among clinicians because of its simplified "mistake-free" application technique. Application on smear layer-covered substrates maintains dentin permeability in very low levels,[15] contributing to reduced postoperative sensitivity and lower susceptibility to moisture degradation.[26] Its interaction with subjacent dentin and enamel has been suggested to occur through formation of a hybridized complex and chemical interaction with hydroxyapatite.[27] However, limited information is available with regard to the bonding mechanism and nanoleakage of such products when they are applied to teeth under simulated pulpal pressure.[25,28-30] Within this context, prolonged storage time with simulated pulpal pressure may compromise the sealing ability and mechanical properties of the bonded interface over time.

The aim of this study was to evaluate the effects of simulated pulpal pressure (SPP) on bond strength and nanoleakage in resin-dentin interfaces produced by different cementation strategies after 12 months. The research hypothesis was that bond strength provided by self-adhesive resin cements in indirect restorations is not as susceptible to the outward flow of dentinal fluid as regular resin-luting agents (resin cements/adhesive systems). In addition, it was anticipated that the changes in the nanoleakage patterns at the adhesive interface as a consequence of SPP are similar for multistep and self-adhesive resin cements.

METHODS AND MATERIALS

Tooth Preparation

One hundred sixty-eight recently extracted caries-free third molars stored in 0.1% thymol (Symrise GmbH, Holzminden, Germany) solution at 4°C for no longer than 3 months were used in this study. Teeth were obtained by protocols that were approved (SISNEP/384) by the review board of Guarulhos University (Guarulhos, São Paulo, Brazil). After disinfection and removal of soft tissues, flat middle depth coronal dentin surfaces were exposed with 600-grit SiC paper (3M of Brazil Ltd, Sumare, Brazil) under running water to create a standardized smear layer. Teeth had their roots removed using a diamond saw (ISOMET, Buehler, Lake Bluff, IL) 2 mm below the cement-enamel junction. Pulpal tissue was gently removed so as not to damage the predentin region.

Teeth were assigned to four experimental groups (with or without SPP and immediate or 12 months), which were distributed into 24 experimental subgroups (n=7) according to the luting technique, pulpal pressure, and storage time. Three self-adhesive cements, RelyX Unicem (UN), RelyX U100 (UC), Clearfil SA Luting (SA), and two conventional luting agents, one that uses a two-step etch-and-rinse adhesive (RelyX ARC [RX]) and one that uses a one-step self-etching adhesive (Panavia F [PF]), were used in this study. An additional group included the use of a two-step self-etching primer adhesive system (Clearfil SE Bond) prior to the application of Panavia F (PS). Luting agents were mixed and placed according to manufacturers' instructions (Table 1).

To simulate pulpal pressure on the dentin surface, each tooth was bonded to a Plexiglass platform (3 cm × 3 cm × 0.3 cm) penetrated by an 18-gauge stainless-steel tube and fixed with cyanoacrylate adhesive (Loctite Super Bonder Gel; Henkel, Düsseldorf, Germany). The pulp chamber was filled with distilled water via polyethylene tubing connected to a syringe barrel with 10 mL of distilled water and suspended 15 cm from the tooth crown. Thus, each specimen was connected to a hydraulic pressure device that delivered 15 cm water pressure.[31] The teeth were kept under hydrostatic pressure for 48 hours or 12 months, starting 24 hours before luting procedures.

Luting Procedures for Microtensile Bond Strength (μTBS)

Five teeth of each group were used for the μTBS evaluation. Four-millimeter-thick composite resin discs 12 mm in diameter were prepared by layering 2-mm-thick increments of a microhybrid composite resin (Filtek Z250, shade A1; 3M ESPE) into a silicone mold. Each increment was light activated (700 mW/mm^2) for 40 seconds with a halogen light (Optilux 501; Kerr Corp, Orange, CA). One side of the composite resin discs was abraded with 600-grit SiC paper under water cooling to create a flat surface with standardized roughness. The composite surface was airborne-particle abraded with 50-μm aluminum oxide particles (Asfer Indústria Química Ltda, São Caetano do Sul, Brazil) for 10 seconds. Before luting procedures were performed, the composite resin discs were ultrasonically cleaned in distilled water for 10 minutes, rinsed with running water, air dried, and silanated (RelyX Ceramic Primer; 3M ESPE).

The cementation procedures were randomly processed. Excess water was removed with cotton pellets. Care was taken not to dehydrate dentin surfaces. The application of adhesive systems (when necessary) and luting agents was made according to the manufacturer's instructions, and the composite resin disc was pressed on the cement using digital pressure, which was sustained until light curing was performed from the buccal and lingual sides. Specimens were exposed to light from the same halogen curing unit for 40 seconds on the buccal, lingual, and occlusal directions. Bonded specimens were stored in distilled water for 24 hours or 12 months, and the specimens submitted to SPP were kept under constant pulpal pressure during the same storage time.

Luting Procedures for Nanoleakage Analysis

Two specimens were prepared for each group. After luting agents were mixed and applied onto flat dentin surfaces, a polyester strip was placed over the luting agent and was used to apply proper digital pressure while the luting agent was light activated for 40 seconds. Afterward, a thin layer of a low-viscosity resin composite (Clearfil Majesty Flow, Kuraray Med. Inc, Kurashiki, Okayama, Japan) was applied and light activated for 40 seconds. After similar storage conditions described above (24 hours or 12 months, with or without SPP), teeth were sectioned perpendicular to the adhesive-tooth interface into 0.9-mm-thick slabs using a diamond saw (Isomet 1000; Buehler).

Bonded slabs were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. To rehydrate specimens and avoid desiccation artifacts,[32] they were immersed in distilled water for 20 minutes prior to immersion in the tracer solution of ammoniacal silver nitrate for 24 hours. Tooth slabs were placed in the tracer solution in total darkness for 24 hours, rinsed thoroughly in distilled water, and immersed in a photo-developing solution for eight hours under a fluorescent light to reduce

Table 1: *Cements, Lot Number, Manufacturers, Delivery System, Composition, and Application Technique*[a]

Type	Manufacturers (Lot Number)	Delivery System (Cement)	Composition	Application Technique
Dual-polymerizing resin cement + two-step etch-and-rinse adhesive system	RelyX ARC (GEHG) + Adper Single Bond 2 (7MY); 3M ESPE, St Paul, MN	Automatic dispenser, two pastes, hand mixed for 10 s	Cement: bis-GMA, TEGDMA polymer, zirconia/silica filler	a (15 s); b (15 s); c; d; e; i (10 s); mix cement; apply mixture
			Etchant: 35% H_3PO_4	
			Adhesive: bis-GMA, HEMA, UDMA, dimethacrylates, ethanol, water, camphorquinone, photoinitiators, polyalkenoic acid copolymer, 5-nm silica particles	
Dual-polymerizing resin cement + 1-step self-etching adhesive	Panavia F (paste A, 00249D; paste B, 0027A) + ED Primer (primer A, 00262A; primer B, 00137A); Kuraray Medical, Inc, Tokyo, Japan	One-step self-etching adhesive + resin cement, dual polymerizing two pastes, hand mixed	Primer A: HEMA, 10-MDP, 5-NMSA, water, accelerator	h (A+B) (leave undisturbed for 60 s); mix cement; apply mixture; i (40s)
			Primer B: 5-NMSA, accelerator, water, sodium benzene sulphinate	
			Paste A: 10-MDP, silanated silica, hydrophobic aromatic and aliphatic dimethacrylate, hydrophilic dimethacrylate photoinitiator, dibenzoyl peroxide	
			Paste B: silanated barium glass, sodium fluoride, sodium aromatic sulfinate, dimethacrylate monomer, BPO	
Dual-polymerizing resin cement + two-step self-etching adhesive system	Panavia F (paste A, 00249D; paste B, 0027A), Clearfil SE Bond (00788A); Kuraray Medical, Inc	Two-step self-etching adhesive + ED Primer + resin cement, dual polymerizing two pastes, hand mixed	Primer: MDP, HEMA, hydrophilic dimethacrylate, dl-camphorquinone, N,N-diethanol p-toluidine, water	f (20 s); e; g; i (10 s); h (ED Primer); e; mix cement; apply mixture; i (40 s)
			Bond: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-camphorquinone, N, N-diethanol p-toluidine, silanated colloidal silica	
			Paste A and Paste B: as described above	
Dual-polymerizing self-adhesive resin cement	RelyX U100 (366321) 3M ESPE	Clicker dispenser two pastes, hand mixed	Base: glass fiber, methacrylated phosphoric acid esters, dimethacrylates, silanated sílica, sodium persulfate	Mix cement; apply mixture; i (40 s)
			Catalyst: glass fiber, dimethacrylates, silanated sílica, p-toluene sodium sulfate, calcium hydroxide	
Dual-polymerizing self-adhesive resin cement	RelyX Unicem (365979) 3M ESPE	Capsules, mechanically mixed for 10 s	*Powder:* glass powder, silica, calcium hydroxide, self-curing initiators, pigments, light-curing initiators, substituted pyrimidine, peroxy compound	Automix cement; apply mixture i (40 s)
			Liquid: methacrylated phosphoric esters, dimethacrylates, acetate, stabilizers, self-curing initiators, light-curing initiators	
Dual-polymerizing self-adhesive resin cement	Clearfil SA luting (008AA), Kuraray Medical, Inc	Dual polymerizing two pastes, hand mixed	Paste A: MDP, Bis-GMA, TEGDMA, Hydrophobic aromatic dimethacrylate dl-Camphorquinone, benzoyl peroxide, initiator, silanated barium glass filler, silanated colloidal silica	Automix cement; apply mixture, i (40 s)
			Paste B: Bis-GMA, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, accelerators, pigments, surface treated sodium fluoride, silanated barium glass filler, silanated colloidal silica	

Abbreviations: 10-MDP, 10-methacryloxydecyl dihydrogen phosphate; 4-META, 4-methacryloyloxyethyl trimellitate anhydride; 5-NMSA, N-methacryloxyl-5-aminosalicylic acid; Bis-GMA, bisphenol A diglycidyl ether methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

[a] *Application technique = a: acid etch; b: rinse surface; c: dry with cotton pellet; d: apply one bottle adhesive; e: gently air dry; f: apply primer; g: apply adhesive; h: apply mixture; i: light polymerize; j: autopolymerize.*

silver ions into metallic silver grains within voids along the interface.

Scanning and Transmission Electron Microscopy

For scanning electron microscopy (SEM) analysis, specimens were fixed in Karnovsky's solution and embedded in epoxy resin (Epoxycure, Buehler). Afterward, they were polished with 400-, 600-, 1200-, and 2400-grit SiC paper and 6-, 3-, 1-, and 0.25-µm diamond paste (Arotec, Cotia, SP, Brazil). Then, specimens were dehydrated in ascending ethanol series and coated with carbon (MED 010, Balzers Union, Balzers, Liechtenstein). Resin-dentin interfaces were observed with an SEM (LEO 435 VP, LEO Electron Microscopy Ltd, Cambridge, UK) operated in the backscattered electron mode. After SEM analysis, representative leakage patterns at the cement-dentin interfaces produced by each system were photographed at 500× magnification.

For transmission electron microscopy (TEM) analysis, specimens were examined with the TEM to compare silver uptake patterns along the resin-dentin interfaces. Undemineralized specimens were fixed in Karnovsky's solution, postfixed in osmium tetroxide, dehydrated in an ascending ethanol series, and embedded in epoxy resin (Dr Spurr, Electron Microscopy Sciences, Hatfield, PA). Representative 90-nm-thick ultrathin sections were prepared with an ultramicrotome (Leica UC6; Leica Microsystems, Wetzlar, Germany) and collected on 100-mesh copper grids. Without additional staining, they were observed in a TEM (Zeiss EM 900; Zeiss, Munich, Germany) operated at 80 kV. Silver deposition patterns were compared among the different luting products and storage conditions. Because of differences in hybrid layer thickness and in the magnifications required to characterize the interfaces, no attempt was made to quantify the silver deposits. Representative images were chosen to depict the most frequently observed aspect of the resin-dentin interfaces in the different testing conditions.

μTBS Evaluation

After the storage time was completed (24 hours or 12 months), the restored teeth were serially sectioned perpendicular to the adhesive-tooth interface into slabs and the slabs into beams with a cross-sectional bonded area of approximately 1 mm^2 using a diamond saw (Isomet 1000; Buehler). Beams were fixed to the grips of a universal testing machine (EZ Test; Shimadzu Corp, Kyoto, Japan) using a cyanoacrylate adhesive (Loctite Super Bonder Gel; Henkel, Düsseldorf, Germany) and tested in tension at a cross-head speed of 1 mm/min until fracture occurred. Maximum tensile load was divided by specimen cross-sectional area to express results in units of stress (MPa). Five beams were selected from each restored tooth, and the average value for each tooth was used in the calculations. Bond strength values were evaluated statistically using three-way analysis of variance (ANOVA) and Tukey post hoc test (p=0.05). Pretest failures were not included in the statistical analysis.[33,34] Statistical analyses were performed using a statistical software program (SAS for Windows V8; SAS Institute, Inc, Cary, NC). Failure modes were determined by examination of fractured specimens with an SEM (LEO 435 VP; LEO Electron Microscopy Ltd). Fractured specimens were mounted on aluminum stubs and gold-sputter coated (MED 010; BAL-TEC AG, Balzers, Liechtenstein) prior to viewing at different magnifications. Failure mode at the fractured interface was classified into one of four types: CD (cohesive failure in Dentin), AD (adhesive failure between hybrid layer and dentin), CC (cohesive failure in the cement), or ADR (adhesive failure between the luting agent and composite resin). Instead of classifying failures as mixed, the area percentage of each type of failure in each specimen was recorded.

RESULTS

μTBS

The mean μTBS values are presented in Table 2. Three-way ANOVA revealed a significant difference for the factor "cements" (p=0.00001), for the factor "pulpal pressure," and for the interaction "cements × pulpal pressure × storage time" (p=0.00028). For RX, SPP significantly reduced μTBS both at 24 hours and 12 months. However, the negative effects of SPP were more pronounced after 12 months. Storage in water for 12 months without SPP did not influence negatively μTBS values of RX. SPP and long-term storage did not affect μTBS values of PS and UN. When SPP was applied, a considerable reduction in μTBS of PF was recorded at both storage times. Even though the self-adhesive cement UC was positively influenced by SPP at 24 hours, the association of long-term storage and SPP was catastrophic to UC and SA, which presented 100% of pretest failures.

Without SPP at 24 hours, RX and PS presented the highest bond strength values (Table 2). PF, UN, UC, and SA did not present significant differences. With SPP at 24 hours, the highest μTBS values were produced by RX, PS, and UC, which did not differ among them. However, no difference in μTBS values was observed among UC, UN, and SA. PF showed the lowest μTBS but with no significant difference from UN and SA. Without SPP at 12 months, RX presented the highest bond strength values, followed by PS. The lowest μTBS at 12 months without SPP were recorded for PF, UN, UC, and SA, which did not differ among them. After 12 months of storage with SPP, the highest μTBS values were recorded for PS. RX, UN, and PF did not differ from each other after 12 months with SPP. UC and SA did not withstand the challenge and could not be tested.

Distribution of failure modes is presented in Figure 1. Cohesive failure in resin cement and

Table 2: *Mean Bond Strength Values in MPa (Standard Deviation) for the Different Resin Cements Applied on Dentin With and Without Simulated Pulpal Pressure and Stored in Water for 24 Hours and 12 Months*[a]

Product Type	Resin Cement	24 Hours		12 Months	
		No Pressure	Hydrostatic Pressure	No Pressure	Hydrostatic Pressure
Two-step etch-and-rinse adhesive/resin cement	RelyX ARC + Single Bond (RX)	53.0 (8.6) Aa	34.8 (11.3)ABb*	67.9 (11.0) Aa*	18.6 (17.9) Bb
Two-step self-etching adhesive/resin cement	Panavia F + Clearfil SE Bond (PS)	45.5 (6.9) Aa	38.0 (10.2) Aa	48.4 (14.6) Ba	57.5 (7.9) Aa*
One-step self-etching adhesive/resin cement	Panavia F (PF)	14.1 (4.6) Ba	7.8 (1.4) Db	22.5 (4.3) Ca	12.0 (4.2) Bb
Self-adhesive cement	RelyX Unicem (UN)	17.5 (7.4) Ba	20.4 (7.6) BCDa	18.5 (8.4) Ca	16.2 (10.5)Ba
Self-adhesive cement	RelyX U100 (UC)	14.2 (5.6) Ba	24.2 (2.3) ABCa*	19.9 (6.3) Ca	0.0 Cb
Self-adhesive cement	Clearfil SA Luting (SA)	13.1 (11.1) Ba	14.3 (5.3) CDa*	23.8 (16.2) Ca	0.0 Cb

[a] *Means followed by different upper case letters (columns), lowercase letters (rows within each hydrostatic pressure condition), and asterisks (rows within each time of storage) are significantly different by Tukey test at the 5% confidence level.*

adhesive failure were predominantly observed in RX at 24 hours and PS at both times, regardless of the application of pulpal pressure. At 12 months, RX presented predominantly adhesive failure (AD). On the other hand, the predominant failure mode for all other groups was adhesive between dentin and the luting agent.

Nanoleakage

SPP increased nanoleakage in all groups, except for the self-adhesive cements UN and UC at 24 hours. The influence of storage time was material dependent. When RX was applied without SPP, silver deposition was observed in some regions within the hybrid layer (Figure 2). RX with SPP revealed silver deposition within the entire thickness of the hybrid layer and tags (Figure 2). After 12 months, increased silver deposition was observed for RX, increasing considerably more when it was submitted to SSP (Figure 2). PS with or without SPP presented small silver deposition at the bottom of the hybrid layer (Figure 3). After 12 months, no increase in silver deposition was observed for PS without SPP, and a moderate increase was observed with SSP. Some regions of the interface produced by PF without SPP presented adhesive layer, hybrid layer, and tags

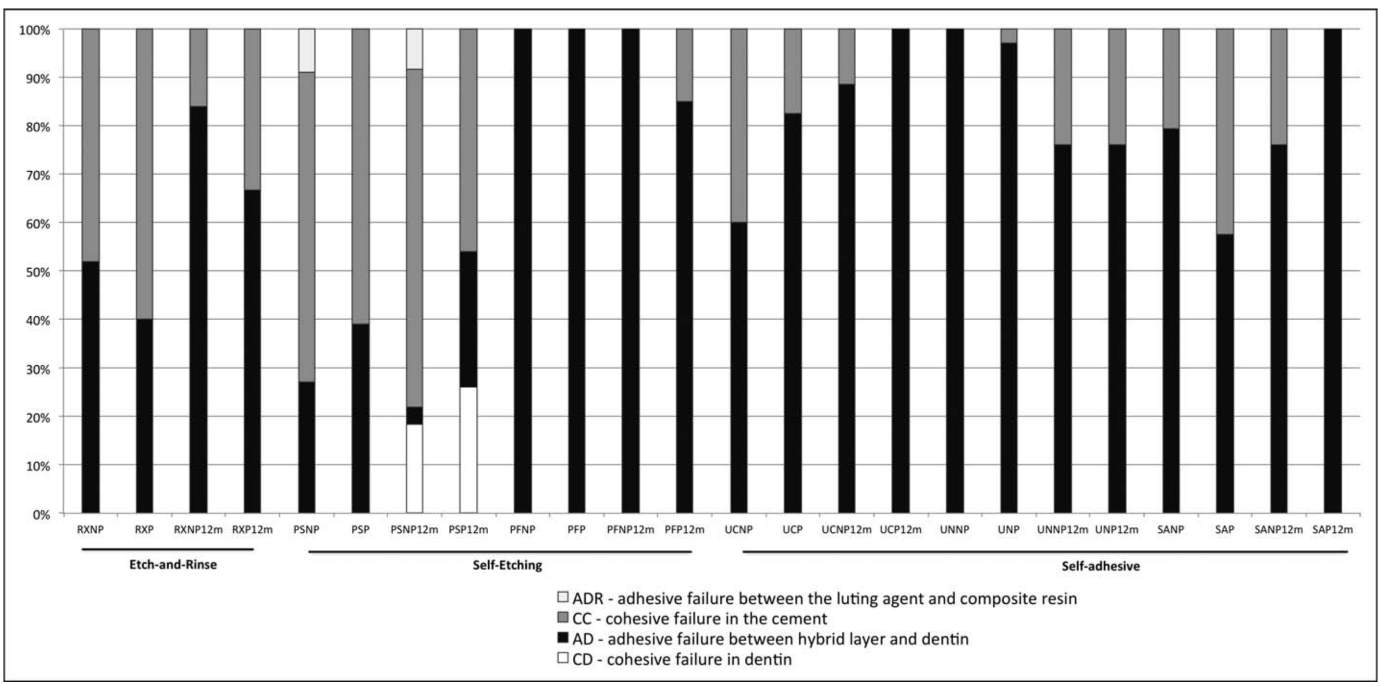

Figure 1. *Distribution of failure modes within groups.*

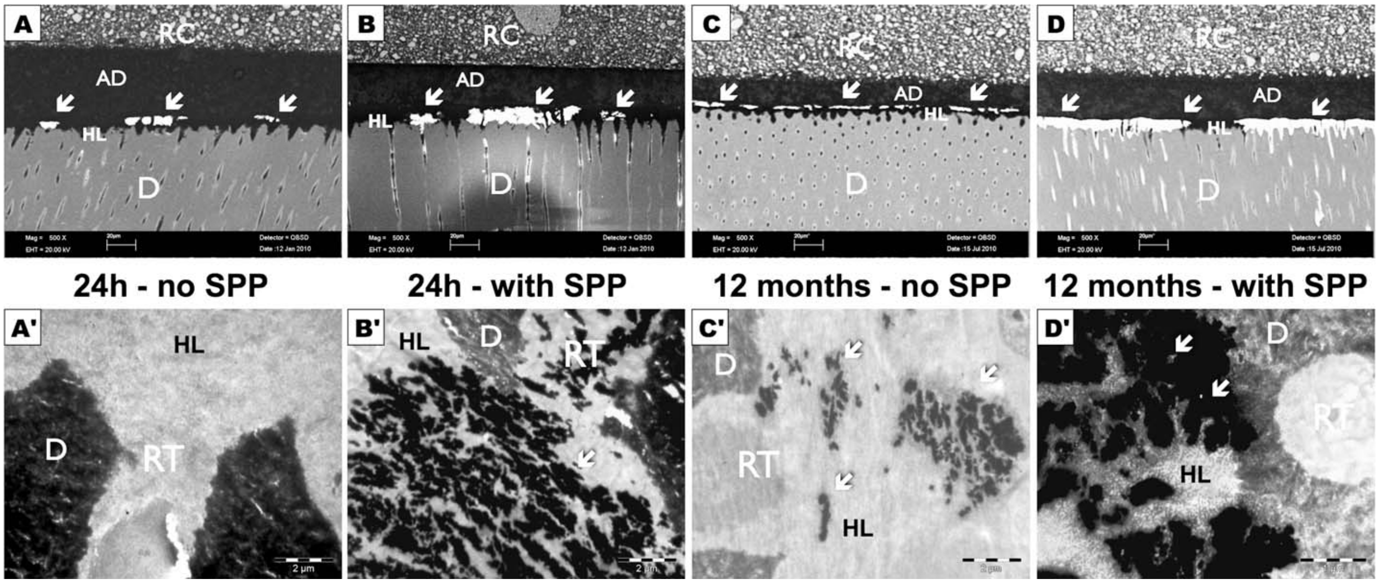

Figure 2. *Representative backscattered SEMs and TEMs of the cement-dentin interfaces produced by the two-step etch-and-rinse system RelyX ARC (RX).*

completely impregnated by silver (Figure 4). PF with SPP presented gaps between the cement layer and dentin (Figure 4). After 12 months, PF with and without SPP presented higher silver deposition than observed at 24 hours.

The self-adhesive cements presented lower silver impregnation than the other cement systems at 24 hours (Figures 5 through 7). UN, UC, and SA presented almost no or very little silver deposition at the cement-dentin interface after 24 hours, with or without SPP (Figures 5 through 7). After 12 months of storage without SPP, a small increase in silver deposition was observed for SA and UC. However, when they were stored with SPP, gaps were frequently observed. The cement-dentin interface could not be observed for SA and UC after 12 months with SPP, because specimens did not survive SEM/TEM preparation procedures.

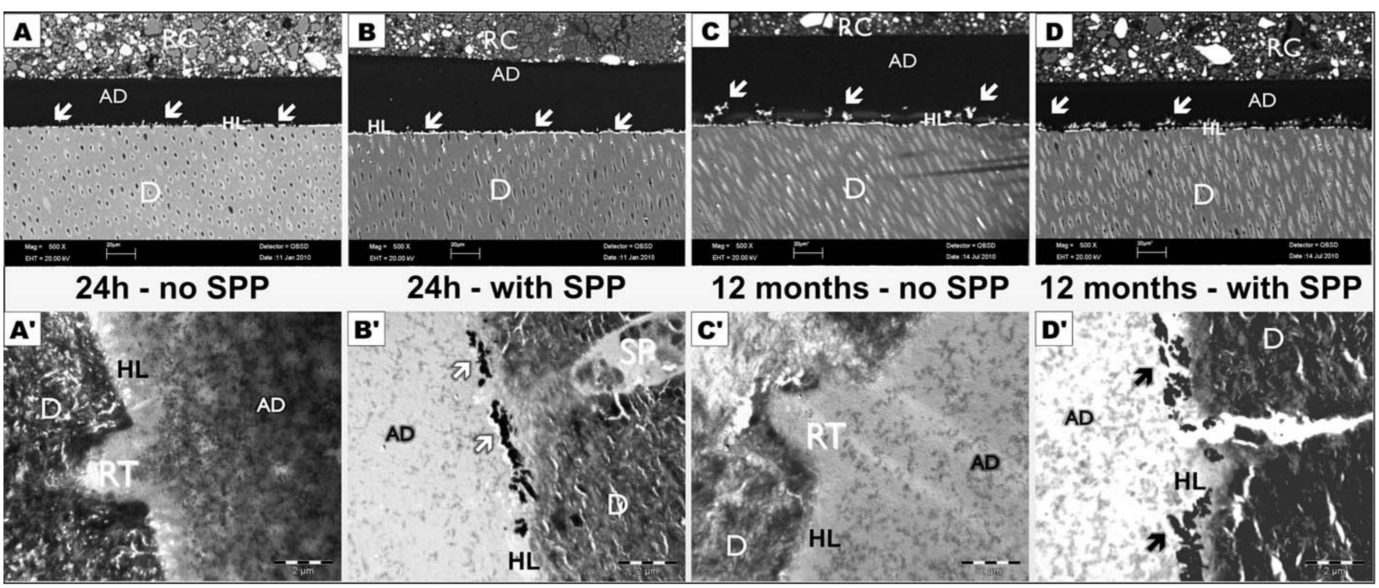

Figure 3. *Representative backscattered SEMs and TEMs of the cement-dentin interfaces produced by the two-step self-etching system Panavia F + Clearfil SE Bond (PS).*

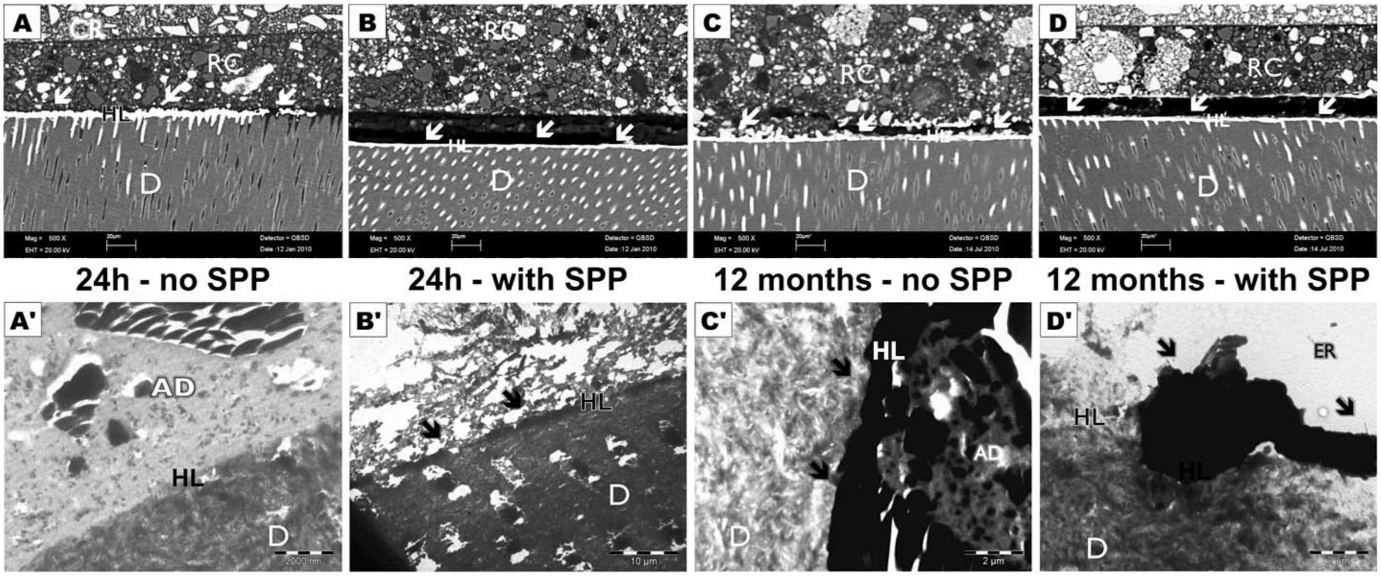

Figure 4. *Representative backscattered SEMs and TEMs of the cement-dentin interfaces produced by the one-step self-etching system Panavia F (PF).*

DISCUSSION

In this study, the etch-and-rinse group presented significantly decreased μTBS when it was submitted to SPP either after 24 hours or 12 months. This finding can be attributed to the diffusion of water into the hybrid layer, as noted by the silver deposition within the hybrid layer (Figure 2). As a consequence, the mechanical properties of the polymer within dentin are compromised by swelling and decreased frictional force between the polymer chains, a process known as plasticization.[5,19,35]

Even in the absence of SPP, all conventional cement systems presented a certain degree of nanoleakage, which was located mainly within the hybrid layer (Figures 2 through 4). The degree of silver deposition depends on the adhesive used, application mode, and composition. The presence of water on the adhesive composition plays an important role in both total- and self-etching techniques, as water-based

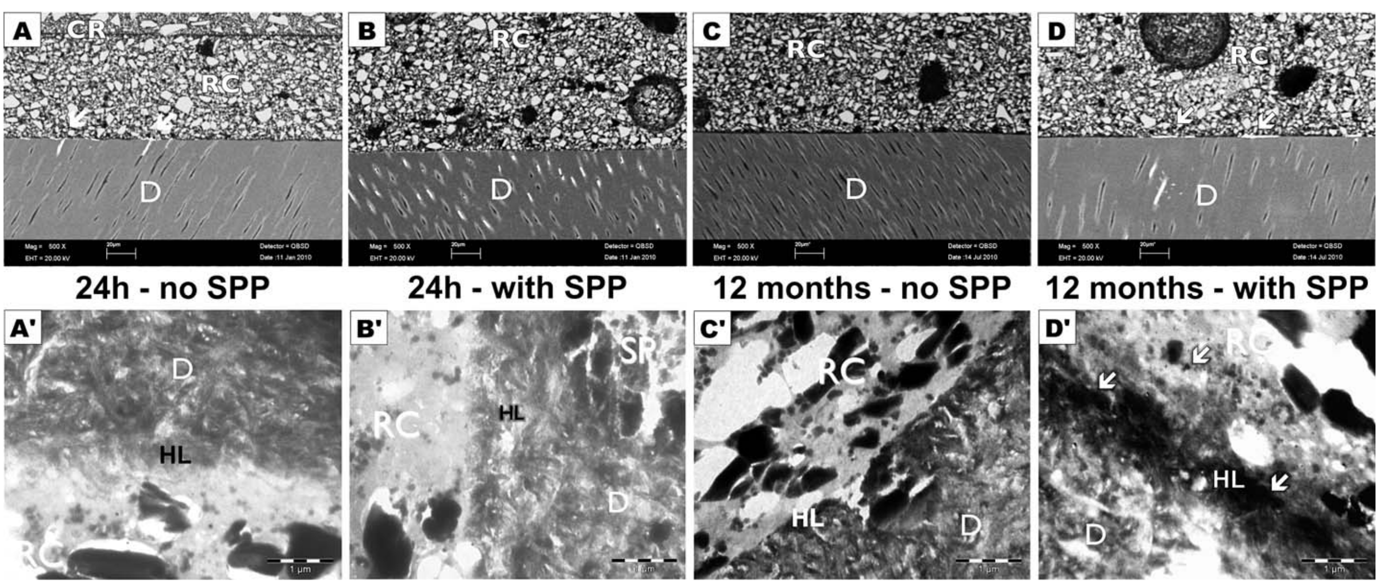

Figure 5. *Representative backscattered SEMs and TEMs of the cement-dentin interfaces produced by the self-adhesive cement RelyX UNICEM (UN).*

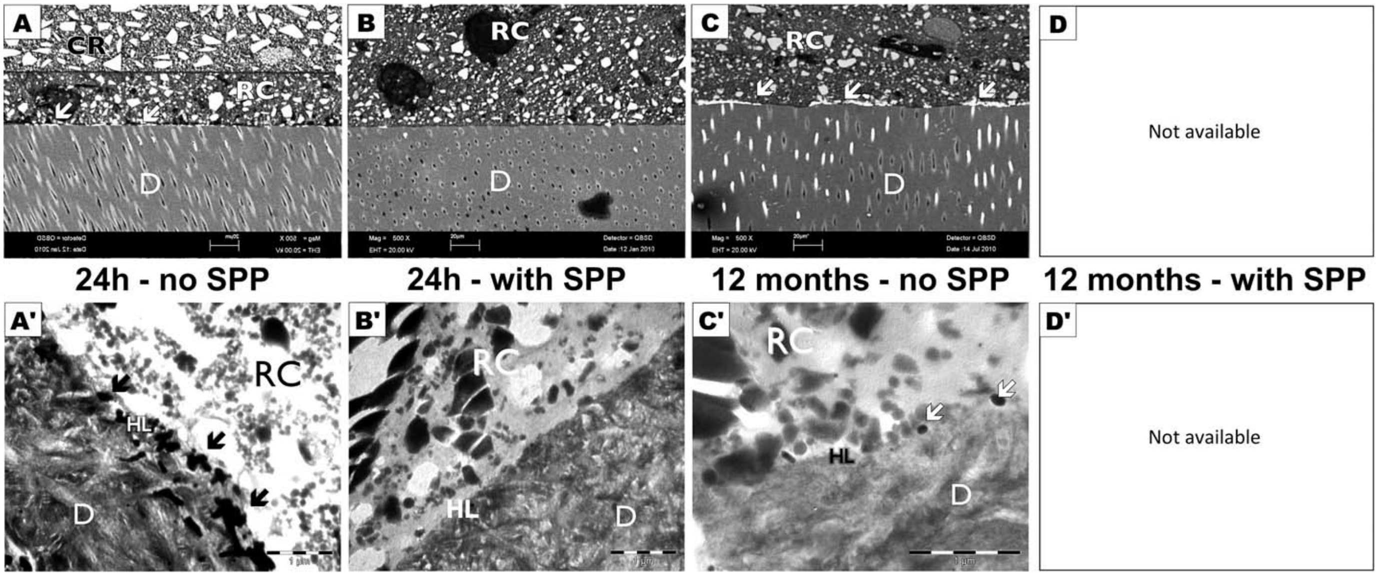

Figure 6. *Representative backscattered SEMs and TEMs of the cement-dentin interfaces produced by the self-adhesive cement RelyX U100 (UC). Figures 6D/6D′ are not presented because specimens did not resist electron microscopy preparation procedures after being stored for 12 months with simulated pulpal pressure.*

adhesives used in total-etch systems can solvate dried matrices and reexpand dentin collagen.[36] Probably, water was not eliminated during the adhesive procedures, producing the nanoleakage patterns observed in Figures 2A and 2A′. Besides the presence of water in their composition, the two-step etch-and-rinse adhesive produces a semipermeable membrane due its high concentration of hydrophilic monomers and solvents.[13,14] Hydrophilic resin monomers attract water molecules and permit their movement from dentin across the adhesive layer through water channels.[16,37] Such a statement is confirmed in Figures 2B and 2D, which show an evident increase in nanoleakage, probably due to fluid flow produced by SPP. This water-filled channel has been considered to be a site of hydrolytic degradation and crack propagation during μTBS testing.[38,39]

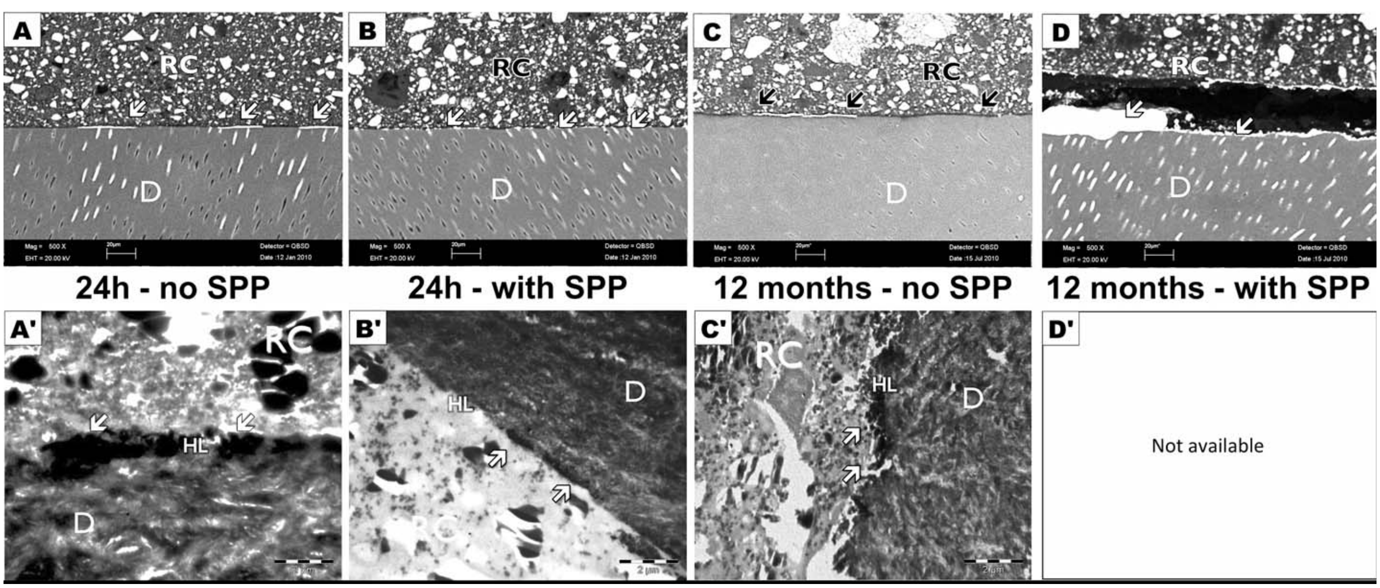

Figure 7. *Representative backscattered SEMs and TEMs of the cement-dentin interfaces produced by the self-adhesive cement Clearfil SA Luting (SA). Figure 7D′ is not presented because specimens did not resist electron microscopy preparation procedures after being stored for 12 months with simulated pulpal pressure.*

Table 3: *Three-Way Analysis of Variance*[a]

Source	df	SS	MS	f	p Value
Cement	5	25383.1963793	5076.6392759	63.6640	0.00001
Pressure	1	2735.7870911	2735.7870911	34.3084	0.00001
Time	1	14.7899862	14.7899862	0.1855	0.67154
Cement × Pressure	5	4084.4360507	816.8872101	10.2442	0.00001
Cement × Time	5	1267.3700452	253.4740090	3.1787	0.01074
Pressure × Time	1	1299.0906566	1299.0906566	16.2914	0.00028
Cement × Pressure × Time	5	2209.5092822	441.9018564	5.5417	0.00031
Error	96	7655.1477991	79.7411229		
Total	119	44649.3272904			

[a] Coefficient of variation = 35.588%.

Another phenomenon that contributes to reduction in μTBS values is water diffusion from the underlying hydrated dentin structure across the polymerized hydrophilic adhesive layer via an osmotic gradient during the slow setting process of these resin cements.[13] According to Hiraishi and others,[15] continuous water uptake via the adhesive layer could result in an unstable porous region, increasing the degradation along the interface between the adhesive and resin cement. As a result, this bonding interface turns into a weak link when pulpal pressure is simulated.[2,40] Porous regions were observed in fractured specimens of RX with SPP, suggesting the presence of water channels (Figure 2E,F,G). At higher magnification, nonuniform globular structures were identified, which might suggest poor polymerization (Figure 2E,F,G).[1] In addition, backscattered SEM micrographs of PF subjected to SPP after 24 hours and 12 months revealed silver deposition between the adhesive cement and resin (Figure 4), suggesting permeability within this system.

When SPP was applied for 24 hours or 12 months, PF presented a significant reduction in μTBS values. Previous studies have reported a negative influence of pulpal pressure for Panavia F.[2,15] This performance is probably associated with a high concentration of hydrophilic and ionic resin monomers in ED Primer, resulting in the formation of a highly permeable layer after polymerization.[13,41] Because of its high permeability,[15] primed dentin allows water to diffuse from dentin across the hybrid layer and form water droplets in the interface (Figure 4), resulting in low μTBS. Hydrophilic monomers, such as HEMA, can attract water, which impairs the cement polymerization, reducing mechanical properties.[41] This statement can be confirmed by the high amount of silver deposition (Figure 4), even without SPP. As previously demonstrated,[42] for an ideal bonding performance, the water concentration must be sufficient to provide adequate ionization of the acidic monomers but without lowering the resin concentration too much to optimize their bonding efficacy to dentin. The high water content at the interface during polymerization setting probably contributed to the reduction in monomer concentration when PF was submitted to SPP. This fact can explain the lower μTBS associated with gap formation at the interface. The failure modes for PF were exclusively adhesive between resin cement and dentin. The fracture of PF groups with SPP occurred frequently on top of the hybrid layer, where it was not possible to visualize the tubular lumen, suggesting a poorly cured adhesive layer. Besides, it could be speculated that inhibition of the polymerization of the luting agent (Panavia F) could occur due to the presence of acidic monomers within the ED Primer composition.[41] However, as light activation was performed immediately upon luting, this effect is probably negligible.[28]

Infiltration of hydrophobic monomers from Clearfil SE Bond into primed dentin along with direct light activation of the adhesive system probably resulted in a better monomer conversion and stronger network polymer within the hybrid and adhesive layers, resulting in the significantly higher μTBS of the PS group in comparison to the values of PF group and in the highest μTBS after 12 months with SPP. This hydrophobic layer can reduce permeability of dentinal fluids between dentin and the luting agent and result in better long-term performance.[2,43,44] In addition, the presence of smear plugs is very important to reduce significantly the rate of fluid flow through the interface even in the presence of intrapulpal pressure, *in vivo* and *in vitro*.[14,16,45]

The self-adhesive resin cements maintained their μTBS when submitted to SPP for 24 hours. Three different self-adhesive materials were used in this study. The self-adhesive cements RelyX U100 (UC) and RelyX Unicem (UN) were developed by the same manufacturer and are marketed under the same name in some countries. According to the manufacturer, the only difference between these products is the delivery system. While UN requires an activator, triturator, and applicator, UC can be hand mixed. Another self-adhesive cement used was Clearfil SA Luting, which needs to be hand mixed. When SPP is applied, water transudation increases the acidic monomers' aggressiveness, improving smear layer dissolution and dentin demineralization.[42] It also optimizes the acid-basic reactions, allowing better setting.[26] The favorable μTBS and very low silver impregnation (Figures 5 through 7) observed for self-adhesive resin cements have been attributed to the micromechanical retention and chemical interaction between monomer acidic phosphate groups and dentin-enamel hydroxyapatite.[15,25,26] This possible improvement produced by water was evidenced by the μTBS values and favorable nanoleakage patterns observed when UC and UN were initially submitted to SPP (Figures 5 and 6).

However, despite the favorable μTBS results observed at 24 hours with and without SPP, and after 12 months without SPP, long-term storage with SPP resulted in debonding of the indirect restorations cemented with UC and SA prior to testing. The low initial pH of UN and UC ($pH<2$ in the first minute, according to the manufacturer) and SA (pH 2-3 in the first minute, according to the manufacturer), produces almost no demineralization and hybrid layer formation on dentin surface.[18,28,46-48] The present findings demonstrated that this interaction was not strong enough to resist 12 months of storage with SPP for UC and SA.

During polymerization of the self-adhesive resin cements (UN and UC), an increase in pH from 1 to 7 is observed as a consequence of the reaction between phosphate groups and both alkaline filler particles and hydroxyapatite from enamel and dentin, to neutralize resin acidity.[49,50] The presence of calcium hydroxide can also act in pH neutralization. The pH neutralization results in water formation and a more hydrophilic cement, which enhances the cement's wetting ability on the dentin surface and the cement tolerance to water. Water is crucial for self-adhesive luting agents to release hydrogen ions required for smear layer demineralization[51] and is also reused in the reaction between multifunctional acidic phosphate monomers and alkaline filler particles. Such a phenomenon is speculated to be responsible for a change in the nature of the cement from hydrophilic to hydrophobic, which is thought to improve adhesive stability. This hydrophobic characteristic is desired for bonded interfaces, because it can prevent hydrolytic degradation and consequently improve the long-term durability of indirect restorations. After 12 months of storage with SPP, UN demonstrated bond strength stability and very low silver deposition throughout the study.

Except for the materials that presented a high number of pretest failures, all groups presented acceptable bond strengths throughout the evaluation period. However, the groups that presented the highest bond strength values are more indicated for cementation of indirect restoration in less-retentive cavity preparations.

CONCLUSION

Based on the results of this study, it was concluded that the hydrostatic pulpal pressure effect was material dependent. Storage in water for 12 months without simulated pulpal pressure did not affect the bond strength for all materials. However, long-term storage with simulated pulpal pressure influenced the performance of the etch-and-rinse adhesive system RelyX and the self-adhesive cements U100 and Clearfil SA luting regarding μTBS and nanoleakage patterns. Panavia F+ Clearfil SE Bond and Unicem presented bond strength stability and low silver deposition throughout the study regardless of the application of simulated pulpal pressure.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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