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Two-year clinical evaluation of proanthocyanidins added to a two-step etch-and-rinse adhesive

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ABSTRACT

Objective: To compare the clinical behavior of Proanthocyanidins (PA)-free and PA-containing two-step etch-and-rinse adhesive used underneath resin composite restorations in non-carious cervical lesions (NCCLs) over a 6- (6 M) and 24-month (24 M) period.

Methods: 135 restorations were randomly placed in 45 subjects. The NCCLs were conditioned (37% phosphoric acid for 15 s) and distributed into 3 groups: Control (EX0) - ExciTE F (Ivoclar Vivadent) adhesive applied following the manufacturer's recommendations; EX2 and EX5 – 2 wt% and 5 wt% of PA were added to ExciTE F, respectively, and applied as in EX0. Resin composite was placed incrementally and light-cured. The restorations were evaluated at baseline, 6 M and 24 M, using FDI and USPHS criteria. Statistical analyses were performed using Friedman and Wilcoxon tests ($\alpha = 0.05$).

Results: The retention rates were 98% (95% confidence interval 88–99%) for EX0, 92% (80–97%) for EX2; and 85% (72–93%) for EX5 at 6 M. A significant difference was found only for EX5 at 6 M when compared with the respective baseline findings ($p = 0.03$) and when compared with EX0 and EX2 ($p = 0.001$) at 6 M. After 24 M, the retention rates were 98% (88–99%) for EX0, 73% (59–84%) for EX2, and 71% (56–82%) for EX5. Only EX0 did not result in significant difference in retention rate at 24 M when compared with baseline but showed a significant higher retention rate when compared with those of EX2 and EX5 ($p = 0.001$).

Conclusion: Adding proanthocyanidins to the adhesive solution jeopardized the retention of composite resins restorations in non-carious cervical lesions after 24 months.

Clinical relevance: In spite of being user-friendlier than when used separately, the incorporation of proanthocyanidins into the adhesive solution impairs the longevity of composite restorations.

1. Introduction

A resistant long-term resin-dentin bond is fundamental for the success of adhesive restorations [1]. Ideally, adhesive monomers must thoroughly infiltrate and encapsulate exposed collagen fibrils, creating the hybrid layer. However, the penetration of adhesive into the demineralized dentin does not occur optimally [2]. As a result, collagen fibrils in the hybrid layer are partially exposed and susceptible to deterioration [3]. The degradation mechanism of dentin collagen and adhesive resin is triggered by a variety of physical and chemical factors, including hydrolysis and catalytic action of enzymes, such as host-derived matrix metalloproteinases and cysteine cathepsins [4–6]. Therefore, strengthening the collagen fibrils might increase the resistance of the resin-dentin interface to degradation. The use of cross-linking agents to increase mechanical properties of collagen fibrils and decrease enzymatic degradation has become important in restorative dentistry [7–9].

Several strategies have been developed to decrease the collagen degradation using enzymatic inhibitors, as well as, increasing the

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collagen resistance against the degradation process [9–11]. The association of these two treatments may improve the stability of resin–dentin bonded interface, which has been the main purpose of incorporating collagen cross-linkers into the bonding process [9]. The substances most frequently used, *in vitro*, for this purpose are proanthocyanidins (PA) [9,12,13].

Several studies have shown that PA improve the durability of the resin–dentine bonds when used as primer [12–15]. However, PA as primer add an extra step to the bonding protocol, challenging the clinicians' preference for simplification. Thus, the addition of PA into adhesive system seems more clinically acceptable [16].

Although promising results has been reported in laboratory studies when PA-containing adhesive were tested [16–19], to extent of our knowledge no clinical trials have been conducted to evaluate the effect of two concentrations of PA added to the adhesive on the clinical performance of composite restorations in non-carious cervical lesions (NCCLs). Therefore, the aims of this double-blind, randomized equivalence clinical trial were to compare the retention rates of PA-free and 2% PA- and 5% PA-containing 2-step etch-and-rinse adhesive (ExciTE F) on the clinical behavior of composite restorations in NCCLs over 6 and 24 months, using two evaluation criteria: World Dental Federation (FDI) and United States Public Health Service (USPHS) criteria. The null hypothesis tested was that bonding to NCCLs with PA-free or 2% PA- and 5% PA-containing 2-step etch-and-rinse adhesive yield similar clinical performance over 6- and 24-month of clinical service.

2. Materials and methods

The description of the experimental design followed the Consolidated Standards of Reporting Trials (CONSORT) statement [20].

2.1. Ethics approval

The local Ethics Committee on Investigations Involving Human Subjects reviewed and approved the protocol and issued a consent form for this study (protocol 640.695). Written informed consent was obtained from all patients prior to starting the treatment.

2.2. Protocol registration

This clinical trial was registered in a national clinical trial registry system under protocol RBR-366 MBJ. All participants were informed about the nature and objectives of the study.

2.3. Trial design, settings and location of data collection

This was a double-blind, equal allocation rate, split-mouth randomized clinical trial. The study was carried out in the clinics of the School of Dentistry at the local University from November 2014 to January 2017.

2.3.1. Recruitment

Subjects were recruited as they sought treatment in the Dental Clinics of the local university. No advertisement was made for participant recruitment. Subjects were recruited in the order in which they reported for the screening session, thus forming a sample of convenience.

2.3.2. Eligibility criteria

A total of 69 participants were examined by two calibrated dental graduate students to check if the participants met the inclusion and exclusion criteria (Fig. 1). The evaluations were performed using a mouth mirror, an explorer, and a periodontal probe. Participants had to

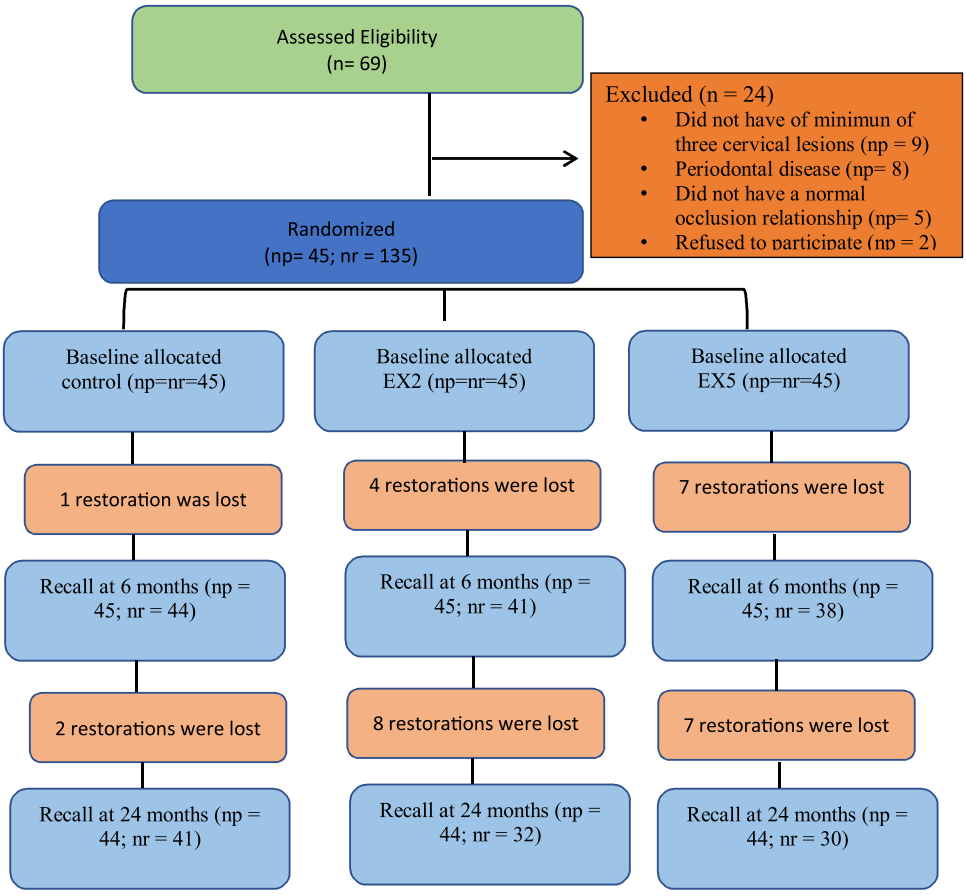

Fig. 1. Flow diagram. Np: number of patients, Nr: number of restorations. EX2 = 2% proanthocyanidins incorporated into the adhesive system; EX5 = 5% proanthocyanidins incorporated into the adhesive system.

be in good general health, older than 18 years old, have an acceptable oral hygiene level, and present at least 20 teeth under occlusion.

Participants were required to have at least three NCCLs to be restored in three different teeth. These lesions had to be non-retentive, deeper than 1 mm, and involving both the enamel and dentin of vital teeth without mobility. The cavosurface margin could not involve more that 50% of enamel [21]. Patients with extremely poor oral hygiene, severe or chronic periodontitis, or heavy bruxism habits were excluded from the study as they would receive other treatments before restorative intervention.

2.4. Sample size calculation

The 2-step etch-and-rinse adhesive ExciTE F (Ivoclar Vivadent AG, Schaan, Liechtenstein) was used in the present study. The percentage of retention of the ExciTE F adhesive system is 73% after 5 years of clinical evaluation in NCCLs [22]. Considering a 5% alpha, an 80% power and a two-sided test, the minimum sample size was 43 restorations per group to find a 22% difference between the tested groups.

2.5. Random sequence generation and allocation concealment

The randomization was done on an intra-individual basis so that each subject ended up with three restorations. These randomization schemes were performed using software available at http://www.sealedenvelope.com.

A staff member not involved in the research protocol performed the randomization process with computer-generated tables. Details of the allocated groups were recorded on cards contained in sequentially numbered, opaque and sealed envelopes. Opening the envelope immediately before of the restorative procedure guaranteed the concealment of the random sequence. In all cases, the tooth with the highest tooth number received the treatment described first, while the tooth with the next number in sequence received the treatment mentioned second and the next tooth received the treatment mentioned third.

2.6. Interventions: restorative procedure

Forty-five subjects were selected for this study and all received dental prophylaxis with a suspension of pumice and water in a rubber cup and signed an informed consent before the restorative procedures.

The degree of sclerotic dentin from the NCCLs was measured according to the criteria described by Swift et al. [23] (Table 1). The cavity dimensions in millimeters (height, width, and depth), the geometry of the cavity (evaluated by profile photograph and labeled at $< 45^{\circ}$, 45°-90°, $90^{\circ} < 135^{\circ}$, and $> 135^{\circ}$), [24] the presence of an antagonist, and the presence of attrition facets were observed and recorded. Pre-operative sensitivity was also evaluated by applying air-blast for 10 s from a dental syringe placed 2 cm from the tooth surface and with a dental explorer. These features were recorded to allow comparison of the baseline features of the dentin cavities among experimental groups.

To calibrate the operator for the restorative procedure, the study director placed one restoration of each group in one patient not involved in this trial to identify all steps involved in the application technique. Then, one operator with over five years of clinical experience in operative dentistry placed three restorations, one of each group, under the supervision of the study director in a clinical setting. All potential shortcomings due to the restorative procedure were shown to the operator prior to starting the study. At this point, the operator was considered calibrated to perform the restorative procedures. All subjects received three restorations, one of each experimental group in different lesions previously selected according to the inclusion criteria.

The operator cleaned all lesions with pumice and water in a rubber cup, followed by rinsing and drying prior to starting the restorative procedures. Then, shade selection was made using a shade guide (Ivoclar Vivadent AG, shade guide, Schaan, Liechtenstein). The tooth to be restored was isolated with cotton rolls and a light-cured gingival barrier (Top Dam, FGM, Joinville, SC, Brazil), following the ADA guidelines [25]. The operator did not prepare any additional retention features or bevel in the NCCL.

The adhesive (ExciTE F, Ivoclar Vivadent, Schaan, Liechtenstein) (Table 2) was used as control. For experimental groups, the same adhesive was modified by the incorporation of 2 mg of proanthocyanidins (PA) (Vitis vinifera, Meganatural Gold, Madera, CA, USA) to 98 mg of the adhesive or the incorporation of 5 mg PA (Vitis vinifera, Meganatural Gold, Madera, CA, USA) to 95 mg of the adhesive to form a mixture with PA concentrations of 2.0 wt% or 5.0 wt%, respectively. The teeth were distributed to these 3 groups and the adhesives were applied as described below. The materials, compositions and application modes are described in Table 2.

- *EXO (Control)*– 37% phosphoric acid gel (Condac 37, FGM, Joinville, SC, Brazil) was applied for 15 s and rinsed for 15 s, keeping dentin visible moist slightly with absorbent paper. One coat of adhesive was gently scrubbed on the entire enamel and dentin surface for approximately 10 s, according to the respective manufacturer's recommendations (Table 2). Then, the solvent was evaporated with a gentle air stream for 5 s and light cured for 10 s at 1.250 mW/cm^2 (Emitter A FIT Schuster Equipamentos Odontológicos, Santa Maria, RS, Brazil).

- *EX2* – After etching with 37% phosphoric acid gel for 15 s, rinsing for 15 s, and removing the excess of water with absorbent paper as in EX0, one coat of ExciTE F adhesive modified with 2% PA was applied and gently scrubbed on the entire enamel and dentin surface for approximately 10 s. Then, the solvent was evaporated with a gentle air stream for 5 s and light cured for 10 s at 1.250 mW/cm^2 (Emitter A FIT Schuster Equipamentos Odontológicos, Santa Maria, RS, Brazil) as in EX0.

- *EX5* - After etching with 37% phosphoric acid gel for 15 s, rinsing for 15 s, and removing the excess of water with absorbent paper as in EX2, ExciTE F adhesive modified with 5% PA were applied and light-cured as in EX2.

The resin composite Empress Direct (Ivoclar Vivadent, Schaan, Liechtenstein) was used in up to three increments, each one being lightly cured for 20 s at 1.250 mW/cm^2 (Emitter A FIT Schuster Equipamentos Odontológicos, Santa Maria, RS, Brazil). The restorations were finished immediately with fine and extra-fine #3195 diamond burs (KG Sorensen, Barueri, SP, Brazil) under constant water-cooling. After one-week, each one was finished and polished with slow-speed polishing points (Jiffy Polishers, Ultradent, South Jordan, UT, USA).

Table 1
Dentin sclerosis scale.[a]

CATEGORY	CRITERIA
1	No sclerosis present; dentin is light yellowish or whitish, with little discoloration; dentin is opaque, with little translucency or transparency
2	More sclerosis than in category 1 but less than halfway between categories 1 and 4
3	Less sclerosis than in category 4 but more than halfway between categories 1 and 4
4	Significant sclerosis present; dentin is dark yellow or even discolored (brownish); glassy appearance, with significant translucency or transparency evident

[a] Adapted from Swift and colleagues[24] with permission from Elsevier.

Table 2
Materials, composition and application mode.

Materials	Composition (*)	Application Mode (**)
Condac 37 phosphoric acid (FGM, Joinville, SC, Brazil)	37% phosphoric acid, thickening agents and pigments.	1. Prepare the region to be etched by cleaning 2. Drying it 3. Apply Condac 37 to the area to be etched and wait for a period of 15 seconds 4. Wash the surface with plenty of water 5. Dry the cavity in such a manner that the dentin does not become dehydrated.
ExciTE F adhesive system (Ivoclar Vivadent, Schaan, Liechnstein)	HEMA, dimethacrylate, Bis-GMA, UDMA, phosphonic acid acrylate, highly dispersed silicone dioxide, initiators, stabilizers and potassium fluoride in an ethanol solution.	1. Apply to the enamel and dentin and agitate the adhesive on the prepared surfaces for at least 10 seconds. Make sure that all the cavity walls are completely covered 2. Disperse to a thin layer with a weak stream of air, thereby removing any excess. 3. Polymerize for 10 seconds at a light intensity of more than 500 mW/cm^2
IPS Empress Direct resin composite (Ivoclar Vivadent, Schaan, Liechnstein)	Dimethacrylates (20-21.5 wt%, opalescent shade 17 wt%). The fillers contain barium glass, ytterbium trifluoride, mixed oxide, silicon dioxide and copolymer (77.5-79 wt%, opalescent shade 83 wt%). Additional contents: additives, initiators, stabilizers and pigments (< 1.0 wt%). The total content of inorganic fillers is 75-79 wt% or 52-59 vol% (opalescent shade 60.5 wt% or 45 vol%). The particle size of the inorganic fillers is between 40 nm and 3 μm with a mean particle size of 550 nm.	1. Apply IPS Empress Direct Effect in layers of max. 2 mm thickness. 2. Polymerize each layer for 20 s and keep the light emission window as close as possible to the surface of the restorative material

(*) HEMA = 2-hydroxyethyl methacrylate Bis-GMA = bisphenol glycidyl methacrylate; UDMA = urethane dimethacrylate.
(**) According to the manufacturer's instructions.

2.7. Calibration procedures for clinical evaluation

For training purposes, two experienced and calibrated examiners observed 10 photographs that were representative of each score for each criterion. They evaluated 10 patients each on two consecutive days. These patients had cervical restorations but were not part of this project. An intra-examiner and inter-examiner agreement of at least 85% was necessary before beginning the evaluation [26]. In case of disagreement between the examiners, consensus was obtained.

2.7.1. Blinding

The examiners, who were not involved with the restoration procedures and therefore blinded to the group assignment, performed the clinical evaluation. Subjects were also blinded to group assignment in a double-blind randomized clinical trial design.

2.7.2. Clinical evaluation

An individual standardized paper case report form was used for each evaluator at each recall time so that evaluators were kept blinded to earlier evaluations during the follow-up recalls. Intraoral color photographs were collected at baseline and at the recall appointments to aid in the evaluation, if necessary. Clinical photographs consisted of digital images obtained using a Nikon D90X camera with a Nikon Micro-Nikkor 105-mm lens (Nikon Inc., Melville, NY, USA).

The restorations were evaluated using the World Federation criteria (FDI) [27] and the classical United States Public Health Service (USPHS) criteria (adapted by Bittencourt et al. [28] and Perdigão et al. [29] at baseline, after 6 months (6 M) and 24 months (24 M) of clinical service. Only the clinically relevant measures for evaluation of adhesive performance were used and scored (Tables 3 and 4). The primary clinical endpoint was restoration retention/fracture, but the following secondary endpoints were also evaluated: marginal staining, marginal adaptation, postoperative sensitivity, and recurrence of caries.

These variables were ranked according to FDI criteria into clinically very good, clinically good, clinically sufficient/ satisfactory, clinically unsatisfactory but repairable, and clinically poor (replacement required), [27] while in the USPHS criteria were ranked into Alfa, Bravo and Charlie [28]. Both examiners evaluated all the restorations once and independently. When disagreements occurred during the evaluations, they had to reach a consensus before the subject was dismissed. The restoration retention rates were calculated according to the ADA guidelines [25]. Cumulative failure percentage = [(PF + NF) / (PF + RR)] X 100%, where PF is the number of previous failures before the current recall, NF is the number of new failures during the current recall, and RR is the number of currently recalled restorations.

2.8. Statistical analysis

The statistical analyses followed the intention-to-treat protocol according to CONSORT (Consolidated Standards of Reporting Trials) suggestion [20]. Descriptive statistics were used to describe the distributions of the evaluated criteria. Statistical analysis for each individual item was performed for each evaluation criteria (FDI and USPHS criteria).

The differences in the ratings of the three groups after 6 M and 24 M were tested with the Friedman repeated-measures analysis of variance by rank, and differences in the ratings of each group at baseline and after 6 and 24 months were evaluated using the Wilcoxon test. Cohen's kappa statistics was used to test inter-examiner agreement. In all statistical tests the level of significance was 5%.

3. Results

The restorative procedures were implemented exactly as planned and no modification was performed. Twenty-four out of 69 subjects were not enrolled in the study because they did not fulfill the inclusion criteria (Fig. 1). Thus, 45 subjects were selected. All baseline details relative to the research subjects and characteristics of the restored lesions are displayed in Table 5. All research subjects were evaluated at the baseline and at 6 M evaluation and the reason for drop-off was because only one patient with three restorations did not attend the 24 M recall rate (Fig. 1), because this patient moved to another city.

3.1. Retention

Twelve restorations were lost at 6 M. According to FDI and USPHS criteria, the 6 M retention rates (95% confidence interval) were 98% (88–99%) for EX0; 92% (80–97%) for EX2; and 85% (72–93%) for EX5. Twenty-nine restorations were lost at 24 M. According to FDI and USPHS criteria, the 24 M retention rates (95% confidence interval)

Table 3
World Dental Federation (FDI) criteria used for clinical evaluation [27].

	Esthetic Property	Functional Properties		Biological Properties	
	1. Staining margin	2. Fractures and retention	3. Marginal adaptation	4. Postoperative (hyper-) sensitivity	5. Recurrence of caries
1. Clinically very good	1.1 No marginal staining	2.1 Restoration retained, no fractures / cracks	3.1 Harmonious outline, no gaps, no discoloration.	4.1 No hypersensitivity.	5.1 No secondary or primary caries
2. Clinically good (after correction very good)	1.2 Minor marginal staining, easily removable by polishing.	2.2 Small hairline crack	3.2.1 Marginal gap (50 μm). 3.2.2 Small marginal fracture removable by polishing	4.2 Low hypersensitivity for a limited period of time	5.2 Very small and localized demineralization. No operative treatment required
3.Clinically sufficient / satisfactory (minor shortcomings with no adverse effects but not adjustable without damage to the tooth)	1.3 Moderate marginal staining, not esthetically unacceptable.	2.3 Two or more or larger hairline cracks and/or chipping (not affecting the marginal integrity).	3.3.1 Gap < 150 μm not removable 3.3.2. Several small enamel or dentine fractures	4.3.1 Premature / slightly more intense 4.3.2 Delayed/weak sensitivity; no subjective complaints, no treatment needed.	5.3 Larger areas of demineralization, but only preventive measures necessary (dentine not exposed)
4. Clinically unsatisfactory (repair for prophylactic reasons)	1.4 Pronounced marginal staining; major intervention necessary for improvement	2.4 Chipping fractures which damage marginal quality; bulk fractures with or without partial loss (less than half of the restoration).	3.4.1 Gap > 250 μm or dentine/base exposed 3.4.2. Chip fracture damaging margins 3.4.3 Notable enamel or dentine wall fracture	4.4.1 Premature/ very intense 4.4.2 Extremely delayed/weak with subjective complaints 4.4.3 Negative Sensitivity; Intervention necessary but not replacement	5. 4 Caries with cavitation (localized and accessible and can be repaired)
5. Clinically poor (replacement necessary)	1.5 Deep marginal staining not accessible for intervention.	2.5 (Partial or complete) loss of restoration.	3.5 Filling is loose but in situ.	4.5 Very intense, acute pulpitis or non vital. Endodontic treatment is necessary and restoration has to be replaced.	5.5 Deep secondary caries or exposed dentine that is not accessible for repair of restoration.
Acceptable or not acceptable (n, % and reasons)	Aesthetic criteria	Functional criteria		Biological criteria	

Table 4
Modified United States Public Health Service (USPHS) criteria according to Bittencourt et al. [28] and Perdigão et al. [29].

	Marginal staining	Retention	Fracture	Marginal adaptation	Postoperative sensitivity	Recurrence of caries
Alfa	No discoloration along the margin	Retained	None	Restoration is continuous with existing anatomic form.	No postoperative sensitivity directly after the restorative process and during the study period	No evidence of caries contiguous with the margin
Bravo	Slight and superficial staining (removable, usually localized)	Partially retained	Small chip, but clinically acceptable	Detectable V-shaped defect in enamel only. Catches explorer going both ways.	–	–
Charlie	Deep staining cannot be polished away	Missing	Failure due to bulk restorative fracture	Detectable V-shaped defect to dentin-enamel junction	Sensitivity present at any time during the study period	Evidence of presence of caries

were 98% (88–99%) for EX0; 73% (59–84%) for EX2; and 71% (56–82%) for EX5. When the data from the 6 M recall in each group were compared with their baseline findings, a significant difference was found only for EX5 ($p = 0.03$; Tables 6 and 7). When the data from the 24 M recall in each group were compared with their baseline findings, a significant difference was found for EX2 and EX5 ($p = 0.03$; Tables 6 and 7). After 6 M, the retention rates of EX0 and EX2 were significantly higher than those of EX5 ($p = 0.001$; Tables 6 and 7), while EX0 showed significantly higher retention rate after 24 M than those of EX2 and EX5 ($p = 0.001$; Tables 6 and 7).

3.2. Post-operative sensitivity

None of the restorations showed post-operative sensitivity immediately after restorative procedures according to the FDI and USPHS criteria. After 6 M and 24 M, no restoration showed post-operative sensitivity using both the FDI and USPHS criteria (Tables 6 and 7).

3.3. Marginal adaptation

According to the FDI criteria, 93 restorations at the 6 M recall were considered to have some discrepancies in marginal adaptation. After 24 M, 126 restorations were considered to have some discrepancies in marginal adaptation. No significant difference was detected between any pair of groups at 6 M and at 24 M for both criteria ($p = 0.45$; Tables 6 and 7).

However, a significantly worse marginal adaptation was observed within all groups over time, mainly after 24 M ($p = 0.001$; Tables 6 and 7). Despite the high number of the restorations with marginal discrepancy in the FDI criteria, none of them were considered to have clinically relevant discrepancies (clinically unsatisfactory) in the marginal adaptation even after 24 M (Table 6).

When the USPHS criteria were used, only 7 restorations were scored as *Bravo* for marginal adaptation at 6 M compared to baseline ($p = 0.51$). After 24 M, 10 restorations were scored as *Bravo* for marginal adaptation ($p = 0.62$). No significant difference was detected between any pair of groups at 6 M and 24 M and between recall times within group ($p > 0.51$).

3.4. Marginal discoloration

For the FDI criteria, 18 restorations at 6 M were considered to have minor discrepancies (clinically good and satisfactory). After 24 M, 57 restorations were considered to have minor discrepancies (clinically good and satisfactory).

A significant difference between baseline vs. 6 M was observed for the group EX5 using the FDI criteria ($p = 0.002$; Tables 6). However, a significant difference between baseline vs. 24 M was observed for all groups using the FDI criteria ($p = 0.003$; Tables 6). It worth mentioning that EX5 showed significantly higher marginal staining at 24 M when compared with EX0 and EX2 ($p = 0.001$; Tables 6 and 7).

When the USPHS criteria were used, only eight restorations at 6 M were scored as *Bravo* for marginal staining ($p > 0.05$). After 24 M, thirty restorations were scored as *Bravo* for marginal staining. A significant difference between baseline vs. 24 M was observed for all groups using USPHS criteria ($p = 0.01$; Tables 6). However, after 24 M, only EX5 showed a significantly higher marginal staining when compared with EX0 ($p = 0.001$; Tables 6 and 7).

3.5. Recurrence of caries

No restoration showed recurrent caries lesion at 6 M and 24 M using the FDI and the USPHS criteria.

Table 5
Distribution of noncarious cervical lesions according to research subject (gender and age) and characteristics of Class V lesions (shape, cervicoincisal size of the lesion, degree of sclerotic dentin, presence of antagonistic, presence of attrition facets, presence of preoperative sensitivity, and tooth and arch distribution).

Characteristics of research subjects	Number of lesions
Gender distribution	
Male	28
Female	17
Age distribution (years)	
20-29	06
30-39	11
40-49	9
> 49	19

Characteristics of Class-V lesions	Number of lesions		
	EX0	EX2	EX5
Shape (degree of angle)			
< 45	1	1	1
45-90	11	10	13
90-135	19	20	23
> 135	14	14	8
Cervico-incisal height (mm)			
< 1.5	2	7	5
1.5-2.5	28	20	21
> 2.5	15	18	19
Degree of sclerotic dentin			
1	22	19	18
2	13	15	18
3	9	10	8
4	1	1	1
Presence of antagonist			
Yes	45	45	45
No	00	00	00
Attrition facet			
Yes	43	43	43
No	2	2	2
Pre-operative sensitivity (spontaneous)			
Yes	00	00	00
No	45	45	45
Pre-operative sensitivity (air dry)			
Yes	24	24	25
No	21	21	20
Tooth distribution			
Anterior			
Incisor	4	10	8
Canine	9	6	3
Posterior			
Premolar	30	27	32
Molar	2	2	2
Arc distribution			
Maxillary	20	20	23
Mandibular	25	25	22

Table 6
Number of evaluated restorations for each experimental group (EX0 [adhesive without PA], EX2 [2% PA incorporated into the adhesive] and EX5 [5% PA incorporated into the adhesive]) classified according to the World Dental Federation (FDI) criteria [27].

Timer		Baseline			6 months			24 months		
FDI Criteria	(*)	EX0	EX2	EX5	EX0	EX2	EX5	EX0	EX2	EX5
Marginal adaptation	VG	45	45	45	15	06	07	05	01	–
	GO	–	–	–	27	33	28	33	28	26
	SS	–	–	–	02	02	02	03	03	04
	UN	–	–	–	–	–	01	–	–	–
	PO	–	–	–	–	–	–	–	–	–
Marginal staining	VG	45	45	45	41	38	25	27	15	04
	GO	–	–	–	01	02	08	07	07	13
	SS	–	–	–	02	01	04	07	10	13
	UN	–	–	–	–	–	01	–	–	–
	PO	–	–	–	–	–	–	–	–	–
Fractures and retention	VG	45	45	45	44	41	38	41	32	30
	GO	–	–	–	–	–	–	–	–	–
	SS	–	–	–	–	–	–	–	–	–
	UN	–	–	–	–	–	–	–	–	–
	PO	–	–	–	01	04	07	03	12	14
	VG	45	45	45	44	41	38	41	32	30
Post-operative sensitivity	GO	–	–	–	–	–	–	–	–	–
	SS	–	–	–	–	–	–	–	–	–
	UN	–	–	–	–	–	–	–	–	–
	PO	–	–	–	–	–	–	–	–	–
Recurrence of caries	VG	45	45	45	44	41	38	41	32	30
	GO	–	–	–	–	–	–	–	–	–
	SS	–	–	–	–	–	–	–	–	–
	UN	–	–	–	–	–	–	–	–	–
	PO	–	–	–	–	–	–	–	–	–

(*) VG for clinically very good; GO for clinically good; SS for clinically sufficient/satisfactory; UN for clinically unsatisfactory and; PO for clinically poor.

3.6. General overview

When the FDI criteria for 'acceptable' *vs*. 'not acceptable' restorations were applied, only twenty-nine restorations were ranked as 'not acceptable', the majority from EX2 and EX5 groups (Table 8).

4. Discussion

Having shown promising results in laboratory studies [16–18], the use of PA as cross-links agents in the present study aimed at evaluating the clinical performance of PA-containing simplified etch-and-rinse adhesive, focusing on the retention rate. In fact, there was a decrease in retention rate for the experimental groups after 6-month for EX5 and after 24-month for both EX2 and EX5, which leads to rejection of the null hypothesis.

The use of PA as an additional primer applied prior to the adhesive has shown good results in vitro. In fact, PA enhance the degree of collagen cross-linking, protect the exposed collagen fibrils in the hybrid layer, and increase the resistance to biodegradation to collagenase, as well as the longevity of bond strengths [12,13,30,31]. However, the additional step extends the application clinical time, which goes against the tendency for simplification of adhesive materials and techniques.

To simplify the use of PA in clinical situations, some studies have incorporated PA into the adhesive solution, [14,16,17] reducing the number of bonding steps. This might be appealing to clinicians provided that it improves the durability of resin–dentin adhesive restorations [32].

The idea of adding PA to adhesives is to allow a sustained release of PA from the cured resin into the surrounding collagen fibrils to exert its collagen cross-linking and protease inhibitory, not only in the immediate time, but also long-term [19]. The present study evaluated two different concentrations of PA, because it has been shown that the in vitro inhibitory effect of PA against matrix metalloproteinases and cysteine cathepsins is proportional to the respective concentration [33]. Also,

Table 7
Number of evaluated restorations for each experimental group (EX0 [adhesive without PA], EX2 [2% PA incorporated into the adhesive] and EX5 [5% PA incorporated into the adhesive]) classified according to the adapted United States Public Health Service (USPHS) criteria [28,29].

Timer		Baseline			6 months			24 months		
USPHS Criteria		EX0	EX2	EX5	EX0	EX2	EX5	EX0	EX2	EX5
Marginal adaptation	Alfa	45	45	45	42	39	35	38	29	26
	Bravo	–	–	–	02	02	03	03	03	04
	Charlie	–	–	–	–	–	–	–	–	–
Retention	Alfa	45	45	45	44	41	38	41	32	30
	Charlie	–	–	–	01	04	07	03	12	14
Marginal staining	Alfa	45	45	45	42	40	33	34	22	17
	Bravo	–	–	–	02	01	05	07	10	13
	Charlie	–	–	–	–	–	–	–	–	–
Post-operative sensitivity	Alfa	45	45	45	44	41	38	44	41	38
	Charlie	–	–	–	–	–	–	–	–	–
Recurrence of caries	Alfa	42	45	45	44	41	38	41	32	30
	Charlie	–	–	–	–	–	–	–	–	–

Epasinghe et al. [18] showed that the amount of PA released was increased with higher concentrations of PA incorporated into the adhesive.

Although several advantages were observed in previous in vitro studies, PA-containing adhesives resulted in worse clinical parameters in our study. A reduction in the retention rate of 15% at 6 M was observed for the EX5 group. After 24 M an even greater reduction of 27% and 29% were observed for EX2 and EX5, respectively, which is quite significant for a dental adhesive. The ADA guidelines require a 90% retention rate for full acceptance at 24 months [25].

These results suggest that the PA added to the adhesive negatively affect the adhesive interface for both concentrations evaluated. PA have a free radical-scavenging potential [34,35]. When involved in radical polymerization, PA donate hydrogen atoms to the free radicals and inhibit the initiation and propagation of the chain reaction of polymerization [36]. However, this inhibition is proportional to the PA concentration in the adhesive. For instance, Liu and Wang (2013) [36] reported that 2.5% PA did not interfere with the degree of conversion and polymerization rate of adhesives when compared with adhesive without PA. On the other side, a concentration of PA of ≧5%, alone or added to the adhesive, interferes with the polymerization reaction of adhesive systems [16,36].

This suboptimal polymerization of the monomers inside resin-dentin hybrid layer may lead to greater nanoleakage, due to some unreacted monomers that leach out of the adhesive and hybrid layers, thereby increasing the porosity of the bonded interface. According to Epasinghe et al. [17], this also explains the higher number of adhesive failures and lower bond strength when a higher PA concentrations (EX5) were evaluated in their study.

These findings may explain the higher number of restorations with marginal discoloration for EX5 after 6 M and 24 M. However, PA present a dark brown color, which might cause an esthetic problem, mainly high concentration as in EX5. Dentin treated with PA solutions shows a brownish color *in vitro* [37]. The presence of high molecular weight polymer polyphenols may be responsible for the color change, as well as the increase in marginal staining for experimental groups [9,38,39]. Nonetheless, this marginal discoloration was not associated with any gap between restoration/tooth, as no restoration was considered to have clinically unsatisfactory marginal discoloration [40–42].

The clinical performance of EX5 in terms of retention rate was worse after 6 M. Moreover, a lower retention rate was observed for both PA groups after 24 M compared to the adhesive without PA, with no significant difference between EX2 and EX5.

The explanation for the results obtained with EX2 is not clear. We hypothesize that, as PA contain the oligomeric proanthocyanidin molecule, the higher molecular size may further interrupt chain propagation by separating the monomer molecules from the continuing polymer

Table 8
Restorations acceptable or not acceptable according to the Federation Dental International (FDI) criteria after 24 months [27].

Properties	Aesthetic			Functional						Biological					
	Marginal staining			Fractures and retention			Marginal adaptation			Postoperative (hyper-) sensitivity			Recurrence of caries		
	EX0	EX2	EX5	EX0	EX2	EX5	EX0	EX2	EX5	EX0	EX2	EX5	EX0	EX2	EX5
Acceptable	41	32	30	41	32	30	41	32	30	41	32	30	41	32	30
Not acceptable	–	–	–	03	12	14	–	–	–	–	–	–	–	–	–
Reasons				Total loss of restorations: 29											

chain, thereby negatively impacting the mechanical properties of the adhesive layer [18]. In fact, a recent paper [18] showed that the addition of 1.5–2% PA significantly reduced flexural strength, modulus of elasticity and Vickers hardness, as well as, solubility in water, when compared with adhesive without PA.

Interestingly, no significant changes in water sorption was observed when different concentrations of PA were used in Epasinghe's study [18]. The presence of water softens the polymer by swelling the network, which reduces the frictional forces between polymer chains (i.e., plasticizes resins). After the relaxation process, the elution of unreacted monomers previously separated by PA, but still trapped in the polymer network, is facilitated [43]. All these mechanisms significantly increase the solubility of the adhesive material itself [44–46], decreasing the mechanical properties of the adhesive and, consequently leading to degradation of the resin-dentin bonds over time. However, water sorption is a slow process, which may have been responsible for the significant decrease in retention rate of EX2 only after 24-month of clinical use.

It worth mentioning that the composition of the adhesive tested might have influenced the results. The interaction between PA and several components of the ExciTE F may have caused the loss of retention and marginal discrepancy observed in the present study. Therefore, future studies need to be carried out evaluating the addition of PA to other adhesive systems with different photo-initiation formulations, as recommended by Liu and Wang [36].

In terms of marginal adaptation, 93 restorations exhibited some marginal discrepancy at 6 M and 126 restorations at 24 M. All the groups showed a significantly worse marginal adaptation over time, mainly after 24 M when the FDI criteria were used. The marginal discrepancies of a composite restoration are common and develop rather rapidly [41,47–49]. However, this appears to cause no relevant clinical change, because most of the marginal defects were considered small and clinically acceptable [50]. Simply re-polishing the defects improves these discrepancies without causing any damage to the integrity of the restoration [47,51].

When USPHS criteria were used, only 7 restorations after 6 M and 10 restorations after 24 M were deemed to have some marginal discrepancies, with no significant difference between groups. This difference between FDI and USPHS criteria is because the FDI criteria are more sensitive than the USPHS criteria for identifying small variations in the clinical outcomes when evaluating restorations of NCCLs [41,47].

Although the application of PA has been advocated for etch-and-rinse adhesive systems in several in vitro studies, the present study showed that PA added into ExciTE F negatively affected the clinical performance after 24-month, especially with the highest concentration used (5%). With this in mind, future studies should evaluate the addition of lower percentages of PA (1% or less) into adhesive systems. In virtue of promising in vitro results, another alternative in future clinical trials would be to apply PA as an additional primer [12–15] or PA incorporated in the etchant [52,53].

5. Conclusion

The clinical behavior of resin composite restorations in non-carious cervical lesions over a 24-month follow-up was jeopardized when 2% and 5% proanthocyanidins were added to the simplified etch-and-rinse adhesive system evaluated.

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