Journal of Dentistry 90 (2019) 103219

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

18-month clinical evaluation of a copper-containing universal adhesive in non-carious cervical lesions: A double-blind, randomized controlled trial

Thalita P. Matos[a], Mario F. Gutiérrez[a,b,c], Taise A. Hanzen[a], Pâmela Malaquias[a], Alexandra M. de Paula[a], Jullian J. de Souza[a], Viviane Hass[d], Eduardo Fernández[e,f], Alessandra Reis[g], Alessandro D. Loguercio[g,*]

[a] School of Dentistry, State University of Ponta Grossa, Ponta Grossa, Brazil
[b] Institute for Research in Dental Sciences, Faculty of Dentistry, University of Chile, Santiago, Chile
[c] Faculty of Dentistry, Finis Terrae University, Santiago, Chile
[d] Postgraduate Program in Dentistry, University of Northern Parana, Londrina, Brazil
[e] Department of Restorative Dentistry, Faculty of Dentistry, University of Chile, Santiago, Chile
[f] Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Providencia, Chile
[g] Department of Restorative Dentistry, State University of Ponta Grossa, Ponta Grossa, Brazil

ARTICLE INFO

Keywords:
Universal adhesive system
Copper nanoparticles
Non-carious cervical lesion
Clinical trial
Longevity

ABSTRACT

Objective: This study aimed to evaluate the addition of copper nanoparticles (CuNp) on the clinical performance of a universal adhesive system used as etch-and-rinse (ER) and self-etch (SE).
Methods: 216 restorations were randomly placed in 36 subjects according to the following groups: ER*cu* = etch-and-rinse with 0.1% CuNp; ER*ct* = etch-and-rinse without CuNp; SE*cu* = self-etch with 0.1% CuNp; SE*ct* = self-etch without CuNp. Resin composite was placed incrementally and light-cured. The restorations were evaluated at baseline and 6, 12 and 18 months using the FDI and USPHS criteria. Statistical analyses were performed using appropriate tests ($\alpha = 0.05$).
Results: The addition of CuNp did not increase the clinical performance (FDI / USPHS) of the universal adhesive tested after 18-month when applied in the ER mode ($p > 0.05$). The addition of CuNp in SE restorations increased the retention rate significantly and decreased the marginal discrepancies after 18 months ($p < 0.05$).
Conclusion: The clinical performance of universal adhesive was significantly increased when applied in the SE mode with the addition of copper nanoparticles.
Clinical relevance: This is the first study that demonstrates a slight improvement in the clinical performance of universal adhesive systems in non-carious cervical lesions when added CuNp in lower concentration.

1. Introduction

Resin composites are the most-used dental restorative material, representing 65% of the restorations currently used in the United States [1,2]. However, concerns about their limited in vivo durability exist because the replacement of failed restorations accounts for nearly 70% of all restorative dentistry [3]. Secondary caries is responsible for 60% of all replacement restorations in typical dental practices [4].

The high accumulation of dental biofilm around resin-dentin interfaces likely causes the resin-based restorations to degrade faster. This degradation is mainly attributed to an association between the resin-dentin interface's failure and increased levels of cariogenic bacteria at the perimeter of the resin-composite restorations [3,5]. The production of acids [6] and esterases [7] may also accelerate the degradation of the resin-dentin interface, an essential factor in the premature failure of moderate-to-large composite restorations [3,8]. Recent evidence also indicates that matrix metalloproteinases (MMPs) are involved in dentinal caries progression due to their crucial role in dentin collagen breakdown, during caries lesions [9,10].

There are continuous efforts to improve the antimicrobial and anti-MMP properties of adhesive systems in these contexts [11,12]. Many studies focused on preserving the adhesive interfaces via antimicrobial and anti-MMP agents incorporated into the adhesive protocols or systems to solve problems, such as secondary caries [11–17].

* Corresponding author at: Universidade Estadual de Ponta Grossa, Departamento de Odontologia, Avenida Carlos Cavalcanti, 4748 – Uvaranas, Ponta Grossa, Paraná, CEP: 84030-900, Brazil.
E-mail address: aloguercio@hotmail.com (A.D. Loguercio).

https://doi.org/10.1016/j.jdent.2019.103219
Received 21 May 2019; Received in revised form 19 September 2019; Accepted 15 October 2019

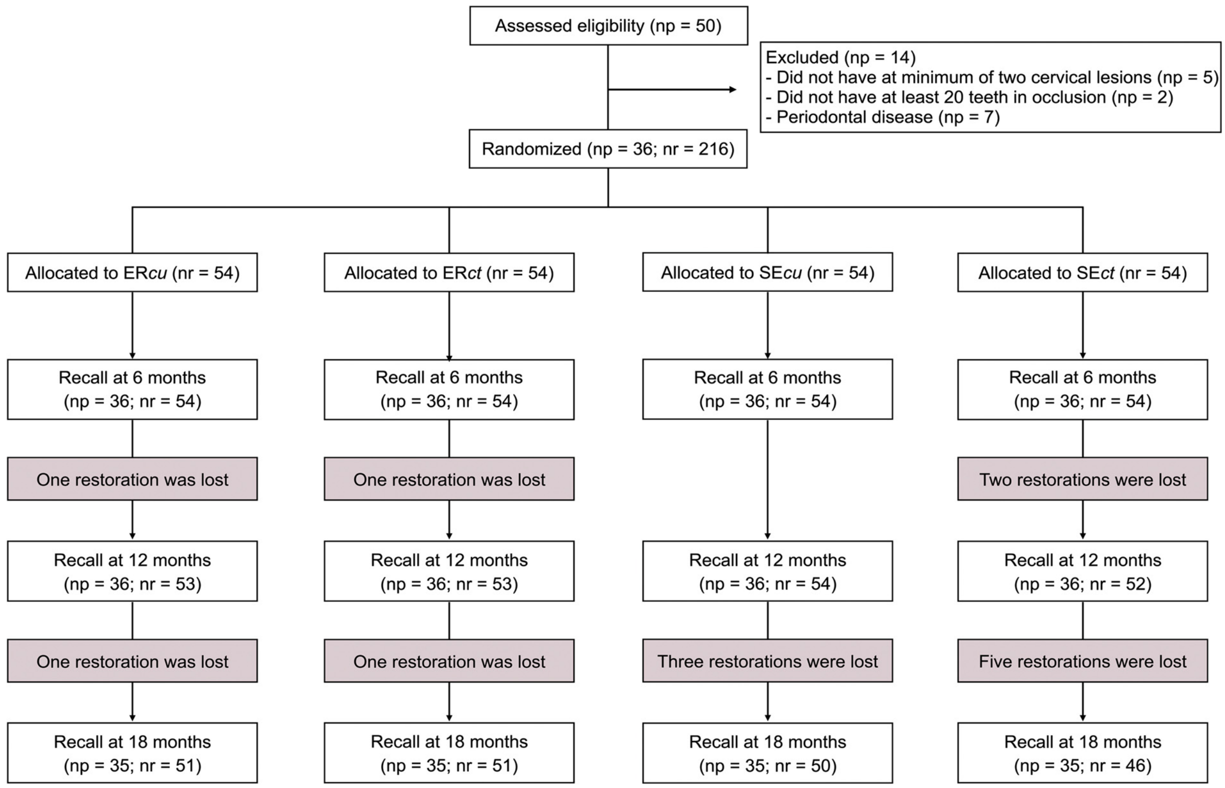

Fig. 1. Participant flow diagram in the different phases of the study design. Abbreviations: Np – number of participants; Nr – number of restorations.

A simple and effective alternative is to add metal nanoparticles, such as copper nanoparticles (CuNp) [13,18] as they are effective against gram-negative and gram-positive bacteria [19]. Also, it seems to be a potent inhibitor of MMPs [20,21]. Although CuNp's antibacterial effects are not fully understood, several mechanisms have been proposed [13,18]. A more recent theory is called "Trojan horse effect." In this theory, the acidic lysosomal environment (pH 5.5) can promote nanoparticles' degradation and corrosion, which converts core metals to ions and toxic substances. Inside the mitochondria, a high volume of reactive oxygen is released, causing malfunction of other organelles [22].

Besides, copper is cheap, and, because it oxidizes to form copper oxide nanoparticles, it easily blends with polymers or macromolecules because of its relatively stable chemical and physical properties [14,20,21]. Recent studies show that adding CuNp in concentrations up to 0.1 wt% in an adhesive system provided antimicrobial properties without reducing the mechanical and optical properties of the adhesive formulations [16,17]. Both studies also show that adding copper nanoparticles in concentrations up to 1% may be an alternative to preserving the bond to dentin after two years of water storage [16,17]. To the authors' knowledge, however, no clinical study has evaluated the clinical performance of a CuNp-containing universal adhesive system.

Therefore, this double-blind, randomized controlled trial aims to evaluate the influence of incorporating CuNp in a concentration of 0.1 wt% on the clinical performance of a universal adhesive system. The adhesive system is applied in etch-and-rinse and self-etch modes and is used underneath resin composite restorations in non-carious cervical lesions (NCCLs) for 18 months. Two evaluation criteria were used: the World Dental Federation (FDI) and the US Public Health Service (USPHS) criteria. The null hypothesis was that bonding to NCCLs using CuNp-containing universal adhesives, applied in both etch-and-rinse and self-etch modes, generate similar clinical performances over 18 months when compared with a CuNp-free universal adhesive.

2. Materials and methods

2.1. Ethics approval and protocol registration

The local Ethics Committee on Involving Human Subjects reviewed and approved the protocol and consent form for this study (protocol 19670/95). Written informed consent was obtained from all participants before starting the treatment. The experimental design followed the Consolidated Standards of Reporting Trials (CONSORT) statements [23].

This study was a randomized, double-blind controlled trial, registered in the Brazilian Registry of Clinical Trials (REBEC) by the number RBR-65zy7 x. The study was carried out in the local university from October 2016 to March 2017. The participants were informed about the nature and the objectives of the study, but they were not aware of what tooth received the specific treatments under evaluation.

2.2. Participant recruitment

Subjects were recruited while seeking treatment in the local university's dental clinics. No advertisement was made for participant recruitment. Subjects were recruited in the order they reported for the screening session, thus forming a convenient sample.

2.3. Sample size

The primary outcome of this study was the retention rate. The sample size calculation was made using an online software that is available at http://www.sealedenvelope.com, based on the retention rate of universal adhesive that was reported to be 94% at 18-month follow-up [24]. A minimal sample size of 50 restorations was required to detect a difference of 20% among the tested groups, with an α of 0.05, and a power of 80%.

2.4. Eligibility criteria

A total of 50 participants were examined by two pre-calibrated dentists to ensure the subjects met the inclusion and exclusion criteria. Following these examinations, 14 subjects were excluded, and 36 were recruited after accepting the terms of the research (Fig. 1). The evaluations were performed using a mouth mirror, an explorer, and a periodontal probe.

Participants had to be in good general health, be older than 18 years, have an acceptable level of oral hygiene according to the Simplified Oral Hygiene Index [25], and present at least 20 teeth under occlusion. They had to have at least four NCCLs in four different teeth that needed restoration. These lesions had to be non-carious, non-retentive, and deeper than 1 mm and they had to involve both the enamel and dentin of vital teeth without mobility. The cavosurface margin could not involve more than 50% of the enamel [26].

All subjects were given oral hygiene instructions before performing the operative treatment. Subjects with inferior oral hygiene, severe or chronic periodontitis, heavy bruxism habits or use of orthodontic devices were excluded from the study.

2.5. Randomization sequence generation and allocation concealment

The randomization process was performed (using software available at http://www.sealedenvelope.com) by a staff member not involved in the research protocol. The allocated group's details were recorded on cards in sequentially numbered, opaque and sealed envelopes. These were prepared by a staff member not involved in any of the clinical trial phases. The allocation assignments were revealed by opening the envelope immediately before the restorative procedure to guarantee the concealment of the random sequence and prevent selection bias. The participants and the examiners were blinded to the group assignments.

2.6. Baseline characteristics of the selected teeth

The same two trained and calibrated dentists involved in the selection of participants carried out the restorative procedures. The features of the NCCLs were evaluated before the placement of the restorations. The degree of dentin sclerosis was evaluated according to an earlier scoring system [27] modified by Swift et al. [28] (Table 1), and the lesion dimensions in mm (height, width, and depth) and the geometry of the lesion (evaluated by photograph profile and labeled at < 45°, 45°-90°, 90°-135°, > 135°) were recorded [29]. Other features, such as the presence of attrition facets [30], were also observed and recorded. Pre-operative sensitivity was evaluated prior to examination (spontaneous), by applying air stream for 10 s from a dental syringe placed 2 cm from the tooth surface (air dry), and with an explorer (touch).

For the calibration procedure step, the study director placed one restoration of each group in order to identify all steps involved in the protocol. Then, two operators placed another four restorations of each group under the supervision of the study director in a clinical setting. Any discrepancies of the restorative protocol were identified and discussed with the operator before starting the study. At this point, the operators were considered calibrated to perform the restorative procedures. The calibrated operators restored all teeth under the supervision of the study director.

2.7. Interventions: restorative procedure

A detailed protocol standardized the interventions, briefly summarized following. A preliminary dental prophylaxis of the tooth surface was performed with pumice and water in a rubber cup to remove the salivary pellicle and any remaining dental plaque, followed by rinsing and drying. The proper shade of the resin composite was determined using a shade guide. Local anesthesia was applied with a 3% mepivacaine solution (Mepisv, Nova DFL, Rio de Janeiro, RJ, Brazil), and all restorations were placed under rubber dam isolation. Following the guidelines of the American Dental Association (ADA) [31] the operators did not prepare any additional retention or bevel.

The Ambar Universal (batch# 200716; FGM Prod. Odont.Ltda, Joinville, SC, Brazil) were used to restored all NCCLs according to the following groups: 1) ER*cu* = Etch-and-rinse with the universal adhesive containing copper nanoparticles; 2) ER*ct* = Etch-and-rinse with the universal adhesive without copper nanoparticles (commercial adhesive); 3) SE*cu* = Self-etch with the universal adhesive containing copper nanoparticles; 4) SE*ct* = Self-etch with the universal adhesive without copper nanoparticles (commercial adhesive). For the groups without copper (ER*ct* and SE*ct)* the adhesive system Ambar Universal was used without none modification (as available commercially). For the groups containing copper nanoparticles (ER*cu* and SE*cu),* the adhesive system Ambar Universal suffer the addition of copper nanoparticles (99.9% pure, SkySpring Nanomaterials, Inc., Houston, TX, USA; www.ssnano.com) in a concentration of 0.1 wt%, as described in previous studies [16,17].

All adhesive system bottles for all experimental groups were pre-coded by a staff member not involved in the research protocol to provide blinding of the study team as well as the patient. The adhesive was applied on the entire dentin surface in all groups for approximately 20 s (two separate coats of 10 s each), according to the manufacturer's recommendations, followed by gentle air for 10 s and finally light-curing for 20 s at 1200 mW/cm^2 (Radii Cal, SDI, Victoria, Australia). For etch-and-rinse groups, a 37% phosphoric acid was applied for 15 s in enamel and dentin (Condac, FGM Prod. Odont. Ltda, Joinville, SC, Brazil) and after rinsing, the dentin was kept moisture before adhesive application.

The operators restored the cervical tooth anatomy by applying three increments of a resin composite (Opallis, FGM Prod. Odont.Ltda, Joinville, SC, Brazil). Each increment was light cured for 10 s at 1200 mW/cm^2 (Radii Cal, SDI, Victoria, Australia). The restorations were finished immediately with fine diamond burs (#3195 F and #3195FF, KG Sorensen, Barueri, SP, Brazil) and polishing with flexible disks (Soflex, 3 M ESPE, St. Paul, MN, USA).

2.8. Clinical evaluation

Two blinded, experienced and calibrated dentists (who specialized in esthetic dentistry with more than 15 years of clinical practice), performed the clinical evaluation. Patients were also blinded to group assignment. An inter-examiner and inter-examiner agreement of at least 85% was necessary before beginning the evaluation [32].

Table 1
Dentin sclerosis scale*.

Category	Criteria
1	No sclerosis present; dentin is light yellowish, with little discoloration; dentin is opaque, with little transparency
2	More sclerosis than 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

* Heymann and Bayne [27], modified by Swift et al [28].

Table 2
World Dental Federation (FDI) criteria used for clinical evaluation [33,34].

	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 (VG)	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) (GO)	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) (SS)	1.3 Moderate marginal staining, not esthetically unacceptable.	2.3 Two or more 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 dentin fractures	4.3.1 Premature/slightly more intense. 4.3.2 Delayed/weak sensitivity; no subjective complains, no treatment needed.	5.3 Large areas of demineralization, but only preventive measures necessary (dentine not exposed).
4. Clinically unsatisfactory (repair for prophylactic reasons) (UN)	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 dentin/base exposed. 3.4.2 Chip fracture damaging margins. 3.4.3 Notable enamel or dentin wall fracture.	4.4.1 Premature/very intense 4.4.2 Extremely delayed/weak with subjective complains. 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) (PO)	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 no 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 3
Modified United States Public Health Service (USPHS) criteria according to Dalton Bittencourt et al. [35] and Perdigão et al. [36].

	Marginal Staining	Retention	Fracture	Marginal adaption	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	None 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

All parameters during evaluation were recorded using a standardized paper case report form. The evaluation paper had to be sent after each observation to the research staff, so that evaluators were blinded to group assignment during follow-up recalls. Two criteria were used for evaluation of the restorations: the FDI [33,34] and USPHS criteria (adapted by Bittencourt et al. and Perdigão et al.) [35,36] immediately after restorative procedure (baseline), and after 6, 12 and 18-month of clinical service.

For either of the two criteria, only the clinically relevant measures of the performance of the adhesives were evaluated (Tables 2 and 3). The primary outcome was retention/fracture, but the following secondary outcomes were also evaluated: marginal staining, marginal adaptation, post-operative sensitivity, and recurrence of caries. The post-operative sensitivity was performed one week after the restorative procedure, by applying an air stream from a dental syringe for 10 s at 2 cm from the tooth surface.

These variables were ranked according to the criteria in the following scores: (1) FDI criteria (clinically very good [VG]; clinically good [GO]; clinically sufficient/satisfactory [SS]; clinically unsatisfactory [UN] and clinically poor [PO]) and (2) USPHS criteria (Alfa = excellent, Bravo = acceptable and Charlie = bad). Both examiners evaluated all the restorations once and independently. In case of disagreements during the evaluations, a consensus had to be reached before the participant was dismissed. The restoration retention rates were calculated according to the ADA guidelines [31]. 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.

2.9. Statistical analysis

The statistician was blinded to the study group types, and the analysis followed the intention-to-treat protocol, according to CONSORT's suggestion [23]. Descriptive statistics were used to describe the distributions of the evaluated criteria. A statistical analysis was performed for each item (the retention/fracture, marginal staining, marginal adaptation, postoperative sensitivity, and recurrence of caries), and for each overall parameter (FDI and USPHS). The differences between the four groups' ratings after 6, 12, and 18 months were tested by Friedman's repeated measures analysis of variance rank ($\alpha = 0.05$). For the primary outcome retention, we also calculated the risk ratio and the relative risk of all approaches relative to the most traditional approach (ER*ct)*. The 95% confidence interval was also reported. Cohen's kappa statistics were used to test the inter-examiner agreement. In all statistical tests, we preset the significance level at 5% (Statistica for Windows 7.0, StatSoft Inc., Tulsa, OK, USA).

3. Results

The restorative procedures were implemented as planned, and no modification was performed. Fourteen out of 50 subjects were not enrolled in the study because they did not fulfill the inclusion criteria (Fig. 1). Thus, 36 subjects with a mean age of 49 ± 9 and a range from 20 to 60 (17 women, 47%) were selected. All baseline details relative to the research subjects and characteristics of the restored lesions are displayed in Table 4. Two hundred and sixteen restorations were placed, 111 in the maxillary arch and 105 in the mandibular arch. Approximately 65% of the restorations were placed in premolars. All restorations received the score of clinically very good (FDI) and Alfa (USPHS) at baseline, for all primary and secondary outcomes. All research subjects were evaluated at the baseline and after 6, 12, and 18 months. One patient was not possible to evaluate, due to wearing an orthodontic appliance at the 18-month evaluation time.

The overall Cohen's kappa statistics showed excellent agreement between the examiners in the 6-month (0.94), 12-month (0.92), and 18-

Table 4
Characteristics of the research subjects and the non-carious cervical lesions (NCCLs) per group.

Characteristic	Number of lesions			
	ER*cu*	ER*ct*	SE*cu*	SE*ct*
Shape (degree of angle)				
< 45	0	0	0	0
45-90	21	22	14	16
90-135	12	15	12	15
> 135	21	17	28	23
Cervico incisal height (mm)				
< 1.5	13	16	7	7
1.5-2.5	23	17	25	21
2.5-4.0	15	19	19	21
> 4.0	3	2	3	5
Score on dentin sclerosis scale				
1	4	4	2	4
2	26	23	27	20
3	18	20	24	26
4	6	7	1	4
Presence of antagonist				
Yes	50	51	47	50
No	4	3	7	4
Attrition facet				
Yes	10	12	10	9
No	44	42	44	45
Pre-operative sensitivity (spontaneous)				
Yes	17	18	20	20
No	37	36	34	34
Pre-operative sensitivity (air dry)				
Yes	20	28	26	26
No	34	26	28	28
Pre-operative sensitivity (touch)				
Yes	18	19	20	20
No	36	35	34	34
Tooth distribution				
Incisor	7	5	7	5
Canine	7	5	5	6
Posterior				
Premolar	34	38	35	35
Molar	6	6	7	8
Arc distribution				
Maxillary	28	29	26	28
Mandibular	26	25	28	26

month (0.92) follow-ups. None of the patients reported postoperative sensitivity, and we did not detect caries at any of the follow-up periods.

3.1. Retention/fracture

No restoration was lost at the 6-month recall. Four restorations were lost at 12 months (1 for ER*cu*, 1 for ER*ct*, and 2 for SE*ct*) (Table 5). According to FDI and USPHS criteria, the 12-month retention rates (95% CI) were 98% (90–100%) for ER*cu;* 98% (90–100%) for ER*ct*; 100% for SE*cu*; and 96% (87–99%) for SE*ct* (Table 5). Fourteen restorations were lost at 18 months (2 for ER*cu,* 2 for ER*ct*, 3 for SE*cu,* and 7 for SE*ct*). According to FDI and USPHS criteria, the 18-month retention rates (95% confidence interval) were 96% (87–100%) for ER*cu;* 96% (87–100%) for ER*ct*; 94% (85–98%) for SE*cu*; and 87% (75–93%) for SE*ct* (Table 5). The lost restorations at 12 months ($n = 4$), and 18 months ($n = 10$) were counted in the evaluation of the other parameters. Three restorations were fractured at 18 months (1 for SE*cu* clinically sufficient/satisfactory and 2 for SE*ct* clinically unsatisfactory).

No significant difference was found when different groups were compared after the 6 and 12-month recalls for both criteria (Tables 5 and 6). However, according to the FDI criteria, a significantly higher retention rate was found at the 18-month recall in the SE*cu,* ER*ct,* and ER*cu* groups when compared with the SE*ct* group ($p < 0.05$).

3.2. Marginal staining

For the FDI criteria, two restorations at 6 months (one for SE*cu* and one for SE*ct)*, nine restorations at 12 months (one for ER*ct*, five for SE*cu,* and three for SE*ct),* and ten restorations at 18 months (one for ER*cu*, two for ER*ct*, four for SE*cu,* and three for SE*ct*) were considered to have minor discrepancies (clinically good and satisfactory) but no statistical difference was found ($p > 0.05$) (Table 5). When the USPHS criteria were used, only two restorations at 12 months were scored as Bravo for marginal staining (2 for SE*cu*) ($p > 0.05$). After 18 months, three restorations were scored as Bravo for marginal staining (3 for SE*cu*) ($p > 0.05$) (Table 6), with no significant difference between any pair of groups in each time comparison ($p > 0.05$).

3.3. Marginal adaptation

According to the FDI criteria, 24 restorations at 6 months (four for ER*cu,* three for ER*ct*, 8 for SE*cu,* and 9 for SE*ct*), 42 restorations at 12 months (six for ER*cu*, three for ER*ct*, 16 for SE*cu,* and 17 for SE*ct*) and 39 restorations at 18 months (six for ER*cu*, four for ER*ct*, 11 for SE*cu,* and 18 for SE*ct*) were considered to have minor discrepancies regarding marginal adaptation (clinically good and satisfactory). According to the FDI criteria, significantly lower marginal adaptation was found at the 12- and 18-month recalls in the SE*cu* and SE*ct* groups, when compared with the ER*ct* and ER*cu* groups ($p < 0.05$). Also, after 18-month recalls, there is a significantly lower marginal adaptation in the SE*ct* when compared to SE*cu* ($p < 0.05$). 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 6, 12, and 18 months (Table 5).

When USPHS criteria was used one restoration at 6 months (one for SE*ct*), three restorations at 12 months (one for SE*cu* and 2 for SE*ct*), and seven restorations at 18 months (one for ER*cu*, one for ER*ct*, two for SE*cu,* and three for SE*ct*) were scored as Bravo for marginal adaptation compared to the baseline ($p > 0.05$). No significant difference was detected between any pair of groups at the 6-, 12-, and 18-month recalls ($p > 0.05$) (Table 6).

3.4. Postoperative sensitivity

None of the restorations showed postoperative sensitivity immediately after the restorative procedures, according to the FDI and USPHS criteria. None of the restorations showed postoperative sensitivity at any recall time for either the FDI or USPHS criteria.

3.5. Recurrence of caries

No restoration showed recurrence of caries at any recall time using the FDI and the USPHS criteria.

3.6. General overview

When the FDI criteria for “acceptable” vs. “not acceptable” restorations were applied, only 14 restoration were ranked as “not acceptable” after 18 months (two for ER*cu*, two for ER*ct*, three for SE*cu* and seven for SE*ct*), the majority where from the SE*ct* group.

4. Discussion

This study was the first to evaluate the clinical performance of a

Table 5
Number of Evaluated Restorations for Each Experimental Group Classified According to the World Dental Federation (FDI) Criteria [33,34].

FDI Criteria	(*)	Baseline				6 months				12 months				18 months			
		ER*cu*	ER*ct*	SE*cu*	SE*ct*	ER*cu*	ER*ct*	SE*cu*	SE*ct*	ER*cu*	ER*ct*	SE*cu*	SE*ct*	ER*cu*	ER*ct*	SE*cu*	SE*ct*
Marginal staining	VG	54	54	54	54	54	54	53	53	53	52	49	49	50	49	46	43
	GO	–	–	–	–	–	–	1	1	–	1	3	3	1	2	1	3
	SS	–	–	–	–	–	–	–	–	–	–	2	–	–	–	3	–
	UN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	PO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Retention and Fractures	VG	54	54	54	54	54	54	54	54	53	53	54	52	51	51	49	43
	GO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
	SS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–
	UN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
	PO	–	–	–	–	–	–	–	–	1	1	–	2	2	2	3	7
Marginal adaptation	VG	54	54	54	54	50	51	46	45	47	50	38	35	45	47	39	27
	GO	–	–	–	–	4	3	8	8	6	3	15	15	5	3	9	16
	SS	–	–	–	–	–	–	–	1	–	–	1	2	1	1	2	3
	UN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	PO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Postoperative (hyper-) sensitivity	VG	54	54	54	54	54	54	54	54	53	53	54	52	51	51	50	46
	GO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	SS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	UN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	PO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Recurrence of caries	VG	54	54	54	54	54	54	54	54	53	53	54	52	51	51	50	46
	GO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	SS	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	UN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	PO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

ER*cu*, etch-and-rinse, copper-containing adhesive; ER*ct*, etch-and-rinse, commercial adhesive; SE*cu*, self-etch, copper-containing adhesive; SE*ct*, self-etch, commercial adhesive.

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

Table 6
Number of evaluated restorations for each experimental group according to the modified United States Public Health Service (USPHS) criteria [35,36].

USPHS Criteria	(*)	Baseline				6 months				12 months				18 months			
		ER*cu*	ER*ct*	SE*cu*	SE*ct*	ER*cu*	ER*ct*	SE*cu*	SE*ct*	ER*cu*	ER*ct*	SE*cu*	SE*ct*	ER*cu*	ER*ct*	SE*cu*	SE*ct*
Marginal staining	Alfa	54	54	54	54	54	54	54	54	53	53	52	52	51	51	47	46
	Bravo	–	–	–	–	–	–	–	–	–	–	2	–	–	–	3	–
	Charlie	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Retention	Alfa	54	54	54	54	54	54	54	54	53	53	54	52	51	51	50	46
	Bravo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Charlie	–	–	–	–	–	–	–	–	1	1	–	2	2	2	3	7
Fractures	Alfa	54	54	54	54	54	54	54	54	53	53	54	52	51	51	49	44
	Bravo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–
	Charlie	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
Marginal adaptation	Alfa	54	54	54	54	54	54	54	53	53	53	53	50	50	50	48	43
	Bravo	–	–	–	–	–	–	–	1	–	–	1	2	1	1	2	3
	Charlie	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Postoperative sensitivity	Alfa	54	54	54	54	54	54	54	54	53	53	54	52	51	51	50	46
	Bravo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Charlie	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Recurrence of caries	Alfa	54	54	54	54	54	54	54	54	53	53	54	52	51	51	50	46
	Bravo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Charlie	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

ER*cu*, etch-and-rinse, copper-containing adhesive; ER*ct*, etch-and-rinse, commercial adhesive; SEcu, self-etch, copper-containing adhesive; SEct, self-etch, commercial adhesive.

CuNp-containing universal adhesive system applied in the etch-and-rinse and self-etch modes of NCCL restorations. In the current study, adding copper nanoparticles in the self-etch mode significantly improved the retention rate compared to the self-etch control group, which leads to the partial rejection of the null hypothesis. However, the same effect was not observed in the etch-and-rinse strategies, whether CuNp was added or not.

The universal adhesives provide dentists with a choice of adhesion strategy (etch-and-rinse or self-etch) according to the dentist's preference [37,38]. Although no official definition exists for a universal adhesive, the literature describes it as a single-bottle adhesive that performs equally well with any adhesion strategy and bonds adequately to the tooth structure and various direct and indirect restorative materials [39,40]. Universal adhesives are similar to the simple one-step self-etch adhesives, but they contain specific functional monomers to better bond with the dental hard substrates. The best-known of these monomers is 10-methacryloyloxydecyl dihydrogen phosphate (MDP). Several studies showed that MDP ionic bonds to dentin, forming stable calcium salts on hydroxyapatite (nanolayer). MDP also promotes a more effective and stable bond in water than other functional monomers do [41–43].

Several concerns exist regarding the bond with dentin when simplified self-etch adhesives containing MDP are applied. Some studies showed a scarce nanolayer formation when several MDP-containing adhesives were applied to dentin in the self-etch mode [44,45], attributed to the presence of other hydrophilic monomers or photoinitiators, which may inhibit and or compete with the MDP and decrease the nanolayer formation. Other studies indicate that the nanolayer formation is directly proportional to the MDP concentration [44,45]. Unfortunately, the exact concentrations of MDP added to the universal adhesives available on the market are unknown. All these factors could impair the bonding interaction with dentin when the Ambar Universal adhesive was applied in the self-etch mode. However, adding CuNp to the Ambar Universal adhesives when applied as a self-etch increased their clinical performance.

The faster degradation of the adhesive interfaces may be associated with higher accumulation of esterases from cariogenic bacteria and saliva [3,5,7,46,47]. These factors, along with an MMP activity, contribute to an increase in caries formation, caries progression, and premature restoration failure [9,10,48]. The MMPs present in dentin are produced by odontoblasts [49] during secretion of dentin matrix and are suggested to be involved in dentin formation. After mineralization of the collagen matrix, the inactive proforms of MMPs remain trapped within the calcified matrix [50], where they can be re-exposed and potentially activated by the acidic environment created by bacterial acids during the dentin caries process.

MMP-8 and MMP-9 are the most abundant salivary MMPs and predominate in dentin caries lesions [51], especially in the outer caries compared with the inner caries (caries-affected) layer [52]. Although no clinically visible secondary caries could be observed in these contexts, there was a subclinical degradation of the adhesive interfaces. The collagenolytic and gelatinolytic activity may explain this phenomenon on the partially demineralized dentin after application of self-etch adhesives. By activating the MMPs [53,54] and the cysteine-cathepsins (CTs) [55,56], a self-degradation of the partially infiltrated collagen fibrils [57] occurs with reduction of bond strength values over time [58].

In this sense, copper can inhibit the MMPs [20,21] and, on the other hand, act as a collagen cross-linker [55,56] that may make the substrate less susceptible to the effects of proteolytic enzymes, such as MMPs and CTs [55,56]. Adding copper nanoparticles in the universal adhesive may significantly reduce the activity of the dentin MMPs because of copper's inhibitory potential (mainly on the MMP's subtypes -2 and -9) [59,60]. This strengthens the idea that including CuNp in the protocol adhesive may be an interesting alternative in reducing enzymatic activity.

The copper addition can also increase the strength of the collagen network, one component of the hybrid layer, because the collagen cross-linking enzyme, lysyl oxidase (LOX), is copper-dependent [55,56]. Thus, copper indirectly acts effect as a cross-linking agent. Both effects (anti-enzymatic and cross-linking) help prevent the degradation of the collagen fibrils, which can improve the immediate and long-term results of dentin bonding [16,17]. Notably, adding CuNp increases the bonding layer's immediate and long-term mechanical properties [16], making it less susceptible to solubility after water storage [17]. These processes may all act together for a slight improvement in the retention rate of the self-etch copper-containing group.

Unfortunately, the same phenomenon was not observed for the etch-and-rinse strategy. Several recently published clinical studies evaluating the application of the other MMP inhibitors and cross-linking agents during the protocol application of etch-and-rinse adhesives did not show improvement of the clinical performance [61–64]. It is unclear to the present study's authors why there was no improvement. Future long-term clinical studies need to be done to evaluate the clinical performance of MMP inhibitors and cross-linking agents applied to the etch-and-rinse adhesives.

Regarding marginal defects in general, there was a significant difference in the adhesive technique between the etch-and-rinse and self-etch groups at the 18-month recall, which agrees with a previous study [65–67]. This has already been reported in the literature. A reduction of the enamel bonding's effectiveness was observed when universal adhesives were applied to the enamel as self-etch adhesives [37,68,69]. The increased surface area on intact and ground enamel after applying the self-etch adhesives is lower than that with the phosphoric acid etching [70]. Consequently, the in vitro performance of the simplified self-etch adhesives (as universal adhesives applied in the self-etch mode) could be significantly improved with preliminary phosphoric acid etching [71,72] in the clinical studies [73].

Despite the high number of restorations with a marginal discrepancy in the FDI criteria, none of them had clinically relevant discrepancies (clinically unsatisfactory) in the marginal adaptation, even after 6, 12 and 18 months.

Some of the present study's limitations need to be described. The main idea for adding CuNp into dental adhesive systems was to provide more extended periods of antimicrobial and anti-MMP properties [15–17,20,21]. Cu has already been shown as still present in the adhesive interface formed by Cu-containing adhesives, even after two years of in vitro water storage, with a high enough concentration to produce antimicrobial and anti-MMP effects [16,17]. However, as none secondary caries were observed, the antimicrobial effect of Cu can not be observed. Future long-term follow-ups need to be done to prove this hypothesis.

An important issue to mention when metallic nanoparticles are incorporated into adhesive systems is the cytotoxicity of these nanoparticles. Several studies have shown the potential cytotoxicity of CuNp [18,74], but little attention has been given to the effects of these nanoparticles incorporated in the polymer matrix of the adhesive systems. Based on that, viability tests for adhesive solutions are important in these cases. Uncured components of adhesive systems may diffuse across the subjacent dentinal tubules and in deep cavities, reach the pulp chamber, and modify pulp cell metabolism [75,76].

In a recent viability study [77], zinc oxide and copper nanoparticles were added in 0 wt% (control), 0.1 wt%, and 0.2 wt% concentrations in the same adhesive used in this study. The authors [77] showed higher cytotoxicity of adhesives, irrespectively of the presence of the metallic nanoparticles. This means that the adhesive solution itself is cytotoxic, and this cytotoxicity was not worsened with copper addition. Monomers, like 10-MDP monomer, presented in Ambar Universal, is as cytotoxic as other diluent monomers such as TEGDMA, inducing odontoblastic differentiation and inflammatory response in human dental pulp cells [78].

Releasing copper in vitro may differ from a complex cavity's release of copper as non-carious cervical lesions, thus preventing us from extrapolating the present data into a clinical scenario, but this slow release over time may supplement the antimicrobial and anti-MMP activity of the adhesive apart from maintaining the expected sealing and retention. However, this hypothesis was only partially observed in the present study through a slight improvement potentially related to adding the Cu nanoparticle when universal adhesive was applied in the self-etch mode. Thus, future long-term clinical studies are needed to evaluate the antimicrobial and anti-MMP potential of incorporating CuNp into the universal adhesive systems.

5. Conclusion

The present study demonstrated that adding copper nanoparticles to a universal adhesive system in a concentration of 0.1 wt% is a feasible approach to maintain or even improve the clinical performance of cervical restorations. However, only a slight improvement was potentially related to adding Cu nanoparticles, mainly when the universal adhesive was applied in the self-etch application mode.

Declaration of Competing Interest

None.

Acknowledgments

This study was performed by Thalita de Paris Matos as partial fulfillment of his MSc degree at the State University of Ponta Grossa (UEPG), Ponta Grossa, PR, Brazil. This study was partially supported by the National Council for Scientific and Technological Development (CNPq) under grants 305588/2014-1, 303332/2017-4and in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and by the Fondecyt under grant 1170575 – Chile.

References

[1] V. Moraschini, C.K. Fai, R.M. Alto, G.O. Dos Santos, Amalgam and resin composite longevity of posterior restorations: a systematic review and meta-analysis, J. Dent. 43 (2015) 1043–1050, https://doi.org/10.1016/j.jdent.2015.06.005.
[2] G.J. Christensen, Should resin-based composite dominate restorative dentistry today? J. Am. Dent. Assoc. 141 (2010) 1490–1493, https://doi.org/10.14219/jada.archive.2010.0112.
[3] P. Spencer, Q. Ye, J. Park, E.M. Topp, A. Misra, O. Marangos, Y. Wang, B.S. Bohaty, V. Singh, F. Sene, J. Eslick, K. Camarda, J.L. Katz, Adhesive/dentin interface: the weak link in the composite restoration, Ann. Biomed. Eng. 38 (2010) 1989–2003, https://doi.org/10.1007/s10439-010-9969-6.
[4] N.J. Opdam, F.H. van de Sande, E. Bronkhorst, M.S. Cenci, P. Bottenberg, U. Pallesen, P. Gaengler, A. Lindberg, M.C. Huysmans, J.W. van Dijken, Longevity of posterior composite restorations: a systematic review and meta-analysis, J. Dent. Res. 93 (2014) 943–949, https://doi.org/10.1177/0022034514544217.
[5] R. Hickel, J. Manhart, Longevity of restorations in posterior teeth and reasons for failure, J. Adhes. Dent. 3 (2001) 45–64.
[6] M.A. Borges, I.C. Matos, L.C. Mendes, A.S. Gomes, M.S. Miranda, Degradation of polymeric restorative materials subjected to a high caries challenge, Dent. Mater. 27 (2011) 244–252, https://doi.org/10.1016/j.dental.2010.10.009.
[7] M. Bourbia, D. Ma, D.G. Cvitkovitch, J.P. Santerre, Y. Finer, Cariogenic bacteria degrade dental resin composites and adhesives, J. Dent. Res. 92 (2013) 989–994, https://doi.org/10.1177/0022034513504436.
[8] I. Nedeljkovic, W. Teughels, J. De Munck, B. Van Meerbeek, K.L. Van Landuyt, Is secondary caries with composites a material-based problem? Dent. Mater. 31 (2015) e247–77, https://doi.org/10.1016/j.dental.2015.09.001.
[9] A. Mazzoni, L. Tjaderhane, V. Checchi, R. Di Lenarda, T. Salo, F.R. Tay, D.H. Pashley, L. Breschi, Role of dentin MMPs in caries progression and bond stability, J. Dent. Res. 94 (2015) 241–251, https://doi.org/10.1177/0022034514562833.
[10] C. Chaussain-Miller, F. Fioretti, M. Goldberg, S. Menashi, The role of matrix metalloproteinases (MMPs) in human caries, J. Dent. Res. 85 (2006) 22–32, https://doi.org/10.1177/154405910608500104.
[11] L. Chen, H. Shen, B.I. Suh, Antibacterial dental restorative materials: a state-of-the-art review, Am. J. Dent. 25 (2012) 337–346.
[12] A.R. Cocco, W.L. Rosa, A.F. Silva, R.G. Lund, E. Piva, A systematic review about antibacterial monomers used in dental adhesive systems: current status and further prospects, Dent. Mater. 31 (2015) 1345–1362, https://doi.org/10.1016/j.dental.2015.08.155.
[13] H. Palza, Antimicrobial polymers with metal nanoparticles, Int. J. Mol. Sci. 16 (2015) 2099–2116, https://doi.org/10.3390/ijms16012099.
[14] C. Sabatini, A.S. Mennito, B.J. Wolf, D.H. Pashley, W.G. Renne, Incorporation of bactericidal poly-acrylic acid modified copper iodide particles into adhesive resins, J. Dent. 43 (2015) 546–555, https://doi.org/10.1016/j.jdent.2015.02.012.
[15] L. Argueta-Figueroa, R.J. Scougall-Vilchis, R.A. Morales-Luckie, O.F. Olea-Mejia, An evaluation of the antibacterial properties and shear bond strength of copper nanoparticles as a nanofiller in orthodontic adhesive, Aust. Orthod. J. 31 (2015) 42–48.
[16] M.F. Gutierrez, P. Malaquias, T.P. Matos, A. Szesz, S. Souza, J. Bermudez, A. Reis, A.D. Loguercio, P.V. Farago, Mechanical and microbiological properties and drug release modeling of an etch-and-rinse adhesive containing copper nanoparticles, Dent. Mater. 33 (2017) 309–320, https://doi.org/10.1016/j.dental.2016.12.011.
[17] M.F. Gutierrez, P. Malaquias, V. Hass, T.P. Matos, L. Lourenco, A. Reis, A.D. Loguercio, P.V. Farago, The role of copper nanoparticles in an etch-and-rinse adhesive on antimicrobial activity, mechanical properties and the durability of resin-dentine interfaces, J. Dent. 61 (2017) 12–20, https://doi.org/10.1016/j.jdent.2017.04.007.
[18] A.P. Ingle, N. Duran, M. Rai, Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review, Appl. Microbiol. Biotechnol. 98 (2014) 1001–1009, https://doi.org/10.1007/s00253-013-5422-8.
[19] A.M. Schrand, M.F. Rahman, S.M. Hussain, J.J. Schlager, D.A. Smith, A.F. Syed, Metal-based nanoparticles and their toxicity assessment, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2 (2010) 544–568, https://doi.org/10.1002/wnan.103.
[20] A.P. de Souza, R.F. Gerlach, S.R. Line, Inhibition of human gingival gelatinases (MMP-2 and MMP-9) by metal salts, Dent. Mater. 16 (2000) 103–108, https://doi.org/10.1016/S0109-5641(99)00084-6.
[21] A. Simeon, H. Emonard, W. Hornebeck, F.X. Maquart, The tripeptide-copper complex glycyl-L-histidyl-L-lysine-Cu2+ stimulates matrix metalloproteinase-2 expression by fibroblast cultures, Life Sci. 67 (2000) 2257–2265, https://doi.org/10.1016/S0024-3205(00)00803-1.
[22] S. Sabella, R.P. Carney, V. Brunetti, M.A. Malvindi, N. Al-Juffali, G. Vecchio, S.M. Janes, O.M. Bakr, R. Cingolani, F. Stellacci, P.P. Pompa, A general mechanism for intracellular toxicity of metal-containing nanoparticles, Nanoscale 6 (2014) 7052–7061, https://doi.org/10.1039/c4nr01234h.
[23] K.F. Schulz, D.G. Altman, D. Moher, CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials, Trials 11 (2010), https://doi.org/10.1186/1745-6215-11-32 32–32.
[24] J. Perdigao, C. Kose, A.P. Mena-Serrano, E.A. De Paula, L.Y. Tay, A. Reis, A.D. Loguercio, A new universal simplified adhesive: 18-month clinical evaluation, Oper. Dent. 39 (2014) 113–127, https://doi.org/10.2341/13-045-c.
[25] J.C. Greene, J.R. Vermillion, The simplified oral hygiene index, J. Am. Dent. Assoc. 68 (1964) 7–13, https://doi.org/10.14219/jada.archive.1964.0034.
[26] A.D. Loguercio, A. Reis, A.N. Barbosa, J.F. Roulet, Five-year double-blind randomized clinical evaluation of a resin-modified glass ionomer and a polyacid-modified resin in noncarious cervical lesions, J. Adhes. Dent. 5 (2003) 323–332.
[27] H.O. Heymann, S.C. Bayne, Current concepts in dentin bonding: focusing on dentinal adhesion factors, J. Am. Dent. Assoc. 124 (1993) 26–36.
[28] E.J. Swift Jr., J. Perdigao, H.O. Heymann, A.D. Wilder Jr., S.C. Bayne, K.N. May Jr., J.R. Sturdevant, T.M. Roberson, Eighteen-month clinical evaluation of a filled and unfilled dentin adhesive, J. Dent. 29 (2001) 1–6.
[29] T.R. Da Costa, A.D. Loguercio, A. Reis, Effect of enamel bevel on the clinical performance of resin composite restorations placed in non-carious cervical lesions, J. Esthet. Restor. Dent. 25 (2013) 346–356, https://doi.org/10.1111/jerd.12042.
[30] A.O. Oginni, A.A. Adeleke, Comparison of pattern of failure of resin composite restorations in non-carious cervical lesions with and without occlusal wear facets, J. Dent. 42 (2014) 824–830, https://doi.org/10.1016/j.jdent.2014.04.003.
[31] American Dental Association Council on Scientific Affairs, Acceptance Program Guidelines, Dentin and Enamel Adhesive Materials, Chicago (2001).
[32] S.J. Pocock, The size of a clinical trial, in: S.J. Pocock (Ed.), Clinical Trials: A Practical Approach, John Wiley & Sons, Chinchester, 1983, pp. 123–141.
[33] R. Hickel, J.F. Roulet, S. Bayne, S.D. Heintze, I.A. Mjor, M. Peters, V. Rousson, R. Randall, G. Schmalz, M. Tyas, G. Vanherle, Recommendations for conducting controlled clinical studies of dental restorative materials. Science Committee Project 2/98–FDI World Dental Federation study design (Part I) and criteria for evaluation (Part II) of direct and indirect restorations including onlays and partial crowns, J. Adhes. Dent. 9 (Suppl. 1) (2007) 121–147.
[34] R. Hickel, A. Peschke, M. Tyas, I. Mjor, S. Bayne, M. Peters, K.A. Hiller, R. Randall, G. Vanherle, S.D. Heintze, FDI World Dental Federation – clinical criteria for the evaluation of direct and indirect restorations. Update and clinical examples, J. Adhes. Dent. 12 (2010) 259–272, https://doi.org/10.3290/j.jad.a19262.
[35] D. Dalton Bittencourt, I.G. Ezecelevski, A. Reis, J.W. Van Dijken, A.D. Loguercio, An 18-months' evaluation of self-etch and etch & rinse adhesive in non-carious cervical lesions, Acta Odontol. Scand. 63 (2005) 173–178, https://doi.org/10.1080/00016350510019874.
[36] J. Perdigao, M. Dutra-Correa, C.H. Saraceni, M.T. Ciaramicoli, V.H. Kiyan, C.S. Queiroz, Randomized clinical trial of four adhesion strategies: 18-month results, Oper. Dent. 37 (2012) 3–11, https://doi.org/10.2341/11-222-c.
[37] M. Hanabusa, A. Mine, T. Kuboki, Y. Momoi, A. Van Ende, B. Van Meerbeek, J. De Munck, Bonding effectiveness of a new' multi-mode' adhesive to enamel and dentine, J. Dent. 40 (2012) 475–484, https://doi.org/10.1016/j.jdent.2012.02.012.
[38] M.A. Munoz, I. Luque, V. Hass, A. Reis, A.D. Loguercio, N.H. Bombarda, Immediate bonding properties of universal adhesives to dentine, J. Dent. 41 (2013) 404–411, https://doi.org/10.1016/j.jdent.2013.03.001.
[39] S. Nagarkar, N. Theis-Mahon, J. Perdigao, Universal dental adhesives: current status, laboratory testing, and clinical performance, J. Biomed. Mater. Res. B Appl. Biomater. (2019), https://doi.org/10.1002/jbm.b.34305.
[40] A.B. Matos, L.T. Trevelin, B. Silva, L.F. Francisconi-Dos-Rios, L.K. Siriani, M.V. Cardoso, Bonding efficiency and durability: current possibilities, Braz. Oral Res. 31 (2017) e57, https://doi.org/10.1590/1807-3107BOR-2017.vol31.0057.
[41] K. Yoshihara, Y. Yoshida, N. Nagaoka, S. Hayakawa, T. Okihara, J. De Munck, Y. Maruo, G. Nishigawa, S. Minagi, A. Osaka, B. Van Meerbeek, Adhesive interfacial interaction affected by different carbon-chain monomers, Dent. Mater. 29 (2013) 888–897, https://doi.org/10.1016/j.dental.2013.05.006.
[42] Y. Yoshida, K. Yoshihara, N. Nagaoka, S. Hayakawa, Y. Torii, T. Ogawa, A. Osaka, B.V. Meerbeek, Self-assembled nano-layering at the adhesive interface, J. Dent. Res. 91 (2012) 376–381, https://doi.org/10.1177/0022034512437375.
[43] Y. Yoshida, K. Nagakane, R. Fukuda, Y. Nakayama, M. Okazaki, H. Shintani, S. Inoue, Y. Tagawa, K. Suzuki, J. De Munck, B. Van Meerbeek, Comparative study on adhesive performance of functional monomers, J. Dent. Res. 83 (2004) 454–458, https://doi.org/10.1177/154405910408300604.
[44] F. Tian, L. Zhou, Z. Zhang, L. Niu, L. Zhang, C. Chen, J. Zhou, H. Yang, X. Wang, B. Fu, C. Huang, D.H. Pashley, F.R. Tay, Paucity of nanolayering in resin-dentin interfaces of MDP-based adhesives, J. Dent. Res. 95 (2016) 380–387, https://doi.org/10.1177/0022034515623741.
[45] K. Yoshihara, Y. Yoshida, S. Hayakawa, N. Nagaoka, M. Irie, T. Ogawa, K.L. Van Landuyt, A. Osaka, K. Suzuki, S. Minagi, B. Van Meerbeek, Nanolayering of phosphoric acid ester monomer on enamel and dentin, Acta Biomater. 7 (2011) 3187–3195, https://doi.org/10.1016/j.actbio.2011.04.026.
[46] C.A. Stewart, Y. Finer, Biostable, antidegradative and antimicrobial restorative systems based on host-biomaterials and microbial interactions, Dent. Mater. 35

(2019) 36–52, https://doi.org/10.1016/j.dental.2018.09.013.
[47] R. Pinna, P. Usai, E. Filigheddu, F. Garcia-Godoy, E. Milia, The role of adhesive materials and oral biofilm in the failure of adhesive resin restorations, Am. J. Dent. 30 (2017) 285–292.
[48] R. Gitalis, L. Zhou, M.Q. Marashdeh, C. Sun, M. Glogauer, Y. Finer, Human neutrophils degrade methacrylate resin composites and tooth dentin, Acta Biomater. 88 (2019) 325–331, https://doi.org/10.1016/j.actbio.2019.02.033.
[49] H. Palosaari, C.J. Pennington, M. Larmas, D.R. Edwards, L. Tjaderhane, T. Salo, Expression profile of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in mature human odontoblasts and pulp tissue, Eur. J. Oral Sci. 111 (2003) 117–127.
[50] A.R. Hannas, J.C. Pereira, J.M. Granjeiro, L. Tjaderhane, The role of matrix metalloproteinases in the oral environment, Acta Odontol. Scand. 65 (2007) 1–13, https://doi.org/10.1080/00016350600963640.
[51] L. Tjaderhane, H. Larjava, T. Sorsa, V.J. Uitto, M. Larmas, T. Salo, The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions, J. Dent. Res. 77 (1998) 1622–1629, https://doi.org/10.1177/00220345980770081001.
[52] Y. Shimada, S. Ichinose, A. Sadr, M.F. Burrow, J. Tagami, Localization of matrix metalloproteinases (MMPs-2, 8, 9 and 20) in normal and carious dentine, Aust. Dent. J. 54 (2009) 347–354, https://doi.org/10.1111/j.1834-7819.2009.01161.x.
[53] Y. Nishitani, M. Yoshiyama, B. Wadgaonkar, L. Breschi, F. Mannello, A. Mazzoni, R.M. Carvalho, L. Tjaderhane, F.R. Tay, D.H. Pashley, Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives, Eur. J. Oral Sci. 114 (2006) 160–166, https://doi.org/10.1111/j.1600-0722.2006.00342.x.
[54] J. De Munck, P.E. Van den Steen, A. Mine, K.L. Van Landuyt, A. Poitevin, G. Opdenakker, B. Van Meerbeek, Inhibition of enzymatic degradation of adhesive-dentin interfaces, J. Dent. Res. 88 (2009) 1101–1106, https://doi.org/10.1177/0022034509346952.
[55] R.B. Rucker, T. Kosonen, M.S. Clegg, A.E. Mitchell, B.R. Rucker, J.Y. Uriu-Hare, C.L. Keen, Copper, lysyl oxidase, and extracellular matrix protein cross-linking, Am. J. Clin. Nutr. 67 (1998) 996s–1002s, https://doi.org/10.1093/ajcn/67.5.996S.
[56] B. Marelli, D. Le Nihouannen, S.A. Hacking, S. Tran, J. Li, M. Murshed, C.J. Doillon, C.E. Ghezzi, Y.L. Zhang, S.N. Nazhat, J.E. Barralet, Newly identified interfibrillar collagen crosslinking suppresses cell proliferation and remodelling, Biomaterials 54 (2015) 126–135, https://doi.org/10.1016/j.biomaterials.2015.03.018.
[57] D.H. Pashley, F.R. Tay, C. Yiu, M. Hashimoto, L. Breschi, R.M. Carvalho, S. Ito, Collagen degradation by host-derived enzymes during aging, J. Dent. Res. 83 (2004) 216–221, https://doi.org/10.1177/154405910408300306.
[58] L. Breschi, A. Mazzoni, A. Ruggeri, M. Cadenaro, R. Di Lenarda, E. De Stefano Dorigo, Dental adhesion review: aging and stability of the bonded interface, Dent. Mater. 24 (2008) 90–101, https://doi.org/10.1016/j.dental.2007.02.009.
[59] H. Guo, J.D. Lee, H. Uzui, K. Toyoda, T. Geshi, H. Yue, T. Ueda, Effects of copper and zinc on the production of homocysteine-induced extracellular matrix metalloproteinase-2 in cultured rat vascular smooth muscle cells, Acta Cardiol. 60 (2005) 353–359, https://doi.org/10.2143/ac.60.4.2004982.
[60] A.P. Souza, R.F. Gerlach, S.R. Line, Inhibition of human gelatinases by metals released from dental amalgam, Biomaterials 22 (2001) 2025–2030.
[61] N. Sartori, S.C. Stolf, S.B. Silva, G.C. Lopes, M. Carrilho, Influence of chlorhexidine digluconate on the clinical performance of adhesive restorations: a 3-year follow-up, J. Dent. 41 (2013) 1188–1195, https://doi.org/10.1016/j.jdent.2013.09.004.
[62] M. Dutra-Correa, C.H. Saraceni, M.T. Ciaramicoli, V.H. Kiyan, C.S. Queiroz, Effect of chlorhexidine on the 18-month clinical performance of two adhesives, J. Adhes. Dent. 15 (2013) 287–292, https://doi.org/10.3290/j.jad.a29533.
[63] M. Favetti, T. Schroeder, A.F. Montagner, M.B. Correa, T. Pereira-Cenci, M.S. Cenci, Effectiveness of pre-treatment with chlorhexidine in restoration retention: a 36-month follow-up randomized clinical trial, J. Dent. 60 (2017) 44–49, https://doi.org/10.1016/j.jdent.2017.02.014.
[64] L.C. de Souza, N.S. Rodrigues, D.A. Cunha, V.P. Feitosa, S.L. Santiago, A. Reis, A.D. Loguercio, T.P. Matos, V.P.A. Saboia, J. Perdigao, Two-year clinical evaluation of proanthocyanidins added to a two-step etch-and-rinse adhesive, J. Dent. 81 (2019) 7–16, https://doi.org/10.1016/j.jdent.2018.12.012.
[65] N.C. Lawson, A. Robles, C.C. Fu, C.P. Lin, K. Sawlani, J.O. Burgess, Two-year clinical trial of a universal adhesive in total-etch and self-etch mode in non-carious cervical lesions, J. Dent. 43 (2015) 1229–1234, https://doi.org/10.1016/j.jdent.2015.07.009.
[66] A.D. Loguercio, E.A. de Paula, V. Hass, I. Luque-Martinez, A. Reis, J. Perdigao, A new universal simplified adhesive: 36-month randomized double-blind clinical trial, J. Dent. 43 (2015) 1083–1092, https://doi.org/10.1016/j.jdent.2015.07.005.
[67] A.D. Loguercio, D.D. Bittencourt, L.N. Baratieri, A. Reis, A 36-month evaluation of self-etch and etch-and-rinse adhesives in noncarious cervical lesions, J. Am. Dent. Assoc. 138 (2007) 507–514 quiz 535-7.
[68] J. Perdigao, A.D. Loguercio, Universal or multi-mode adhesives: why and how? J. Adhes. Dent. 16 (2014) 193–194, https://doi.org/10.3290/j.jad.a31871.
[69] M.F. de Goes, M.S. Shinohara, M.S. Freitas, Performance of a new one-step multi-mode adhesive on etched vs non-etched enamel on bond strength and interfacial morphology, J. Adhes. Dent. 16 (2014) 243–250, https://doi.org/10.3290/j.jad.a32033.
[70] D.H. Pashley, F.R. Tay, Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel, Dent. Mater. 17 (2001) 430–444.
[71] K.L. Van Landuyt, M. Peumans, J. De Munck, P. Lambrechts, B. Van Meerbeek, Extension of a one-step self-etch adhesive into a multi-step adhesive, Dent. Mater. 22 (2006) 533–544, https://doi.org/10.1016/j.dental.2005.05.010.
[72] J. Perdigão, M.M. Lopes, G. Gomes, In vitro bonding performance of self-etch adhesives: II—Ultramorphological evaluation, Oper. Dent. 33 (2008) 534–549, https://doi.org/10.2341/07-133.
[73] A. Szesz, S. Parreiras, A. Reis, A. Loguercio, Selective enamel etching in cervical lesions for self-etch adhesives: a systematic review and meta-analysis, J. Dent. 53 (2016) 1–11, https://doi.org/10.1016/j.jdent.2016.05.009.
[74] Y. Wang, X.Y. Zi, J. Su, H.X. Zhang, X.R. Zhang, H.Y. Zhu, J.X. Li, M. Yin, F. Yang, Y.P. Hu, Cuprous oxide nanoparticles selectively induce apoptosis of tumor cells, Int. J. Nanomed. 7 (2012) 2641–2652, https://doi.org/10.2147/ijn.s31133.
[75] E.A. Koulaouzidou, M. Helvatjoglu-Antoniades, G. Palaghias, A. Karanika-Kouma, D. Antoniades, Cytotoxicity of dental adhesives in vitro, Eur. J. Dent. 3 (2009) 3–9.
[76] A. Sengun, M. Yalcin, H.E. Ulker, B. Ozturk, S.S. Hakki, Cytotoxicity evaluation of dentin bonding agents by dentin barrier test on 3-dimensional pulp cells, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 112 (2011) e83–8, https://doi.org/10.1016/j.tripleo.2011.02.023.
[77] M.F. Gutierrez, L.F. Alegria-Acevedo, L. Mendez-Bauer, J. Bermudez, A. Davila-Sanchez, S. Buvinic, N. Hernandez-Moya, A. Reis, A.D. Loguercio, P.V. Farago, J. Martin, E. Fernandez, Biological, mechanical and adhesive properties of universal adhesives containing zinc and copper nanoparticles, J. Dent. 82 (2019) 45–55, https://doi.org/10.1016/j.jdent.2019.01.012.
[78] E.C. Kim, H. Park, S.I. Lee, S.Y. Kim, Effect of the acidic dental resin monomer 10-methacryloyloxydecyl dihydrogen phosphate on odontoblastic differentiation of human dental pulp cells, Basic Clin. Pharmacol. Toxicol. 117 (2015) 340–349, https://doi.org/10.1111/bcpt.12404.