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Clinical Research

Catechin-based Dentin Pretreatment and the Clinical Performance of a Universal Adhesive: A Two-year Randomized Clinical Trial

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Clinical Relevance

At 24 months, the dentin pretreatment with epigallocatechin-3-gallate did not impair the clinical performance of the adhesive Single Bond Universal regardless of the bonding strategy used.

SUMMARY

Purpose: To evaluate the two-year effect of dentin pretreatment with epigallocatechin-3-gallate (EGCG) on the clinical performance of restorations of noncarious cervical lesions (NCCLs) with Single Bond Universal, applied in two different modes (self-etch and etch-and-rinse).

Methods and Materials: In this randomized clinical trial, 33 volunteers were selected, and 156 NCCLs were assigned to four groups: ER, etch-and-rinse; ER-EGCG, 0.1% EGCG dentin pretreatment + etch-and-rinse; SE, self-etch; and SE-EGCG, 0.1% EGCG dentin pretreatment + self-etch. The NCCLs were restored with a nanofilled resin composite and evaluated at baseline and at six, 12, 18, and 24 months using FDI criteria for retention, marginal staining, marginal adaptation, caries, and postoperative sensitivity. Two evaluators were blinded to the treatments performed, and impressions were taken for resin replicas to allow indirect observations. Statistical analyses were performed with Kruskal-Wallis and McNemar tests with a significance level of 5%.

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Results: Six restorations (one from ER, two from SE, one from ER-EGCG, and two from SE-EGCG) were lost at 24 months with no significant differences ($p>0.05$). The retention rates were 97.0% (ER and ER-EGCG), 94.1% (SE), and 94.2% (SE-EGCG). For marginal adaptation, a significant difference was detected between the baseline and 24 months for the SE group ($p=0.0313$). There were no statistical differences among all other evaluated criteria at 24 months, neither for each group at baseline nor for 24-month comparisons ($p>0.05$).

Conclusions: The pretreatment with EGCG provided no benefit in the clinical performance of the adhesive regardless of the bonding strategy used. In addition, it adds an additional required step to the restorative procedure.

INTRODUCTION

Randomized controlled trials are considered the ideal design for comparing different procedures in health care interventions[1] and the most reliable of evidence-based studies to dental practice.[2] Laboratory studies are widely used and provide faster results, but they do not take into account the complex oral environment.[3] The current research in adhesive dentistry is aimed at improving the quality of the resin-dentin bonds and understanding their degradation mechanisms.[4] Many strategies have been proposed to provide greater longevity of the union of the restorative materials to the dental structure, but few of them were evaluated under clinical conditions.[5]

Since proteolytic degradation of the hybrid layer has been described in the literature[6] and associated with the host enzymes, matrix metalloproteinases (MMP), and cysteine cathepsins (CC), numerous investigations have been conducted with potential enzymatic inhibitors. Promising laboratory results have been achieved regarding the hybrid layer preservation and long-term durability.[7,8] These enzymes are present in human dentin and saliva with the ability to degrade most extracellular matrix proteins, such as collagen type I, the organic component of the hybrid layer.[9,10] Within the hybrid layer, denuded collagen fibrils become vulnerable to degradation by these host-derived proteases with collagenolytic activity.[11] Thus, the use of bioactive substances that inhibit the enzymes may avoid the degradation of exposed collagen fibrils at the adhesive interface.[4]

Chlorhexidine (CHX) is the "gold standard" antimicrobial agent for oral health and has demonstrated inhibition of MMP-2, MMP-8, MMP-9, and CC.[12,13] Laboratory results have proven the benefits in the immediate and long-term bond effectiveness of CHX associated with adhesive systems. However, the use of chlorhexidine as an MMP inhibitor was tested under clinical trials (either as dentin pretreatment or incorporated in the adhesive),[14-17] and the results (up to three-year follow-up)[18] showed that there was no improvement in the clinical durability of adhesive restorations. These results also highlighted the lack of correlation between the results of the laboratory tests and the clinical reality to which the adhesive restorations are exposed.[3]

Other enzymatic inhibitors, mainly natural products, have aroused the interest of the scientific community. Epigallocatechin-3-gallate (EGCG) is the most abundant polyphenol found in green tea with a proven ability to inhibit the expression and action of MMP-2, MMP-9, and CC.[19] Through laboratory studies, the use of EGCG has been recently associated with adhesive procedures and was effective in preserving the resin-dentin bond strength for up to six months when incorporated into the dentin adhesive[20] and up to six and 12 months when used as a dentin pretreatment with etch-and-rinse (ER) and self-etch (SE) adhesive systems, respectively.[21,22] Unlike CHX, EGCG also has an additional cross-linking property with positive effects on the mechanical properties of collagen and its stabilization against proteolytic degradation.[23] It has been shown that galloyl radical monomeric catechins, such as EGCG, are more effective than other catechins without the radical in increasing the modulus of elasticity and reducing collagen biodegradation rates.[23]

In this field, EGCG seems to be a promising bioactive substance that may maintain the integrity and stability of dentin collagen by synergistic effects, with protease inhibition and cross-linking ability in dentin. To the authors' knowledge, there are no randomized controlled trials evaluating the effect of EGCG as a dentin primer in the restorations of noncarious cervical lesions (NCCLs).

Thus, the objective of the present study was to evaluate the influence of dentin pretreatment with EGCG in restorations of noncarious cervical lesions performed with a universal adhesive system in the two adhesive strategies *in vivo*. The null hypotheses tested were that (1) there is no difference between the clinical performance of NCCL restorations

Table 1: *Restorative Materials and Application Procedures According to the Manufacturer's Instructions*

Material	Manufacturer	Composition	Lot Number	Application Procedure
Epigallocatechin-3-gallate	Sigma-Aldrich (St Louis, MO, USA)	≥80% (high-performance liquid chromatography), from green tea	SLBL 1959V	0.1% aqueous solution actively applied for 60 s with a microbrush before adhesive application
Filtek Z350 XT	3M ESPE (St Paul, MN, USA)	Treated silanized ceramics; silane-treated silica; urethane dimethacrylate; bisphenol A polyethylene glycol diether dimethacrylate; bisphenol A-glycidyl methacrylate; ceramics of zirconia; polyethylene glycol; dimethacrylate; triethylene glycol and dimethacrylate	190224, 147907	Up to three increments of composite resin, individually photopolymerized
Single Bond Universal	3M ESPE	Methacryloyloxydecyl dihydrogen phosphate; phosphate monomer; dimethacrylate resins; hydroxyethyl methacrylate; methacrylate-modified polyalkenoic acid copolymer; filler; ethanol; water; initiators; silane	582958	Etch-and-rinse strategy: total etching with 37% phosphoric acid (enamel 30 s, dentin 15 s) followed by rinsing with water and drying with air free of moisture and oil, without drying out. Adhesive application to tooth surface by scrubbing action (20 s), drying of the adhesive (5 s), and light curing (10 s)
				Self-etch strategy: adhesive application to tooth surface by scrubbing action (20 s), drying of the adhesive (5 s), and light curing (10 s)

bonded with the ER strategy associated or not with dentin pretreatment with EGCG and (2) there is no difference between the clinical performance of NCCL restorations bonded with the SE strategy associated or not with dentin pretreatment with EGCG.

METHODS AND MATERIALS

Study Design

This study was a blind, randomized clinical trial with an equal allocation rate among the four adhesive procedures under evaluation, and this report follows the Consolidated Standards of Reporting Trials statement.[24] In the control groups, the multimode adhesive was applied in the ER and SE strategies. In the experimental groups (ER-EGCG and SE-EGCG), a dentin pretreatment with a 0.1% w/v EGCG aqueous solution (Sigma-Aldrich, St Louis, MO, USA) was scrubbed on the teeth for 60 seconds, and then the adhesive system was used in the same ER (ER-EGCG) and SE (SE-EGCG) strategies. Table 1 summarizes the restorative materials and the application procedures used.

Patient Selection

Thirty-three volunteers (22 to 66 years old; 19 male and 14 female) were informed about the goals of the study and the risks and benefits to which they would be exposed, were authorized to participate, and signed a written consent form. Inclusion criteria consisted of being over 18 years of age, having good oral hygiene and no periodontal disease or bruxism, and having at least four NCCLs and at least 20 teeth in occlusion. Exclusion criteria consisted of a previous cervical restorative procedure or caries lesions. All types of noncarious lesions (abrasion, erosion, and abfraction) were included, and all of them were nonretentive with no more than 50% of the margin in enamel and with the cervical margin in dentin.

Sample Size Calculation and Sample Randomization

The sample size was calculated based on the 96% retention rate of Single Bond Universal (3M ESPE, St Paul, MN, USA) in an 18-year clinical follow-up and considering a two-tailed test hypothesis.[25] At least 33 restorations per group were required to determine a 25% difference between groups with a significance level of 5% and a statistical power of 80%. A 20% increase in the sample size was set, considering possible loss during the study.

The assignment was done following one random list created using the website www.sealedenvelope.com, which indicated the control or experimental group for each tooth, without the patient's awareness.

Restorative Procedures

Each patient received at least one restoration per group for a total of 156 restorations. All NCCLs were classified according to cavity dimensions in millimeters (height, width, and depth), shape of the cavity (degree of the angle labeled as <45°, 45° to 90°, 90° to 135°, or >135°), degree of sclerotic dentin,[26] presence of incisal/occlusal wear facets, presence of preoperative sensitivity, and distribution among tooth types (Table 2).

Clinical photographs of the teeth prior to restoration were taken with a digital camera (Canon EOS 60D, Canon Macro EF 100-mm lens, Canon, Tokyo, Japan) in three positions: vestibular, proximal (mesial), and incisal/occlusal. Silicone rubber impressions with heavy- and light-body polyvinylsiloxane material (Adsil, Vigodent, Rio de Janeiro, Brazil) were obtained of each NCCL to make epoxy resin (MC130/FD154, Epoxyfiber, Rio de Janeiro, Brazil) replicas.[27]

The same operator performed all restorations. Twenty-seven patients received four restorations, and six patients received eight restorations. No cavity preparation was carried out, enamel margins were not beveled, and no mechanical retention was placed. Local anesthesia with 1.8 mL of 2% lidocaine hydrochloride and phenylephrine 1:2500 (S.S. White 100, S.S.White, Petrópolis, Brazil), a labial retractor (Arc Flex, FGM, Joinville, Brazil), cotton rolls, and #00 retraction cord (Ultrapak, Ultradent, Indaiatuba, Brazil) were used for all cases. Prior to the restorative procedure, the NCCLs were cleaned with pumice and water (S.S.White), then rinsed and dried.

The adhesive system's application in the control groups (ER and SE) followed the ER and SE protocols suggested by their manufacturers (Table 1). The experimental groups (ER-EGCG and SE-EGCG) followed the same protocols but with a dentin pretreatment with a 0.1% EGCG aqueous solution (Sigma-Aldrich) scrubbed on the lesions for 60 seconds (Table 1). Increments of the resin composite Filtek Z350XT (3M ESPE) (up to three) were inserted and light cured individually for 20 seconds using the LED light-curing unit DB-685 (1100 mW/cm^2, Dabi Atlante, Ribeirão Preto, Brazil).

The initial finishing was performed with 12- and 30-fluted tungsten carbide burs (Microdont, São Paulo, Brazil) and F and FF diamond tips (KG Sorensen, São Paulo, Brazil). Polishing was performed with Sof-Lex Pop-On abrasive discs (3M ESPE). The initial baseline evaluation was performed one week later when another impression was taken of each restoration. New resin replicas from the restoration interface were obtained for observation under a scanning electron microscope (SEM) (Quanta FEG 450, FEI, Thermo Fisher Scientific, Hillsboro, OR, USA).

Table 2: *Noncarious Cervical Lesion (NCCL) Classification According to Shape, Cervico-Incisal Height, Degree of Sclerotic Dentin, Presence of Antagonist, Presence of Incisal/Occlusal Wear Facets, Presence of Preoperative Sensitivity, and Distribution Among Tooth Types*

Characteristics of NCCL	Number of Lesions			
	ER	ER-EGCG	SE	SE-EGCG
Shape (degree of angle)				
<45	1	2	1	3
45-90	7	10	5	4
90-135	20	20	24	19
>135	11	7	9	13
Cervico-incisal height (mm)				
<1.5	5	4	5	4
1.5-2.5	22	18	22	19
2.5-4.0	10	14	10	13
>4.0	2	3	2	3
Degree of sclerotic dentin				
1	17	20	23	22
2	19	14	12	12
3	3	5	4	5
4	0	0	0	0
Presence of incisal/occlusal wear facets				
Yes	32	31	26	33
No	7	8	13	6
Preoperative sensitivity (air dry)				
Yes	12	15	15	17
No	27	24	24	22
Preoperative sensitivity (spontaneous)				
Yes	7	7	6	6
No	32	32	33	33
Tooth distribution				
Anterior				
Incisors	10	9	10	8
Canines	6	3	7	2
Posterior				
Premolar	21	22	19	28
Molar	2	5	3	1
Arch distribution				
Maxillary	20	23	25	15
Mandibular	19	16	14	24

Abbreviations: ER, etch and rinse; ER-EGCG, etch and rinse + 0.1% EGCG pretreatment; SE, self-etch; SE-EGCG, self-etch + 0.1% EGCG pretreatment.

Clinical Evaluation

The study was blinded for the two independent, calibrated examiners who were responsible for the clinical evaluations. FDI World Dental Federation criteria[28,29] were used to evaluate retention, the presence of fractures, marginal discoloration, marginal adaptation, caries, and postoperative sensitivity at baseline and after six, 12, 18, and 24 months. These variables were classified as follows: clinically very good, clinically good (scores 1+2), clinically sufficient/satisfactory (score 3), clinically unsatisfactory (score 4), and clinically poor (score 5).[29] The analysis was done independently and performed with a clinical mirror and dental explorer #5 (EXD56, Hufriedy, Rio de Janeiro, Brazil). In case of disagreement between the investigators, a consensus was reached by reexamination and discussion before the patient was dismissed.[30]

The retention rates were calculated according to the following equation: cumulative failure percentage = $[(PF+NF)/(PF+RR)] \times 100\%$, where PF is the number of previous failures before the current assessment, NF is the number of new failures during the current evaluation, and RR is the number of restorations currently evaluated.[25]

At all evaluation periods (baseline and six, 12, 18, and 24 months), impressions were taken from representative restorations from each group tested and each level score from marginal adaptation criteria.[27] All restorations were also photographed in two positions (vestibular and mesial).

Statistical Methods

The McNemar test was used for intragroup comparisons between the baseline and the other periods. The Kruskal-Wallis test was used for intergroup comparisons between each group in each period. The comparisons were performed for all criteria evaluated with a significance level of 5%. The Cohen kappa statistic was used to measure the agreement between examiners.

RESULTS

Figure 1 shows the patient's flow diagram. Thirty-eight out of 71 patients were not enrolled in the study because they did not fulfill the inclusion criteria. Thus, 33 subjects were selected, and the one hundred and fifty-six lesions were randomly assigned to one of the four experimental groups. Recall rates were 100% for baseline for six, 12, and 18 months. For 24 months, the recall rate was 96.9%, with one patient not returning due to health problems. One patient was eliminated at 12 months and another at 18 months due to orthodontic treatment.

Table 3 presents data for retention/fractures, marginal staining, marginal adaptation, caries, and postoperative sensitivity at baseline and for all evaluation periods. The pooled (1+2) scores and score 3 present the total number of restorations classified as clinically acceptable. Scores 4 and 5 show the number of restorations considered clinically unsatisfactory. Figure 2 presents representative images of clinically satisfactory restorations from each group at baseline and at 24 months.

Four restorations were lost after six months, one restoration was lost at the 12-month recall, and one restoration was lost at the 18-month recall. Thus, a total of six restorations were lost by 24 months (one for ER, two for SE, one for ER-EGCG, and one for SE-EGCG). The 24-month retention rates were 97.1% for ER, 94.4% for SE, 97.1% for ER-EGCG, and 94.6% for SE-EGCG, with no statistical difference between any pair of groups at the 24-month recall and for each group when the baseline and 24-month results were compared ($p>0.05$; Table 3).

Two restorations had an acceptable material chip fracture (one for ER and one for SE), but it did not affect the marginal integrity score of 3. Six restorations had bulk fractures with partial loss for a score of 4 (two each for the SE, ER-EGCG, and SE-EGCG groups) (Figure 3).

Ten restorations were considered to have discrepancies in marginal adaptation at the 24-month recall. Half of them (one for ER and four for SE) were considered minor acceptable discrepancies, but another half (two for SE, one for ER-EGCG, and two for SE-EGCG) were considered unacceptable discrepancies, with severe material fracture (Figure 4). No significant difference was detected between any pair of groups at the 24-month recall ($p>0.05$), but a significant difference was detected when the baseline and 24-month data were compared within the SE group ($p=0.0313$) (Table 3).

Marginal staining was observed in seven restorations (one for SE, four for ER-EGCG, and two for SE-EGCG). Pronounced marginal staining was observed in only one restoration, which was in the SE-EGCG group. However, no significant difference was found between groups at 24 months and within each group when the baseline and 24-month findings were compared ($p>0.05$; Table 3).

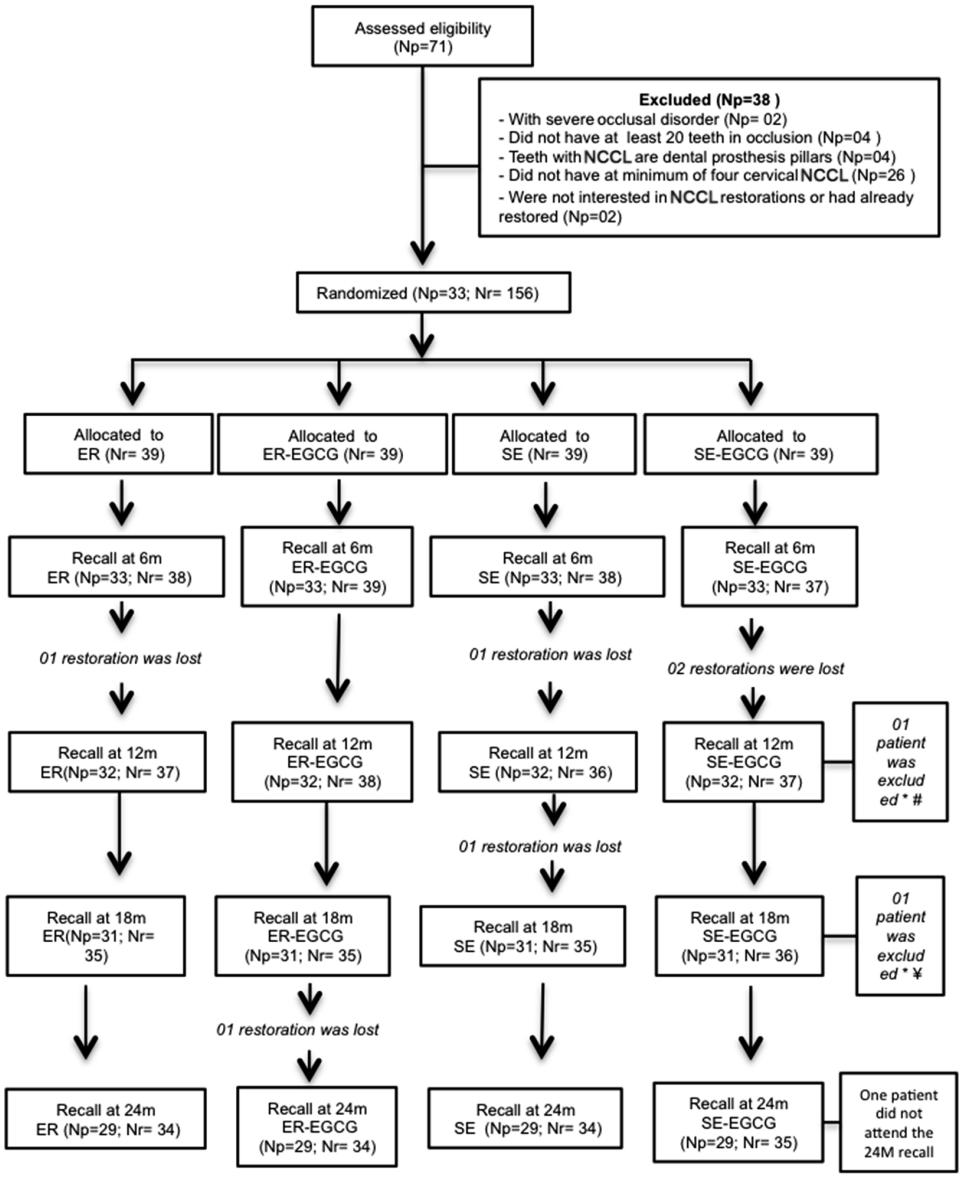

Figure 1. *Patient's flow diagram. Np, number of patients; Nr, number of restorations; NCCL, noncarious cervical lesion; ER, etch-and-rinse strategy; SE, self-etch strategy; EGCG, epigallocatechin-3-gallate; *, the patient was excluded due to orthodontic treatment; #, the excluded patient had already lost the restoration from the SE-EGCG group in the six-month recall; ¥, the excluded patient had eight noncarious cervical lesions restored and had already lost the restoration from the SE and SE-EGCG groups at the 12-month recall.*

Three restorations had postoperative sensitivity one week after the restorative procedures (two for ER and one for SE). Two of them showed very intense sensitivity (score 4), but the sensitivity remained clinically acceptable at six, 12, and 18 months, and no restoration presented postoperative sensitivity at 24 months.

Finally, caries were not observed after 24 months of clinical service, and no tooth became nonvital as a result of the cervical restoration.

DISCUSSION

EGCG is the most abundant catechin found in green tea. It has recently been introduced into the dental field because of several promising benefits, such as its antibacterial effect,[31] protective effects for dental erosion,[32] and effects in preserving the resin-dentin bond strength for up to six and 12 months associated with ER and SE adhesive systems, respectively, in laboratory studies.[20-22]

It has been suggested that EGCG could have a superior clinical application due to its positive effects on both mechanical properties and the stabilization of collagen against proteolytic degradation.[23] To the authors' knowledge, there are no clinical reports regarding the clinical performance of adhesive systems associated with EGCG. Thus, this randomized clinical trial evaluated a universal adhesive on different adhesive strategies, assessing the influence

Table 3: *Number of Evaluated Restorations for Baseline and 6-, 12-, 18-, and 24-Mo Recalls According to the Experimental Groups*[a]

Criteria	Group	Baseline				6-Mo				12-Mo				18-Mo				24-Mo			
		Scores				Scores				Scores				Scores				Scores			
		1+2	3	4	5	1+2	3	4	5	1+2	3	4	5	1+2	3	4	5	1+2	3	4	5
Marginal staining	ER	39	—	—	—	37	1	—	—	36	1	—	—	35	—	—	—	34	—	—	—
	SE	39	—	—	—	38	—	—	—	36	—	—	—	35	—	—	—	33	1	—	—
	ER-EGCG	39	—	—	—	39	—	—	—	37	1	—	—	32	3	—	—	30	4	—	—
	SE-EGCG	39	—	—	—	37	—	—	—	36	1	—	—	35	1	—	—	33	1	1	—
Retention/ fracture	ER	39	—	—	—	38	—	—	1	37	—	—	1	35	—	—	1	33	1	—	1
	SE	39	—	—	—	38	—	—	1	35	—	1	2	34	—	1	2	31	1	2	2
	ER-EGCG	39	—	—	—	39	—	—	—	37	—	1	—	34	—	1	1	32	—	2	1
	SE-EGCG	39	—	—	—	35	—	2	2	35	—	2	2	34	—	2	2	33	—	2	2
Marginal adaptation	ER	39	—	—	—	38	—	—	—	37	—	—	—	35	—	—	—	33	1	—	—
	SE	39[b]	—	—	—	37	—	1	—	35	—	1	—	33	1	1	—	28[b]	4	2	—
	ER-EGCG	39	—	—	—	39	—	—	—	37	—	1	—	34	—	1	—	33	—	1	—
	SE-EGCG	39	—	—	—	36	—	1	—	35	—	2	—	34	—	2	—	33	—	2	—
Caries	ER	39	—	—	—	38	—	—	—	37	—	—	—	35	—	—	—	34	—	—	—
	SE	39	—	—	—	38	—	—	—	36	—	—	—	35	—	—	—	34	—	—	—
	ER-EGCG	39	—	—	—	39	—	—	—	38	—	—	—	35	—	—	—	34	—	—	—
	SE-EGCG	39	—	—	—	37	—	—	—	37	—	—	—	36	—	—	—	35	—	—	—
Postoperative sensitivity	ER	37	1	1	—	38	—	—	—	36	1	—	—	34	1	—	—	34	—	—	—
	SE	38	—	1	—	38	—	—	—	34	2	—	—	35	—	—	—	34	—	—	—
	ER-EGCG	39	—	—	—	38	1	—	—	37	1	—	—	35	—	—	—	34	—	—	—
	SE-EGCG	39	—	—	—	36	1	—	—	37	—	—	—	36	—	—	—	35	—	—	—

Abbreviations: ER, etch and rinse; SE, self-etch; ER-EGCG, etch and rinse + 0.1% EGCG pretreatment; SE-EGCG, self-etch + 0.1% EGCG pretreatment.
[a] *Scores 1+2, clinically very good + clinically good; score 3, clinically sufficient/satisfactory; score 4, clinically unsatisfactory; score 5, clinically poor.*
[b] *Significant difference was detected when baseline and 24-mo data were compared within the SE group (*$p=0.0313$*).*

of the dentin pretreatment with EGCG prior to the adhesive application.

After 24 months, there was no difference in the retention of NCCL restorations bonded with the ER or SE strategy, associated or not with dentin pretreatment with EGCG, which leads to failing to reject the null hypothesis.

In the current study, a 0.1% w/v EGCG aqueous solution was used as the dentin primer since it was the concentration with better laboratory results in preserving the bond strength over time.[21,22] The one-minute application significantly improved the bond strength in research conducted by Zheng and Chen[8] on MMP inhibitors. In the present clinical study, this time of application represented a feasible step to be reproduced in clinical practice.

The clinical efficacy of the four bonding procedures was evaluated by the retention of the restorations as a primary outcome.[3] Retention in NCCLs cannot be associated with any other macromechanical retention, which can directly reflect the quality of the adhesive bonding.[2] In addition, the hypermineralized dentin in NCCLs makes the bonding to this substrate very unpredictable and challenging for adhesive systems.[33]

The high retention rates for all tested groups after 24 months showed that the bond effectiveness of the universal adhesive did not depend on the bonding strategy, in accordance with previous randomized clinical trials.[25,34-37] This retention rate result may be explained by the Single Bond Universal (SU) (3M ESPE) chemical bond mechanism. SU contains 10-methacryloxydecyl dihydrogen phosphate (10-MDP), an acidic phosphate monomer capable of bonding to hydroxyapatite, producing adhesive interfaces with different chemical and morphological characteristics. It also contains a polyalkenoic acid copolymer.[25] These two processes involve micromechanical and chemical bonding to the enamel and dentin, which may have had a direct impact on the retention rate and clinical behavior of SU in the current study after 24 months.

The retention results also showed that the EGCG priming solution did not impair the bond effective-

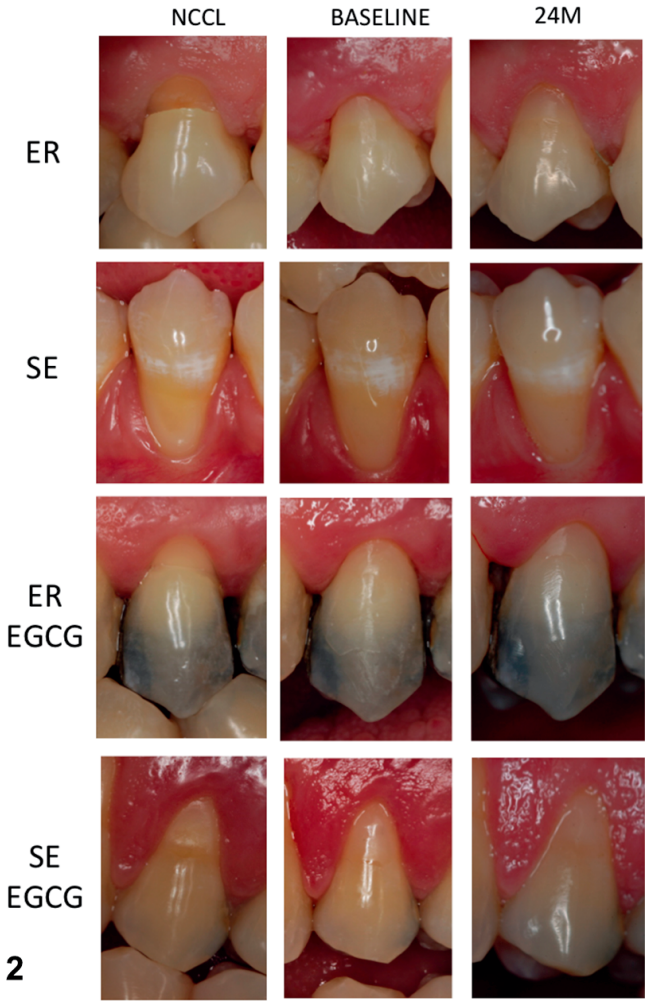

Figure 2. *Noncarious cervial lesions from each group and clinically satisfactory restorations at baseline and 24 months.*

ness and can be associated with both adhesive strategies as reported only by laboratory studies.[21,22] Unlike chlorhexidine, which showed a negative association with a self-etching adhesive,[38] this result highlighted the versatile clinical application of EGCG as a dentin primer in NCCLs.

The scoring system used for the evaluation of the NCCL restorations was the FDI criteria.[29] Four specific secondary outcomes were selected from the total of 16 original parameters. The inclusion of "marginal staining" and "marginal adaptation" was an important measure since they are still shortcomings mainly of cervical restorations and it has been indicated that they may be predictors of future failures.[39] Also, a simplified clinical evaluation was chosen pooling scores 1 and 2 (equivalent to score A from the US Public Health Service criteria);[30] thus, the FDI criteria were expressed with only four scores: two for acceptable (1+2 and 3) and two for

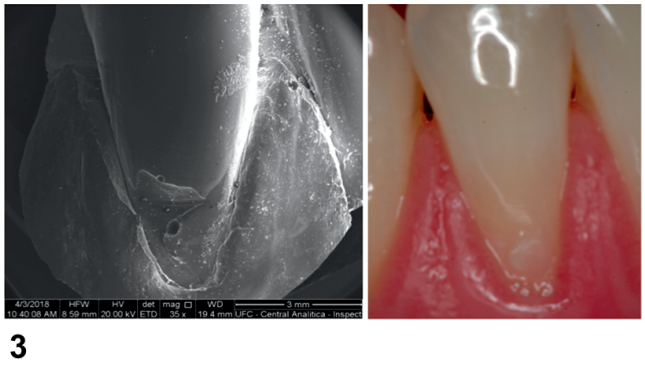

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Figure 3. *On the left, the scanning electron photomicrograph of resin epoxy replicas from the resin fracture. On the right, clinical photograph from the same resin restoration rated as clinically unsatisfactory but repairable (material fracture score 4) at the 24-month recall.*

nonacceptable (4 and 5) for reparable and replacement, respectively.[1,29]

For marginal adaptation, a significant difference was detected when baseline and 24-month data were compared within the SE group (p=0.0313). SU is considered an ultramild SE adhesive (pH~3.0), and, although there is no consensus that pre-etching enamel improves the bond strength of universal adhesives,[40,41] this result may be related to the limited micromechanical retention of the enamel surface conditioned by the SE mode. Surprisingly, the association of EGCG with the SE strategy did not jeopardize the marginal adaptation, and future evaluations could confirm a possible beneficial effect of EGCG priming on enamel.

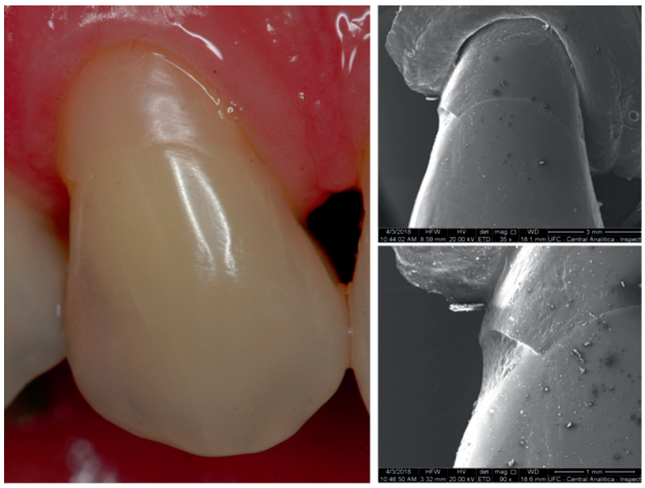

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Figure 4. *On the left, a clinical photograph of resin restoration (self-etch group) rated as clinically unsatisfactory but repairable (marginal adaptation score 4) at the 24-month recall. On the right, scanning electron photomicrographs of resin epoxy replicas from the same restoration (superior right) and an approximated view (inferior right) from the resin fracture.*

After 24 months, five restorations were rated as score 4 (two for SE, one for ER-EGCG, and two for SE-EGCG) with unacceptable discrepancies and severe material fractures. A prophylactic repair was indicated to recover the restoration integrity, avoiding premature replacement of the restoration.[29] These restorations can be viewed as "relative failures," and this result may be associated with heavy occlusal or parafunctional forces since all teeth presented signs of occlusal wear (data not shown). A retrospective clinical study[27] showed that class V restorations of teeth with occlusal wear facets had a 6.65-fold increased risk of failure than those of teeth without wear facets. Clinical covariables shown in Table 2 could determine the bonding effectiveness, such as shape and size of the lesion, degree of sclerosis, presence of wear facets, patient age, and tooth type.

The restorations that were rated unsatisfactory were additionally investigated using digital photographs and epoxy resin replicas under SEM evaluation.[27] The replicas enabled indirect observation and were useful in analyzing changes in discrepancies and/or material fracture in restorations at different periods. This procedure was helpful in determining the need for timely repair or replacement.

Pronounced marginal staining was observed in only one restoration, which was in the SE-EGCG group, and it was associated with a color change along the whole of the restoration margin due to smoking habits and not to the marginal discrepancy.

A meta-analysis and trial sequential analysis[42] evaluated randomized clinical trials comparing degradation inhibitory cavity pretreatment (CHX and quaternary ammonium) prior to the adhesive placement of the resin-based restorations. The authors concluded that there is insufficient evidence to recommend or refute degradation inhibitory cavity pretreatment prior to adhesively placing resin-based restorations. These findings corroborate the current results regarding EGCG since its effects (protease inhibition and cross-linking ability in dentin)[23] could not be proven by this trial. A longer follow-up could be required to validate these experimental strategies aiming to enhance the adhesive interface.

CONCLUSIONS

At 24 months, dentin pretreatment with EGCG did not provide any positive benefit in the clinical performance of the adhesive SU regardless of the bonding strategy used. In addition, including catechin placement prebonding results in an additional required step in the restorative procedure.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Ethics Committee on Investigations involving human subjects of the Federal University of Ceará. The approval code issued for this study is 1.292.593.

Conflict of Interest

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

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REFERENCES

1. Opdam NJM, Collares K, Hickel R, Bayne SC, Loomans BA, Cenci MS, Lynch CD, Correa MB, Demarco F, Schwendicke F, & Wilson NHF (2018) Clinical studies in restorative dentistry: New directions and new demands *Dental Materials* **34(1)** 1-12.
2. Reis A, de Geus JL, Wambier L, Schroeder M, & Loguercio AD (2018) Compliance of randomized clinical trials in noncarious cervical lesions with the CONSORT statement: A systematic review of methodology *Operative Dentistry* **43(3)** E129-E151.
3. Van Meerbeek B, Pneumans M, Poitevin A, Mine A, Van Ende A, & Neves A (2010) Relationship between bond-strength tests and clinical outcomes *Dental Materials* **26(2)** 100-121.
4. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho MR, Carvalho RM, Tay FR, & Pashley DH (2013) Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins *Dental Materials* **29(1)** 116-135.
5. Reis A, Carrilho M, Breschi L, & Loguercio AD (2013) Overview of clinical alternatives to minimize the degradation of the resin-dentin bonds *Operative Dentistry* **38(4)** E1-E25.
6. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, & Ito S (2004) Collagen degradation by host-derived enzymes during aging *Journal of Dental Research* **83(3)** 216-221.
7. Montagner AF, Sarkis-Onofre R, Pereira-Cenci T, & Cenci MS (2014) MMP inhibitors on dentin stability: A systematic review and meta-analysis *Journal of Dental Research* **93(8)** 733-743.

8. Zheng P & Chen H (2017) Evaluate the effect of different MMPS inhibitors on adhesive physical properties of dental adhesives, bond strength and MMP substrate activity *Scientific Reports* **7(1)** 4975-4980.
9. Chaussain C, Boukpessi T, Khaddam M, Tjaderhane L, George A, & Menashi S (2013) Dentin matrix degradation by host matrix metalloproteinases: inhibition and clinical perspectives toward regeneration *Frontiers in Physiology* **1(4)** 308.
10. Tersariol IL, Geraldeli S, Minciotti CL, Nascimento FD, Pääkkönen V, Martins MT, Carrilho MR, Pashley DH, Tay FR, Salo T, & Tjäderhane L (2010) Cysteine cathepsins in human dentin-pulp complex *Journal of Endodontics* **36(3)** 475-481.
11. Perdigão J, Reis A, & Loguercio AD (2013) Dentin adhesion and MMPs: A comprehensive review *Journal of Esthetic and Restorative Dentistry* **25(4)** 219-241.
12. Gendron R, Grenier D, Sorsa T, & Mayrand D (1999) Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine *Clinical and Diagnostic Laboratory Immunology* **6(3)** 437-439.
13. Scaffa PM, Vidal CM, Barros N, Gesteira TF, Carmona AK, Breschi L, & Pashley DH (2012) Chlorhexidine inhibits the activity of dental cysteine cathepsins *Journal of Dental Research* **91(4)** 420-425.
14. Dutra-Correa M, Saraceni CH, Ciaramicoli MT, Kiyan VH, & Queiroz CS (2013) Effect of chlorhexidine on the 18-month clinical performance of two adhesives *Journal of Adhesive Dentistry* **15(3)** 287-292.
15. Sartori N, Stolf SC, Silva SB, Lopes GC, & Carrilho M (2013) Influence of chlorhexidine digluconate on the clinical performance of adhesive restorations: A 3-year follow-up *Journal of Dentistry* **41(12)** 1188-1195.
16. Montagner AF, Perroni AP, Corrêa MB, Masotti AS, Pereira-Cenci T, & Cenci MS (2015) Effect of pretreatment with chlorhexidine on the retention of restorations: A randomized controlled trial *Brazilian Dental Journal* **26(3)** 234-241.
17. Araújo MS, Souza LC, Apolonio FM, Barros LO, Reis A, Loguercio AD, & Saboia VP (2015) Two-year clinical evaluation of chlorhexidine incorporation in two-step self-etch adhesive *Journal of Dentistry* **43(1)** 140-148.
18. Favetti M, Schroeder T, Montagner AF, Correa MB, Pereira-Cenci T, & Cenci MS (2017) Effectiveness of pretreatment with chlorhexidine in restoration retention: A 36-month follow-up randomized clinical trial *Journal of Dentistry* **60** 44-49.
19. Demeule M, Brossard M, Page M, Gingras D, & Beliveau R (2000) Matrix metalloproteinase inhibition by green tea catechins *Biochimica et Biophysica Acta* **1478(1)** 51-60.
20. Du X, Huang X, Huang C, Wang Y, & Zhang Y (2012) Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive *Journal of Dentistry* **40(6)** 485-492.
21. Santiago SL, Osorio R, Neri JR, Carvalho RM, & Toledano M (2013) Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength *Journal of Adhesive Dentistry* **15(6)** 535-540.
22. Neri JR, Yamauti M, Da Silveira F, Mendonça JS, Carvalho RM, & Santiago SL (2016) Influence of dentin biomodification with epigallocatechin-3-gallate on the bond strength of self-etch adhesive: Twelve-month results *International Journal of Adhesion and Adhesives* **71(December)** 81–86.
23. Vidal CM, Aguiar TR, Phansalkar R, McAlpine JB, Napolitano JG, Chen SN, Araújo LS, Pauli GF, & Bedran-Russo A (2014) Galloyl moieties enhance the dentin biomodification potential of plant-derived catechins *Acta Biomaterials* **10(7)** 3288-3294.
24. Schulz KF, Altman DG, Moher D, & CONSORT Group (2011) CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials *International Journal of Surgery* **9(8)** 672-677.
25. Perdigão J, Kose C, Mena-Serrano AP, De Paula EA, Tay LY, Reis A, & Loguercio AD (2014) A new universal simplified adhesive: 18-month clinical evaluation *Operative Dentistry* **39(2)** 113-127.
26. Swift EJ Jr, Perdigão J, Heymann HO, Wilder AD Jr, Bayne SC, May KN Jr, Sturdevant JR, & Roberson TM (2001) Eighteen-month clinical evaluation of a filled and unfilled dentin adhesive *Journal of Dentistry* **29(1)** 1-6.
27. Kim JH, Cho J, Lee Y, & Cho BH (2017) The survival of class V composite restorations and analysis of marginal discoloration *Operative Dentistry* **42(3)** E93-E101.
28. Hickel R, Roulet JF, Bayne S, Heintze SD, Mjör IA, Peters M, Rousson V, Randall R, Schmalz G, Tyas M, & Vanherle G (2007) Recommendations for conducting controlled clinical studies of dental restorative materials *Clinical Oral Investigations* **11(1)** 5-33. Epub 2007 Jan 30. Review. Erratum in (2008) *Clinical Oral Investigations* **12(1)** 97.
29. Hickel R, Peschke A, Tyas M, Mjör I, Bayne S, Peters M, Hiller KA, Randall R, Vanherle G, & Heintze SD (2010) FDI World Dental Federation—Clinical criteria for the evaluation of direct and indirect restorations. Update and clinical examples *Journal of Adhesive Dentistry* **12(4)** 259-272.
30. Cvar JF & Ryge G (2005) Reprint of criteria for the clinical evaluation of dental restorative materials (1971) *Clinical Oral Investigations* **9(4)** 215-232.
31. Xu X, Zhou XD, & Wu CD (2011) The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans Antimicrobial Agents and Chemotherapy* **55(3)** 1229-1236.
32. De Moraes MD, Carneiro JR, Passos VF, & Santiago SL (2016) Effect of green tea as a protective measure against dental erosion in coronary dentine *Brazilian Oral Research* **30** e13.
33. Karan K, Yao X, Xu C, & Wang Y (2009) Chemical profile of the dentin substrate in non-carious cervical lesions *Dental Materials* **25(10)** 1205-1212.
34. Lawson NC, Robles A, Fu CC, Lin CP, Sawlani K, & Burgess JO (2015) Two-year clinical trial of a universal adhesive in total-etch and self-etch mode in non-carious cervical lesions *Journal of Dentistry* **43(10)** 1229-1234.
35. Lopes LS, Calazans FS, Hidalgo R, Buitrago LL, Gutierrez F, Reis A, Loguercio AD, & Barceleiro MO (2016) Six-

month follow-up of cervical composite restorations placed with a new universal adhesive system: A randomized clinical trial *Operative Dentistry* **41(5)** 465-480.

36. Ruschel VC, Shibata S, Stolf SC, Chung Y, Baratieri LN, Heymann HO, & Walter R (2018) Eighteen-month clinical study of universal adhesives in noncarious cervical lesions *Operative Dentistry* **43(3)** 241-249.
37. Loguercio AD, de Paula EA, Hass V, Luque-Martinez I, Reis A, & Perdigão J (2015) A new universal simplified adhesive: 36-month randomized double-blind clinical trial *Journal of Dentistry* **43(9)** 1083-1092.
38. Costa CAGA, Passos VF, Neri JR, Mendonça JS, & Santiago SL (2019) Effect of metalloproteinase inhibitors on bond strength of a self-etching adhesive on erosively demineralized dentin *Journal of Adhesive Dentistry* **21(4)** 337-344.
39. Hayashi M & Wilson NHF (2003) Marginal deterioration as a predictor of failure of a posterior composite *European Journal of Oral Sciences* **111(2)** 155-162.
40. Rosa WL, Piva E, & Silva AF (2015) Bond strength of universal adhesives: A systematic review and meta-analysis *Journal of Dentistry* **43(7)** 765-776.
41. Kaczor K, Gerula-Szymańska A, Smektała T, Safranow K, Lewusz K, & Nowicka A (2018) Effects of different etching modes on the nanoleakage of universal adhesives: A systematic review and meta-analysis *Journal of Esthetic and Restorative Dentistry* **30(4)** 287-298.
42. Göstemeyer G & Schwendicke F. (2016) Inhibition of hybrid layer degradation by cavity pretreatment: Meta- and trial sequential analysis *Journal of Dentistry* **49** 14-21.