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ORIGINAL ARTICLE

24-month clinical evaluation in non-carious cervical lesions of a two-step etch-and-rinse adhesive applied using a rubbing motion

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Abstract The aim of this clinical trial was to evaluate the effects of application mode on the clinical performance of a two-step etch-and-rinse adhesive in class V cavities over 24 months. Forty patients with at least three similar-sized non-carious cervical lesions participated in this study. A total of 120 restorations with Prime & Bond NT were placed, 40 in each group. The adhesive was applied with no rubbing action, with slight rubbing action, or with vigorous rubbing action. The restorations were placed incrementally using the composite resin Esthet-X. The restorations were evaluated at baseline and after 6, 12, and 24 months following the modified United States Public Health Service criteria. Statistical analysis was conducted using Friedman repeated measures analysis of variance by rank and using the Wilcoxon signed-rank test for significance at each pair (α=0.05). The 24-month retention rates of Prime & Bond NT were 82.5% for the no rubbing group, 82.5% for the slight rubbing group, and 92.5% for vigorous rubbing group. No significant difference in the retention rates in each recall period was detected among groups (p>0.05); however, the retention rates in the 24-month recall was statistically lower than the baseline only for no rubbing or slight rubbing groups. The use of a vigorous application mode can be a clinical approach to improve the retention of restorations placed in non-carious cervical lesions.

Keywords Clinical evaluation · Adhesive system · Retention rate · Application mode

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Introduction

Most currently marketed adhesive systems have immediate bond strength that allows clinicians to bond to tooth structure without the use of retentive cavity preparations. Nevertheless, major concerns have been recently expressed regarding interfacial aging due to degradation of the bonding interface. Interestingly, the simplified adhesives produced the least predictable clinical performances when compared with the three-step etch-and-rinse systems [1–3].

The inferior durability of the simplified etch-and-rinse systems can be attributed to several reasons. The most important is that the adhesive layer is intrinsically more hydrophilic as solvents and hydrophilic components from the *primer* are mixed in a single bottle with the hydrophobic monomers from the bonding agents [4]. This hydrophilicity turns the adhesive's semi-permeable membranes even after polymerization [5] with an increased potential to absorb water from the underlying dentin and from the oral cavity [6] and therefore more prone to degradation over time [1].

Various clinical procedures were proposed to optimize bonding and reduce aging, such as prolonged application times of adhesives [7, 8], warm air-dry for solvent evaporation [9], improved impregnation by means of electric impulse-assisted adhesive application [10, 11], use of metaloproteinases inhibitors as chlorhexidine [12–14], and application of selective collagen cross-linkers during adhesive restorative procedures [15]. Besides that, a recent laboratory investigation has demonstrated that simplified etch-and-rinse adhesives can achieve high immediate and 6-month resin–dentin bond strength values when they are vigorously rubbed on the demineralized dentin surface [16, 17].

Whether or not the benefit of this clinical approach can improve the retention rates of simplified etch-and-rinse adhesives when used in non-carious cervical lesions is yet to be determined and needs in vivo validation. Therefore, the aim of the present study was to conduct a 24-month randomized controlled prospective study to evaluate the effect of vigorous rubbing action of a two-step adhesive in non-carious cervical lesions. The null hypothesis to be tested is that no significant difference will be detected among the different modes of application in any of the recall periods.

Materials and methods

The materials employed in this study were Prime & Bond NT, an acetone-based two-step etch-and-rinse adhesive system (Dentsply DeTrey, Konstanz, Germany), and one microhybrid composite resin Esthet-X (Dentsply DeTrey, Konstanz, Germany). Detailed compositions and modes of the application are described in Table 1.

The protocol and consent form for this study were reviewed and approved by the local Committee on Investigations Involving Human Subjects (083/2006). Written informed consent was also obtained from all participants prior to starting the treatment. Patient screening and pretreatment selection of teeth with cervical lesions identified visually or tactilely were performed by four calibrated operators. The patients were screened initially to determine if they met the study entry criteria (described below). Qualified patients were recruited in the order in which they reported for the screening session, thus forming a convenience sample. The investigators carried out the evaluations using a mouth mirror, an explorer, and a periodontal probe. Air from the air–water syringe was used to administer the sensitivity test.

All participants were healthy and had at least 20 teeth. Participants were given oral hygiene instructions before

Table 1 Material, composition, and mode of the adhesive application according to the different groups

Material	Composition	Acid-etching procedure	Groups	Mode of the adhesive application
Prime & Bond NT (Dentsply DeTrey, Konstanz, Germany)	1, acid-etch: 37% phosphoric acid; 2, adhesive: Bis-GMA, BPDM, HEMA, initiators, and acetone	a, acid-etch (15 s); b, rinse (15 s); c, air-dry (2–3 s); d, keep dentin wet (moist technique with air-blowing)	No rubbing action	e, first coat of adhesive systems was only spread over the entire surface for approximately 3 s and left undisturbed for ±10 s; f, air-dry for 10 s at 20 cm; g, second coat of adhesive systems was only spread over the entire surface for approximately 3 s and left undisturbed for ±10 s; h, air-dry for 10 s at 20 cm; i, light-cure (10 s, 600 mW/cm^2)
			Slight rubbing action	e, first coat of adhesive systems was lightly spread on the entire surface for approximately 10 s; however, no intentional manual pressure was exerted on the microbrush; f, air-dry for 10 s at 20 cm; g, second coat of adhesive systems was slightly spread on the entire surface for approximately 10 s; however, no intentional manual pressure was exerted on the microbrush; h, air-dry for 10 s at 20 cm; i, light-cure (10 s, 600 mW/cm^2)
			Vigorous rubbing action	e, first coat of adhesive systems was rigorously rubbed on the entire dentin surface for approximately 10 s.; f, air-dry for 10 s at 20 cm; g, second coat of adhesive systems was rigorously agitated on the entire dentin surface for approximately 10 s; h, air-dry for 10 s at 20 cm; i, light-cure (10 s, 600 mW/cm^2)

operative treatment. Patients with severe or chronic periodontitis or heavy bruxism were not included in the study group as they required other treatment before any restorative intervention. At least three similar-sized cervical lesions (erosion/attrition/abfraction) under occlusion were required per patient.

The lesions had to be expulsive with no undercuts, and no more than 50% of the cavosurface margin could involve enamel. The cervical wall had to be located in cementum. Lesions not classified as criteria 2 and 3 of dentin sclerosis and exhibiting hypersensitivity were excluded from the study [18].

Restorative procedures

All lesions were restored by the same two investigators that participated in the patient screening. Calibration of operators was carried out by one experienced clinician. Operators first observed the detailed application procedures in laboratory models (3× for each protocol) and then performed four repeated restorations (for each protocol) under direct supervision in a clinical set with patients. Questions were addressed and consensus obtained during the calibration session.

Each patient received three restorations, in which the materials were randomly allocated. Randomization of the materials was performed on each patient by the Excel software (Excel 2003, Microsoft Corporatin, One Microsoft Way, Redmond, WA, USA). The lesions were prepared as follows: (1) local anesthesia (Citanest, Dentsply, Petrópolis, RJ, Brazil), (2) cleaning with pumice and water (SS White Prod. Odontol. Ltda, Petrópolis, RJ, Brasil) in a rubber cup (# 8040RA and 8045RA, KG Sorensen, Barueri, SP, Brasil) followed by rinsing and drying, (3) shade selection (Esthet-X shade guide/Dentsply Caulk, Milford, DE, USA), (4) rubber dam isolation (SS White Prod. Odontol. Ltda, Petrópolis, RJ, Brasil), and (5) rinsing with a water/air spray. No additional retention or bevel was performed.

Then the adhesive Prime & Bond NT (Dentsply Caulk, Milford, DE, USA) was applied under three different modes, as described previously by Dal-Bianco et al. [16].

1. *No rubbing action*: In this group, the adhesive was only spread over the entire surface for approximately 3 s and left undisturbed for 7 s. Then, an air stream was applied for 10 s at a distance of 20 cm.
2. *Slight rubbing action*: The adhesive was lightly spread on the entire surface for approximately 10 s; however, no intentional manual pressure was exerted on the microbrush. Before performing the adhesive application, the operator trained on the surface of an analytical balance to determine the equivalent manual pressure that would be placed on the surface of the demineralized dentin (Mettler, type H6; Columbus, OH, USA). For this group, the pressure was equivalent to approximately 4.0 ±1.0 g. An air stream was applied for 10 s at a distance of 20 cm.
3. *Vigorous rubbing action*: The adhesive was rigorously agitated on the entire dentin surface for approximately 10 s. The microbrush was scrubbed on the dentin surface under manual pressure (equivalent to approximately 34.5±6.9 g). An air stream was applied for 10 s at a distance of 20 cm (Table 1).

In all these three groups, a second coat of adhesive layer was applied in the same manner as for the first layer. The time lapse from the beginning of the adhesive application and light curing (QHL75 Lite, Dentsply, Petrópolis, RJ, Brazil; 600 mW/cm^2) was approximately 40 s. The light curing was performed for the respective recommended time (10 s). Before the start of the clinical placement of the restorations, operators were trained in a laboratory setting in order to standardize the material application according to the three different modes. After adhesive application, lesions were incrementally filled with Esthet-X (Dentsply DeTrey, Konstanz, Germany; ±3 increments). The size of the increment was dependent on the cavity size; however, they never exceeded 1.0 mm. Each increment was light cured for 30 s using a VIP light unit set at 600 mW/cm^2 (QHL75 Lite, Dentsply, Petrópolis, RJ, Brazil). All restorations were finished with fine grain diamond burs (# 1190F and 2135F, KG Sorensen, Barueri, SP, Brazil). After 1 week, restorations received a final polishing with Enhance points associated with Prisma Gloss (Dentsply, Petrópolis, RJ, Brazil).

Clinical evaluation

The categories evaluated were retention and marginal discoloration, post-operative sensitivity, and recurrent caries according the US Public Health Service (USPHS) [19] criteria at baseline and after 6, 12, and 24 months. Restoration retention rates were calculated using the ADA Guidelines equation [20].

$$\text{Cumulative failure } \% = [(\text{PF} + \text{NF})/(\text{PF} + \text{RR})] \times 100\%$$

PF is the number of previous failures before the current recall, NF the number of new failures during the current recall, and RR the number of restorations recalled for the current recall. Photographs were taken prior to the beginning of the treatment, at baseline and at each recall period.

Two other experienced and calibrated examiners performed the evaluation using a mirror and an explorer after teeth prophylaxis. The clinicians were unaware of which adhesive protocol was followed. Each examiner evaluated the restoration once and independently. Consensus was

reached when disagreements occurred before dismissing the patient.

Statistical analysis

For sample size calculation, the retention rate of Prime & Bond NT at 24 months was considered to be 98% [18, 21, 22]. Using an α of 0.05, a power of 80%, a one-sided test, the minimal sample size should be 40 restorations in each group in order to detect a difference of 20% between groups [23].

Descriptive statistics were used to describe the frequency distributions of the evaluated criteria. Statistical analysis was made with Friedman repeated measures analysis of variance by rank and using the Wilcoxon signed-rank test for significance at each pair (α=0.05). The Bonferroni correction was applied for corrections of p value for the multiple comparisons performed. Cohen's Kappa statistic was used to test the inter-examiner agreement.

Results

All patients attended the recall periods. Forty out of 118 evaluated patients met the inclusion criteria. One hundred and twenty restorations were placed, 40 for each group. The age and gender distribution of the research subjects are presented in Table 2. Fifty-nine restorations were placed in maxillary teeth and 61 in mandibular teeth. Approximately 62% of restorations were placed in premolars and molars, and 38% were placed in anterior teeth (Table 2).

The overall Cohen's Kappa statistics (0.90) showed excellent agreement between the examiners. All research subjects were evaluated in the 6-, 12-, and 24-month recalls. All patients attended the 24-month recall. A representative clinical case can be seen in Fig. 1.

No restorations presented secondary caries throughout the evaluation period (Table 3). Ten restorations presented post-operative sensitivity in the baseline. After 6 months, nine restorations, three for each group, showed post-operative sensitivity. No post-operative sensitivity was reported in the 12- and 24-month recalls (Table 3).

No interfacial staining was found in the 6-month recall. After 12 months, eight restorations were scored as bravo in marginal discoloration. No significant difference among groups was detected in the 12-month recall (p>0.05) or when compared to their respective baseline scores (p> 0.05). In the 24-month recall, 13 restorations were scored as bravo in marginal discoloration. The application modes were not statistically different with one another (p>0.05) in this recall period. When these figures were compared to their respective baseline records, statistical differences were only observed for no rubbing and slight rubbing groups (p=

Table 2 Distribution of non-carious cervical lesions according to research subject (gender and age) and characteristics of class lesions (shape, cervico-incisal size of the lesion, degree of sclerotic dentin, presence of antagonistic, presence of attrition facets, presence of pre-operative sensitivity, and tooth and arch distribution)

	Number of lesions
Characteristics of research subjects	
Gender distribution	
Male	57
Female	63
Age distribution (years)	
20–29	3
30–39	39
39–49	45
>49	33
Characteristics of class V lesions	
Shape (degree of angle)	
<45	4
45–90	48
90–135	58
>135	10
Cervico-incisal height (mm)	
<1.5	28
1.5–2.5	38
>2.5	54
Degree of sclerotic dentin	
1	74
2	42
3	3
4	1
Presence of antagonist	
Yes	80
No	40
Attrition facet	
Yes	82
No	38
Pre-operative sensitivity (spontaneous)	
Yes	80
No	40
Tooth distribution	
Anterior	
Incisor	16
Canines	30
Posterior	
Premolar	68
Molar	6
Arc distribution	
Maxillary	59
Mandibular	61

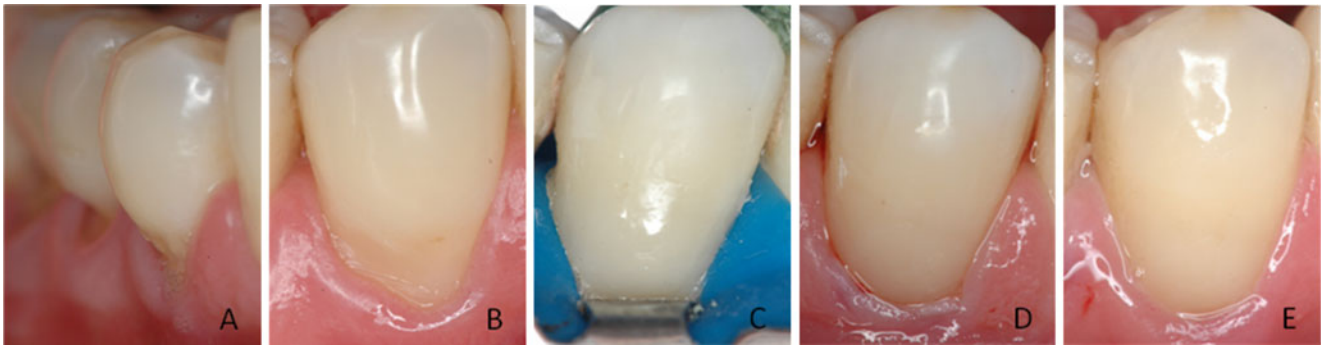

Fig. 1 Lateral (**a**) and frontal view (**b**) of cervical lesion on tooth #14 (5) restored with Prime & Bond NT/Esthet-X applied under vigorous pressure. Observe the aspect before the finished restoration (**c**), after 1-week finishing and polishing (**d**), and after 24-month clinical evaluation (**e**)

0.003; Fig. 2). The marginal discoloration occurred mostly at the enamel margin.

The retention rates of the groups at each recall period are depicted in Fig. 3. No significant difference in the retention rates in each recall period was detected among groups ($p>0.05$). The comparison of 6- and 12-month vs. baseline findings for all groups did not depict any significant difference ($p>0.05$). However, when the 24-month was compared to baseline, significant differences were detected for no rubbing and slight rubbing groups ($p=0.002$ for both comparisons), meaning that the retention rates in the 24-month recall was statistically lower than the baseline only for no rubbing and slight rubbing groups.

Discussion

Based on the results of the present investigation, we need to reject the null hypothesis. The use of a vigorous application mode increased the retention rates of the adhesive tested. Since bonding is created by the impregnation of the dentin substrate by blends of resin monomers, the stability of the bonded interface relies on the creation of a compact and homogenous hybrid layer. In the etch-and-rinse strategy, after the preliminary etching to demineralize the substrate, bonding monomers impregnate the porous etched substrate [4, 24]. Thus, theoretically stable bonds can be achieved if the etched substrate is fully infiltrated by a strong polymer network to avoid different degrees of incomplete impregnation [25, 26] and exposure of collagen fibrils at the base of the hybrid layer [27]. However, it is of widespread knowledge that using manufacturers' protocols, resin monomers, mainly those with high molecular weight from simplified etch-and-rinse adhesives, have limited diffusion into the wet demineralized dentin [25, 26, 28], producing a gradient resin penetration with the highest concentration at the surface of the adhesive, lower concentration in the middle of the hybrid layer, and little resin in deepest portion of the demineralized zone [25, 26, 28].

As bond strength and durability seems to rely on the quality of the hybrid layer (i.e., on the proper impregnation of the dentin substrate), this study investigated the effect of a clinical approach supposed to improve monomer infiltration on the retention rates of an acetone-based simplified

Table 3 Number of evaluated restorations for each experimental group (no rubbing action (NR), slight rubbing action (SR), and vigorous rubbing action (VR)) classified in Alfa, Bravo and Charlie in each item according to the USPHS criteria

Time	↓	Baseline			6 months			12 months			24 months		
USPHS criteria		NR	SR	VR	NR	SR	VR	NR	SR	VR	NR	SR	VR
Retention	A	40	40	40	38	39	39	36	38	38	33	33	37
	C	–	–	–	2	1	1	4	2	2	7	7	3
Marginal discoloration	A	40	40	40	38	39	39	33	34	37	27	27	36
	B	–	–	–	–	–	–	3	4	1	6	6	1
	C	–	–	–	–	–	–	–	–	–	–	–	–
Secondary caries	A	40	40	40	38	39	39	36	38	38	33	33	37
	C	–		–	–	–	–	–	–	–	–	–	–
Post-operative sensitivity	A	37	36	37	35	36	36	36	38	38	33	33	37
	C	3	4	3	3	3	3	–	–	–	–	–	–

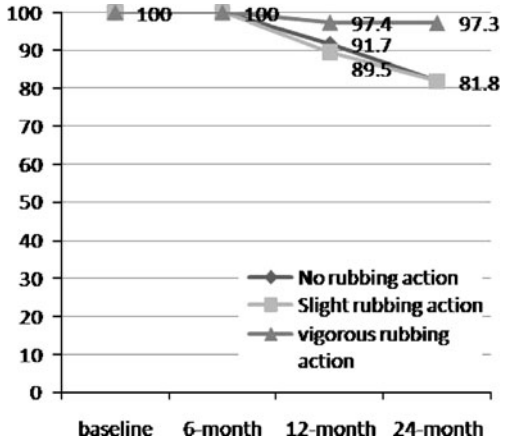

Fig. 2 Percentages of α score (percent) for the item marginal discoloration

etch-and-rinse adhesive. Previous laboratory findings [16, 17] have reported that vigorous rubbing application was capable to increase both the immediate and the 6-month bond strengths compared to slight and inactive applications. A similar trend was observed in the present clinical trial. Although the retention rates of the different application modes did not differ significantly after 24 months, the retention rate of vigorous rubbing group at 24 months (92.5%) was similar to baseline (100%), while they were lower than baseline for the other two techniques (no rubbing (82.5/100%) and slight rubbing (82.5/100%) groups). The range of retention rates found in this study for Prime & Bond NT is very close to what has been previously published after 1 or 2 years of clinical service [18, 29–31].

It is likely that mechanical pressure applied to the demineralized dentin surface during the rubbing action might compress the collagen network like a sponge. As the pressure is relieved, the compressed collagen expands, and the adhesive solution may be drawn into the collapsed

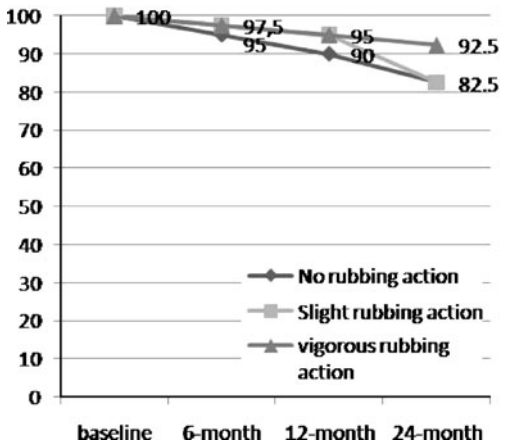

Fig. 3 Retention rates (percent)

collagen mesh [32]. Besides that, the vigorous rubbing action can increase the moieties kinetics and allow better monomer diffusion inward, while solvents diffuse outward. The removal of residual water or solvents increases the mechanical properties of the resin inside the hybrid layer [33–35], and consequently, the mechanical properties of the cured adhesive resin. It was already reported that the ultimate tensile strength of cured adhesives correlates positively with their corresponding resin–dentin bond strengths [36, 37].

The reduction of the amount of solvent retention within the polymer network may also account for reduced water sorption, solubility, and water diffusion coefficients [38]. Water sorption can reduce the durability of a polymer by causing polymer swelling and reduction of the frictional forces between the polymer chains consequently decreasing the mechanical properties of the cured polymer [39, 40].

However, one cannot deny that the vigorous application mode was not capable to completely prevent the degradation of the dentin bonding interface, as some restorations were lost throughout the study period. Although this technique seems to improve the resin impregnation into the demineralized collagen network, it cannot alter the nature of the adhesive, which continues to be intrinsically hydrophilic and therefore still prone to water sorption [6, 41]. Besides that, the vigorous application mode does not inactivate endogenous collagenolytic and gelatinolytic activities derived from acid-etched dentin, which is thought to be responsible for the progressive disintegration of the fibrillar collagen network [12, 13, 42, 43].

Marginal staining is thought to be one of the first clinical signs that a resin composite restoration is prone to failure. Marginal discoloration may be caused by the presence of excess or deficit filling materials at the margin, the formation of gaps [44, 45], and also by retention of microscopic pigments derived from colored beverages and food in the adhesive layer. This discoloration occurred at the enamel margins for the majority of the restorations, which seems to be a common finding in clinical studies [37, 39, 45–48]. It is likely that an over-etching of the enamel in the buccal surfaces would reduce the marginal discoloration observed in this study. The vigorous rubbing group showed less marginal discoloration than the other two application modes owing to the better quality of the polymer network as addressed earlier in this "Discussion" section. It is worth to point out, however, that marginal discoloration does not seem to jeopardize the longevity of the restoration to any significant level as it does not necessarily mean defective restorations. Most of the marginal staining observed in this study appeared to be superficial and could be easily removed by a new finishing and polishing procedure.

All restorations were scored according to the Cvar and Ryge criteria [19]. Meanwhile, it was addressed that the

evaluation of the clinical performance according to Ryge is not precise enough since there are many clinical variables simultaneously involved [49]. They state that, specifically, the clinical evaluation of resin-based restorations requires a more sensitive interpretation, which can easily be compared to other studies, including the habits of patients (such as bruxism) and the existing damage or location and size of the cavity [49]. This was the reason why new criteria were developed and recently published. Unfortunately, the present study was already ongoing by the time the Hickel's criteria were published [49], which prevented us from employing it.

Conclusion

The use of a vigorous application mode can be a viable clinical approach to improve the retention of restorations placed in non-carious cervical lesions using simplified etch-and-rinse adhesives.

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Conflict of interest The authors of this study declare they do not have proprietary, financial, professional, or other personal interest of any nature or kind in the products and/or companies that could be construed as influencing the position presented in this manuscript.

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