

IN VITRO EVALUATION OF MICRO-LEAKAGE OF A UNIVERSAL ADHESIVE USED WITH DIFFERENT ETCHING MODES IN COMPOMER RESTORATIONS

Betul Busra Ursavas¹, Tuğba Bezgin¹ , Mert Ocak², Burak Bilecenoglu³, Ayşe Işıl Orhan⁴

¹Department of Pediatric Dentistry, Faculty of Dentistry, Ankara University, Ankara, Turkey

²Department of Anatomy, Faculty of Dentistry, Ankara University, Ankara, Turkey

³Department of Anatomy, Faculty of Medicine, Ankara Medipol University, Ankara, Turkey

⁴Department of Pediatric Dentistry, Faculty of Dentistry, Ankara Yıldırım Beyazıt University, Ankara, Turkey

ABSTRACT

INTRODUCTION: In order to obtain a good prognosis in resin restorations, a strong adhesion not allowing leakage between tooth tissues and restoration should be provided. Effectiveness of universal adhesives on leakage in primary teeth compomer restorations has not been studied.

OBJECTIVES: The objective of this study was to evaluate sealing ability of a universal adhesive used with different etching modes after 1- and 3-year of artificial aging in compomer restorations of primary teeth.

MATERIAL AND METHODS: Sixty-three extracted mandibular primary second molars were used in the study. Class II box cavities (3 mm × 3 mm × 1.5 mm) were prepared in each tooth. Then, teeth were divided into three groups ($n = 21$), including total-etch, self-etch, and selective-etch modes. All teeth were restored with compomer (Dyract Extra, Dentsply; Konstanz, Germany) after universal adhesive (Prime & Bond Elect Universal, Dentsply; Milford, USA) application. Teeth were divided into three sub-groups ($n = 7$) with the following three different aging protocols: 24 h water storage, 1-year aging, and 3-year aging with chewing simulator (Esetron Chewing Simulator; Ankara, Turkey). 50% silver nitrate solution was applied as a tracer, and its' penetration was analyzed in mm³ using micro-computed tomography system (SkyScan 1275; Kontich, Belgium). Data was analyzed with Kruskal-Wallis H and Friedman tests.

RESULTS: One-year aging significantly increased the leakage in self-etch group of samples (0.197 ± 0.49 mm³) compared to the total-etch (0.001 ± 0.000 mm³) and selective-etch (0.001 ± 0.000 mm³) groups ($p < 0.05$). There was no difference between the groups at baseline and after the 3-year artificial aging ($p > 0.05$).

CONCLUSIONS: Self-etch mode of adhesive revealed higher micro-leakage levels at all examination intervals; however, the difference was only significant after the 1-year aging. It can be concluded that universal adhesives can be used with all etching modes in compomer restorations.

KEY WORDS: compomer, micro-leakage, universal adhesive.

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ADDRESS FOR CORRESPONDENCE: Tuğba Bezgin, Ankara Üniversitesi, Diş Hekimliği Fakültesi, Pedodonti Ana Bilim Dalı, Beşevler, Ankara 06500, Turkey, phone: + 90 312 296 56 60, fax: + 90 312 212 39 54, e-mail: cetintugba@yahoo.com

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INTRODUCTION

A significant progress has been achieved lately in the production of adhesive restorative materials that can bond onto hard tissues of the teeth. Any well-functioning restorative material should provide adhesion to dental hard tissues, support the remaining tooth tissue, provide full sealing, and edge compliance to prevent micro-leakage [1, 2]. Pediatric patients and parents increasingly demand restorations to be both esthetically pleasing and functional [3-5]. The use of tooth-colored restorative materials, together with adhesive systems, is often preferred as it allows for minimal cavity preparation in restoration of the primary teeth. However, despite the continuing developments in resin restorative materials, the desired retention times in such restorations have not been achieved [6-8]. Longer lasting restorations, especially in pediatric dentistry, are needed as increasingly younger children develop dental caries [9-11].

Durability of dental restorations is based on a stable adhesion between resin-based materials and dental tissues [12]. The formation of resin tags into micro-porosities created with acid etching, is an important mechanism for micro-mechanical adhesion of resin-based materials to enamel. Owing to this superior bonding, total-etch systems are still considered the gold standard. However, self-etch adhesives are quick and simple to apply, and are less technique-sensitive than total-etch systems. Short chair-time is an important factor while treating pediatric patients. In addition, it is believed that the risk of moisture contamination decreases with shorter treatment time [13, 14]. However, self-etch systems may prove insufficient adhesion on the enamel surface. Involving the application of acid, total-etch systems work well on the enamel, as it ensures thorough etch patterns resulting in sufficient bonding. Ideally, the acidic resin in self-etch systems should be able to remove the smear layer on the enamel and spread over the tissue. However, high inorganic content of the enamel buffers against acidic resin. Therefore, self-etch adhesives are less successful in enamel than they are in dentin [12].

The relatively new multi-mode universal adhesives are designed to be used via etch-and-rinse technique or self-etch procedure. Manufacturers claim that there is no difference in bonding quality. Selective-etching mode is also possible for this type of adhesive [12, 15].

OBJECTIVES

Although there are studies investigating bonding strength or micro-leakage of universal adhesives with different application techniques [16-19], the effectiveness of this relatively new adhesive on leakage in compomer restorations of primary teeth has not been studied, apart from their common utilization as a direct restorative material in primary teeth [6, 20]. In addition, structural

differences between primary and permanent teeth, such as the thick layer of prismless enamel in primary teeth, is also believed to affect the adhesion efficiency in primary teeth [21-23]. Therefore, the objective of this study was to evaluate the sealing ability of a universal adhesive used with different etching modes after 1- and 3-year artificial aging in class II compomer restorations of primary molar teeth. The null hypothesis tested in the study, included 1) no differences in micro-leakage values exist between different application modes; and 2) no differences in micro-leakage values exist between different aging times.

MATERIAL AND METHODS

Caries-free extracted primary second molar teeth were used in the study. Ethical approval was received from the Institutional Review Board of Ankara University, Faculty of Dentistry (No. 36290600/18), and an informed consents were obtained from participants and their parents. To determine differences between the study groups, power calculation was applied. The results indicated that a minimum of 63 teeth were required with effect size of 0.5, α significance level of 5% (0.05), and β significance level of 20% (0.20) to achieve an 80% power. Until the time of the experiment, the extracted teeth were stored in a glass jar with distilled water at 4°C, for a maximum of 6 months.

PREPARATION OF THE SAMPLES

Standardized class II box cavities (3 mm × 3 mm × 1.5 mm) were prepared in each tooth. Then, teeth were divided into three groups ($n = 21$), each assigned to different etching protocols: total-etch, self-etch, and selective-etch.

TOTAL-ETCH GROUP

Enamel and dentin surfaces of 21 teeth were etched with 37% phosphoric acid (Pentron Clinical; CA, USA) for 30 seconds and 15 seconds, respectively. They were then rinsed for 15 seconds, and dried by using an air spray for 5 seconds. Prime & Bond Elect Universal (Dentsply Caulk Milford; DE, USA) was applied as a thin layer as per manufacturer recommendations, using the disposable micro-brush applicator tip. The applied adhesive was agitated for 20 seconds with the micro-brush. The teeth were dried with a light air spray for 5 seconds to remove excess solvent, and polymerized for 10 seconds with a light source at a power of 1,500 mW/cm² LED (light emitting diode, Rii plus, SDI; Australia). Metal matrix bands (Kerr-Hawe; Bioggio, Switzerland) were placed on all teeth to restore the proximal walls. Compomer (Dyract Extra, Dentsply Caulk Milford; DE,

USA) was placed in homogeneous non-void layers of 1.5 mm using incremental technique. Each layer was polymerized by light curing unit for 20 seconds.

SELECTIVE-ETCH GROUP

After the preparation of 21 teeth cavities, enamel surfaces only were etched with 37% phosphoric acid for 30 seconds. Cavities were rinsed for 15 seconds, and dried by using an air spray for 5 seconds. The samples were then treated with a universal adhesive agent and compomer restorations, as described in the previous group.

SELF-ETCH GROUP

Following cavity preparation in 21 teeth, Prime & Bond Elect Universal was applied to the entire cavity in a thin layer. Cavities were dried using light air spray and polymerized by light curing unit for 10 seconds at a power of 1,500 mW/cm² LED (light emitting diode, Radii plus, SDI; Australia). Restorations were made as described for other groups.

The teeth were then polished with discs (Sof-Lex, 3M ESPE; St. Paul, MN, USA) and kept in distilled water in an etuve at 37°C for 24 hours. The teeth were divided into three sub-groups ($n = 7$) as per aging protocols: baseline (no aging, control group), 1-year aging, and

3-year aging, with a chewing simulator (Esetron Chewing Simulator, Ankara, Turkey).

AGING PROTOCOLS

To prevent leaks, the root tips of all samples were sealed with the same adhesive and a flowable composite resin (Filtek Supreme XT Flow, 3M ESPE; St. Paul MN, USA). For the chewing simulation, cylindrical plastic molds (3 cm in length, 2.5 cm in diameter) were cast to match with the dedicated slot of the chewing simulator to prepare acrylic blocks where the teeth would be embedded in for aging. The teeth were embedded at the center of acrylic blocks, with occlusal surfaces horizontal and cavities above the surface of acrylic blocks, using auto-polymerizing acrylic.

Twenty-one samples from two groups (42 samples in total) were dynamically loaded in a dual-axle chewing simulator (Esetron Chewing Simulator, Ankara, Turkey), and the simulation was controlled by a computer to mimic cycling chewing forces. Chewing forces of the posterior region were simulated applying a force of 50 N at a frequency of 1.6 Hz. The samples were subjected to 250,000 chewing cycles for each one-year aging period in intra-oral simulation environment, with temperatures ranging between 5°C to 55°C [24].

Table 1 shows the control and study groups. Seven samples from each group were aged worth of one year (21 samples in total) and three years (21 samples in total). Seven samples from each group were designated as the control group, and no aging simulation was done. Samples in the control group were immersed in a penetrating paint (50% silver nitrate solution) after being kept in etuve for 24 hours.

TABLE 1. Control and study groups

Groups	No aging (baseline)	1-year aging (250,000 cycles)	3-year aging (750,000 cycles)
Total-etch ($n = 21$)	7	7	7
Selective-etch ($n = 21$)	7	7	7
Self-etch ($n = 21$)	7	7	7

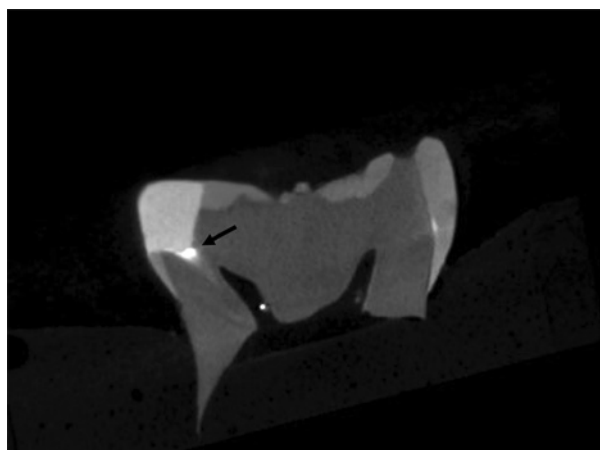


FIGURE 1. Representative image of micro-leakage evaluation obtained from micro-computed tomography. Black arrow indicates the silver nitrate-penetration

MICRO-LEAKAGE EVALUATION

The surface of each sample was painted with two layers of nail polish in different colors for each group, up to 1 mm away from the restoration edges. All samples were kept in the dark for 12 hours in a 50% w/w silver nitrate solution (AgNO₃) (Merckb 101510 Silver Nitrate; Darmstadt, Germany). They were then washed under running water for 2 minutes and kept in photo-enhancing solution (Dental X-Ray Developer, Medley, MDC; Turkey) under fluorescent light for 8 hours. The samples were cleaned with a toothbrush and water.

The volume of silver nitrate penetration was determined in mm³ with a micro-computed tomography system (Micro-CT, SkyScan 1275; Kontich, Belgium) to examine micro-leakage (Figure 1) [25]. The samples were scanned at 100 KVP, 100 mA power, 0.1 mm Cu filtering, and 0.2 step rotation. To reduce ring artifact, the sensor was air-calibrated before each scan. It took about 2 hours to scan each sample. Software packages NRecon (version 1.6.10.4, SkyScan) and CTAn (version

TABLE 2. Comparative evaluation of micro-leakage values according to aging protocols

Group	Kruskal Wallis <i>H</i> test						
	<i>n</i>	Mean	Median	SD	KWH	<i>p</i> -value	
Baseline							
Self-etch	7	0.159	0.001	0.409	3.89	0.142	–
Total-etch	7	0.001	0.001	0.000			
Selective-etch	7	0.001	0.001	0.000			
1-year aging							
Self-etch	7	0.197	0.001	0.490	6.62	0.037	1-2
Total-etch	7	0.047	0.001	0.122			1-3
Selective-etch	7	0.001	0.001	0.000			
3-year aging							
Self-etch	7	0.314	0.060	0.438	3.4	0.183	–
Total-etch	7	0.047	0.001	0.174			
Selective-etch	7	0.021	0.001	0.052			

1.16.1.0, SkyScan) were used to image the samples and perform quantitative measurements. Using NRecon software, radiological artifacts were fixed, and the images collected from the scanner was reconstructed to show two-dimensional slices. For three-dimensional volumetric imaging and measuring the volume of micro-leakage, CTAn software was employed. Two experienced researchers independently assessed each section. When there was a disagreement between the researchers, parts were re-examined until a consensus was reached.

STATISTICAL ANALYSIS

Data analysis was performed using SPSS 21 program. Kruskal-Wallis *H* test was used for comparisons between the groups, and Friedman test was used to calculate group-time interaction differences. The study adopted a significance level of 0.05.

RESULTS

Table 2 shows leakage values derived from penetration volumes of AgNO₃ in the 63 samples investigated in the study. There was no statistically significant difference between the groups at baseline (*p* > 0.05). One-year aging caused significant increase in leakage in the self-etch group (0.197 ± 0.49 mm³) compared to the total-etch (0.001 ± 0.000 mm³) and the selective-etch (0.001 ± 0.000 mm³) groups (*p* < 0.05). There was no difference between the groups at baseline and after 3-year artificial aging (*p* > 0.05) (Table 2).

For each group, leakage values were higher after one-year and three-year aging compared to baseline. However, the increase was not statistically significant (*p* > 0.05) (Tables 3-5).

DISCUSSION

The first null hypothesis that anticipated no significant difference in micro-leakage values between different etching modes was rejected, as the self-etch mode of the universal adhesive revealed statistically higher micro-leakage values after 1-year aging. However, in the 3-year aging group, there was no statistically significant difference between various etching modes. The second null hypothesis that anticipated no significant difference in micro-leakage values among aging times was accepted, because aging did not affect micro-leakage values statistically when groups were evaluated separately.

Due to a lack of data assessing the degree of micro-leakage in compomers used in combination with universal adhesives in primary teeth, comparisons may be drawn with previous *in vitro* and *in vivo* studies using permanent teeth. In several *in vitro* studies, etch-and-rinse mode was found to be better than self-etch mode in terms of bonding strength, where different universal adhesives were used together with composite resins [16-18, 26]. In addition, less marginal staining findings were obtained with etch-and-rinse mode in *in vivo* studies, indicating less leakage in long-term follow-up [27, 28]. This can be explained by the use of phosphoric acid in etch-and-rinse method, which enables better enamel demineralization. This promotes the increase of surface area and wettability, hence increasing micro-mechanical adaptation. However, mild acidity of universal adhesives (pH ≥ 2.5) may reduce enamel bonding when used in self-etch mode [19].

Similar to our findings, Signore *et al.* [19] found statistically significantly more micro-leakage in self-etch mode in a study, where etch-and-rinse and self-etch modes of a universal adhesive in short-term time were compared. However, our study found no difference be-

TABLE 3. Micro-leakage values in mm³ according to aging protocols in total-etch group

Group	Total-etch						Friedman test
	<i>n</i>	Mean	Median	Minimum	Maximum	SD	<i>p</i> -value
Baseline	7	0.001	0.001	0.001	0.001	0.000	0.607
1-year aging	7	0.047	0.001	0.001	0.325	0.122	
3-year aging	7	0.067	0.001	0.001	0.462	0.174	

TABLE 4. Micro-leakage values in mm³ according to aging protocols in selective-etch group

Group	Selective-etch						Friedman test
	<i>n</i>	Mean	Median	Minimum	Maximum	SD	<i>p</i> -value
Baseline	7	0.001	0.001	0.001	0.001	0.000	0.135
1-year aging	7	0.001	0.001	0.001	0.001	0.000	
3-year aging	7	0.021	0.001	0.001	0.139	0.052	

TABLE 5. Micro-leakage values in mm³ according to aging protocols in self-etch group

Group	Self-etch						Friedman test
	<i>n</i>	Mean	Median	Minimum	Maximum	SD	<i>p</i> -value
Baseline	7	0.159	0.001	0.001	1.085	0.409	0.623
1-year aging	7	0.197	0.001	0.001	1.308	0.490	
3-year aging	7	0.314	0.060	0.001	1.089	0.438	

tween etching mode groups in long-term aging. This may be due to the fact that aging initiates leakage in etch-and-rinse and selective-etch groups as well. In line with the results of the present study, Marchesi *et al.* [29] reported that etch-and-rinse mode of a universal adhesive demonstrated better results in short-term evaluation compared to self-etch mode; however, long-term aging resulted in increased leakage expression. The authors explained these results with the fact that universal adhesives contain functional monomers, which help establish a long-term successful chemical bond with dentine collagen scaffold. However, when phosphoric acid is applied to the dentin as in total-etch mode, partially infiltrated collagens would be susceptible to degradation by matrix metalloproteinases in long-term [30, 31]. In this study, although not statistically significant, the selective-etch mode showed less micro-leakage rates than the total-etch group. Better results obtained in this group may be explained by the fact that only the enamel, not the dentin, was etched. Similarly, Antoniazzi *et al.* [17] recommended the selective etching mode in primary teeth in testing micro-shear bond strength of a universal adhesive.

To the best of our knowledge, the only study examining leakage in primary teeth using universal adhesives is the study by Lenzi *et al.* [16]. Even though the researchers used composite resin for restoration, the study presented a comparative evaluation of the bonding per-

formance and nano-leakage values of a universal adhesive used with different etching modes. The authors did not adopt an aging protocol in this *in vitro* study. The amount of nano-leakage after 24-hour water storage for a universal adhesive in self-etch mode was higher than in etch-and-rinse and selective-etch groups, which also showed the lowest bond strength values. Whereas in our study, although the self-etch group demonstrated more micro-leakage after 24 hours in the control group (baseline), there was no statistically significant difference between the groups. This can be explained by the use of different methodology, adhesive agents, and restorative materials.

Clinically, micro-leakage occurs with fatigue. Fatigue is a form of failure in structures subjected to dynamic loads, and is affected by stress, restoration design, condition and configuration of the restoration surface, and environmental factors. Fatigue that occurs as a result of repeated forces causes micro-cracks and adhesive failure on the surface between the tooth and the restoration [32]. At the same time, restorations in the mouth are constantly exposed to changes in temperature and pH. For this reason, *in vitro* studies should include aging protocols that would best simulate the oral cavity. For this purpose, a proper simulation that imitates chewing system should be able to apply predetermined forces to the material, with a certain number of repetitions while moving in a single-

or multiple-axis. The present study utilized a dual-axis chewing simulator for aging in order to assume not only cyclic loading and wet conditions of the oral cavity, but also the thermal changes that these restorations are subjected during daily function [33].

In this study, micro-leakage was evaluated with a micro-CT scan. Micro-CT is preferred because of its' ability to measure the sealing capabilities of dental restorative materials three-dimensionally. Silver nitrate salts, which are radio-opaque and have small ion size, provide satisfactory results with a good penetration capacity for assessing loss of marginal seal [25, 34]. Micro-CT scan has been reported to have a good sensitivity to evaluate pattern of AgNO₃ infiltration at the resin-tooth interface [35].

This study presents a variety of limitations. Our results were obtained under *in vitro* conditions, which enable direct access to the prepared tooth samples. Moreover, a greater sample size would result in more differentiation in leakage analysis. As aging protocol and micro-CT analysis are expensive and time-consuming, the sample size was relatively small in this study. In addition, the longest aging period in this study was 3 years. Higher aging durations would have resulted in more statistically significant differences. There is a need for other *in vitro* studies comparatively evaluating bond strength and micro-leakage total-etch, self-etch, and selective-etch modes of universal adhesives in compomer restorations, and also long-term *in vivo* studies for clinical results.

CONCLUSIONS

The self-etch mode of the universal adhesive showed higher micro-leakage levels at all examination intervals. The addition of an etching step (total-etch or selective-etch) resulted in a significantly less short-term micro-leakage. However, the difference was only significant after 1-year aging. After the long-term aging protocol, with the increase of leakage in etched groups, no statistically significant difference was found between the etching protocols. It can be concluded that universal adhesives are reliable for working under different etching modes in compomer restorations in pediatric dental patients.

ETHICAL APPROVAL

The study has obtained an approval of bioethics committee, with approval number of 36290600/18.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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