

Original Article**Effect of pre-etching on sealing ability of two current self-etching adhesives.***K. Khosravi* , M. Mousavi****ABSTRACT**

Background: We evaluated the effect of phosphoric acid etching on microleakage of two current self-etching adhesives on enamel margins in comparison to a conventional total-etch system.

Methods: Sixty buccal class V cavities were made at the cemento-enamel junction with beveled enamel margins of extracted human premolar teeth and randomly divided into five groups (12 specimens in each group). Group 1 was applying with Clearfil SE bond, Group 2 with 35% phosphoric acid etching of enamel margins plus Clearfil SE bond, Group 3 with I bond, Group 4 with 35% phosphoric acid etching of enamel margins plus I bond and Group 5 with Scotchbond multi-purpose. All groups restored with a composite resins. After 24 hours storage with 100% humidity, the samples were thermocycled, immersed in a dye solution and sectioned buccoligually and enamel margins microleakage were evaluated on a scale of 0 to 2.

Results: The differences between Groups 1 & 3 and Groups 3 & 4 were significant ($P < 0.05$) but no significant differences between Groups 1 & 2 or 1 & 5 were observed.

Conclusion: The findings suggest that all-in-one adhesive systems need pre-etching enamel margins with phosphoric acid for effectively seal.

Key words: Self-Etching Adhesives, Microleakage, Enamel, Total-Etch system

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New adhesive systems continually have been improved. However, one of the great challenges in restorative dentistry is obtaining an effective seal of the tooth / restoration interface¹. If this could be achieved, it would be possible to minimize microleakage and its consequences, such as postoperative sensitivity, pulp inflammation and caries recurrence, which are known to jeopardize the clinical longevity of the restoration^{2,3}. Buonocore introduced acid etching technique at 1994, then reliable bonding of composite resins to the enamel surface became clinically possible⁴. Phosphoric acid creates a porous surface that is penetrated by a low-viscosity resin bonding agent. After polymerization of bonding agent, micromechanical interlocking makes a durable attachment to the enamel surface is achieved⁵. Formation of tag-like resin extensions into the interprismatic and intercrystallite enamel microprossities has been considered as the pre-

dominant mechanism of bonding resin composites to the phosphoric acid-etched enamel^{5, 6}. Bond strength of composite resins to acid-etched enamel are typically in the range of 20-25 Mpa and provide routinely successful retention of resin composite for a variety of clinical applications⁷. Enamel pretreatment could be simplified by self-etching adhesives. They rely on simultaneous etching of dentin and enamel with weaker acids than the traditional phosphoric acid (30-40%) etchants. These alternative acids have not gained popular clinical acceptance because their effect on enamel is not visibly controllable by the dentist⁸. Scanning electron microscopy (SEM) studies indicate that enamel etching pattern caused by self-etching adhesives is less deep and appears less retentive compared to etching pattern of phosphoric acid treatment⁹⁻¹¹. Shallow and less defined etching pattern considered as deficient penetration of self-etching primer into suggested

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that the etching effect is an important factor in the bonding of self-etching adhesives (AD Bond) to enamel [12]. Pradelle et al. also reported that it may be useful to etch enamel margin with an acidic solution before using self-etching adhesives (Opti bond Solo)¹³.

In many studies, self-etching adhesives could seal enamel margins effectively^{14,15}. However, in other studies, self-etching adhesives showed greater scales of microleakage than adhesives using conventional phosphoric acid as etchant^{13,16-18}. There is some concern that manufacturers are sacrificing enamel bond strength for self-etching primers¹⁹. Thus, the clinical use of self-etching adhesives in enamel-to-resin bonding must be confirmed by long-term clinical trials⁷.

The purpose of this invitro study was to evaluate effect of phosphoric acid etching on microleakage of two self-etching adhesives, "Clearfil SE bond" and "I bond" on enamel margins in comparison to "Scotchbond Multi-purpose".

Materials and Methods

Sixty sound human premolars extracted for orthodontic reasons were selected. They stored in a 0.1% thymol solution at room temperature for up to 4 weeks after extraction, consecutively debrided with a slurry of pumice flour and examined to ensure that they were defects free.

In each tooth, buccal V-shaped cavity (4mm wide ×3mm high × 2mm deep) was made with adhesives a cylindrical carbide bur (D&Z 008, Germany) using a turbine with water coolant. The experimental cavity was located at the cementoenamel junction, with two-third of cavity margin in enamel and one-third in root cementum.

Preparation depth was determined with periodontal probe, while an electronic caliper (Mitutoyo, Tokyo, Japan) was used to verify width and height. Occlusal enamel margin was beveled (1.0mm wide, 45 degree) with a flame-shaped diamond bur (D&Z, Germany) Using a turbine with water coolant.

Using Van Meerbeek's classification, the various dentin bonding systems are represented by: Clearfil SE Bond (CSEB): a self-etching adhesives ; i bond (IB): a self-etching priming-bonding; and the control DBS Scotchbond Multi-purpose (SBMP): a three-step total-etch adhesive. RBC (Filtec Z₁₀₀, A₂) were used as filling material.

All teeth were randomly assigned to five groups of 12 teeth . The bonding protocol for each group was as follows:

Group 1: CSEB-primer was applied with brush, left undisturbed 20 seconds, air dried mildly. CSEB-bonding was applied with brush, air thinned gently and light cured 10 seconds.

Group 2: Occlusal enamel margin was etched with 35% phosphoric acid (3M, USA) 15 seconds, rinsed 15 seconds, and dried 2 seconds with filtered compressed air. Then, CSEB was applied as like group 1.

Group 3: IB was applied in 3 layers (start with enamel), left undisturbed 30 seconds, gentle air flowed until now movement, additional dried until glossy surface and light cured for 20 seconds.

Group 4: Occlusal enamel margin was etched with 35% phosphoric acid (3M, USA) 15 seconds, rinsed 15 seconds, dried 2 seconds with filtered compressed air. IB was then used as like group 3.

Group 5: phosphoric acid(35%) was applied on enamel and dentin 15 seconds, rinsed 15 seconds, and dried 2 seconds. SBMP-primer was applied with brush and air thinned 5 seconds. Then, SBMP-bonding was applied with brush and light cured for 10 seconds.

The adhesives and resin-based composites are listed in Table 1.

Table 1: Adhesive and resin-based composite used in the study

<i>Products</i>	<i>Composition</i>	<i>Lot number</i>
<i>Clearfil SE bond</i> (Kuraray, okayama, Japan)	Primer Water; MPD; HEMA; Hydrophilic dimethacrylate; dl-Camphoquinone; N, N-Diethanol-P-toluidine	00434A
	Bond MDP; Bis-GMA; HEMA; Hydrophobic dimethacrylate; dl-Camphorquinone;N,N-Diethanol-P-toluidine; si- lanated colloidal silica	00593A
i Bond (Heraeus Kulzer, Hanau, Ger- many)	Water; acetone; 4-META; Diurethandimethacrylate; 2-Hydroxyethyl methacrylate; 2-(n-Butoxy)ethyl-4-dimethylamino benzoat; 2,3-Bornan dion butyl-hydroxy-touol; Glutaralde- hyde	010062
Scotchbond Multi-purpose (3M, St.Paul, USA)	Etchant 35% phosphoric acid	200 21212 (2GE)
	Primer HEMA; Polyalkenoic aid Copolymer; water	200 21212 (2AB)
Filtek Z100 composite (3M, St.Paul, USA)	Adhesive Bis-GMA;HEMA Bis-GMA; TEGDMA;	200 21212 (2MX)
	Silica , zirconium (66%, 0.01-3.5 μ m)	20040403

Bis-GMA: Bisphenol-glycidyl methacrylate; HEMA: 2-hydroxy ethyl methacrylate, MDP: 10-methacryloxy decyl dihydrogen phosphate; 4-META: Tetra-methacryloxy ethylpyrophosphate.

The composite resins was placed in three increments and each increment was polymerized for 40 seconds using Visilux (3M, USA) curing – light unit in same conditions under indirect light of sun. The power density was monitored with a Dementron radiometer (Demetron Research Corporation, USA) which reading was in the range of 450-500 mw/cm². After the specimens were stored in an environment of 100% humidity for 24 hours, final finishing and polishing were done with medium and fine Sof-lex disks (3M, USA). The specimens were thermo cycled for 1000 cycles between 5 and 55 ml water baths held and 1 minute dwell time. They were sealed using a layer of sticky wax, then all surfaces of the teeth were sealed with two layer of nail varnish, except for one mm around occlusal enamel margins. The specimens were immersed in a 50% silver nitrate

solution and kept in complete darkness for 6 hours and thoroughly rinsed in tap water and immersed for 6 hours in photographic developing solution under fluorescent light to facilitate reduction of silver ions to metallic silver.

The teeth were rinsed and the nail varnish removed with acetone. The samples were embedded in a cold curing epoxy resin (Epofix, USA) and sectioned buccolingually through the center of restoration using diamond discs (Blade XL 12235) at a 250 rpm speed in a Labcut Machine under constant water irrigation. Dye penetration was evaluated through microscope at $\times 50$ magnifications and scored as follow:

0=No dye penetration 1=Dye penetration until half of enamel wall 2=Dye penetration more than half of enamel wall.

A scanned digital image was prepared from each section (Figure 1 & 2), with $\times 40$ magnification (Hewlet packard, USA).

Average values were obtained and results were analyzed using Kruskal-Wallis test, followed by multiple comparisons using Mann-Whitney U test, when necessary.

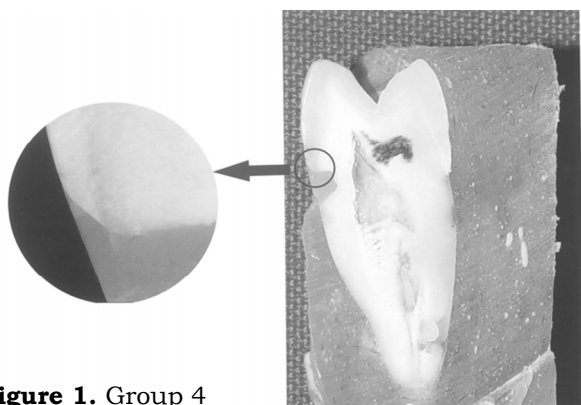


Figure 1. Group 4

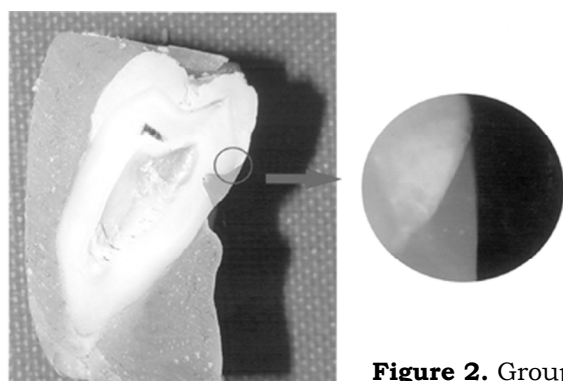


Figure 2. Group 3

Results

The data of micro leakage and the mean of dye penetration scores on occlusal enamel margins are shown in Table 2.

Table 2: The frequency and mean value of micro leakage scores at the enamel margins.

Groups	Microleakage score		
	0	1	2
group1	8	1	3
group2	10	2	0
group3	1	7	4
group4	8	3	1
group5	12	0	0

Kruskal-Wallis test showed significant differences among 5 groups ($P < 0.001$). Mann-Whitney U test showed significant differences between specimens in groups 1 & 3 or between specimens in groups 3 & 4 ($P < 0.05$).

Mann-Whitney U test revealed no statistically significant difference between group 1 and 5 or between specimens in groups 1 & 2, 2 & 5 or 4 & 5 ($P > 0.05$).

Discussion

Sealing performance of adhesive resins is likely to be affected by cavity configuration (C factor), dimensional changes of restorative material such as polymerization shrinkage or thermal/hygroscopic expansion, occlusal stresses and the bonding capacity of resins. A micro leakage test, combined with thermo cycling is a useful In-vitro method to assess sealing performance, especially for large number of thermal cycles through accelerating aging effect to predict the In-vivo longevity of the restorations, since thermal stresses and water exposure continuously work on the restorations in these circumstances¹². Barnes et al reported that In-vitro studies are more prone to dye penetration at the RBC/tooth interface than In-vivo studies, therefore resulting in greater leakage²⁰.

Ferrari et al concluded that In-vitro and In-vivo tests give very similar results²¹.

The silver nitrate method on measuring micro leakage is an acceptable technique. It is however, a sever test because the silver ion has smaller size than a typical bacteria and thus more penetrative. Therefore, it may be assumed that any system that prevents the leakage of silver ion would also prevent leakage of bacteria²².

SBMP system (Group 5) showed the best results on enamel because of its good bonding efficacy^{19, 23,24}, sufficient enamel etching ability of 35% phosphoric acid^{24,25} and presence of polyalkenoic acid copolymers in SBMP-primer that lead to bonding durability in wet environment²⁶.

Moreover, repeated formation of calcium-polyalkenoic acid complex cause continuous stress relaxation²⁷. So, SBMP has been used in many studies as a reference adhesive for enamel micro leakage test^{14, 15, 28, 29}.

CSEB (Group 1) also showed acceptable results on enamel margins. The PH - value of CSEB is 1.9¹⁹. Despite the less distinct pattern of enamel

etching observed in scanning electron microscopy, there is no separation between adhesive resin and enamel²⁴. Further, CSEB contains a functional monomer (MDP) that may also bind to calcium. Recently, Yoshida et al reported that MDP tightly adheres to hydroxyapatite and its calcium salt hardly dissolves in water¹². It is proved that CSEB can bond to enamel effectively^{19, 30, 31}. CSEB also contains silanated colloidal silica nano-fillers, which increases cross linking, strength its resin matrix²⁶ and decreases its polymerization shrinkage³², that may be contributing factors for reducing micro leakage.

In contrast, IB can not prevent micro leakage on enamel margins in comparison with SBMP. PH - value of IB is 2.1, that might explain why the etching pattern of IB is less distinct than that of CSEB and why its enamel micro tensile bond strength is weaker than CSEB¹². Moreover, IB contains 4-META that may bond by a chelating reaction to calcium ions in apatite³³.

In several studies, 4-META containing adhesives showed lower enamel bonding capacity than MDP containing adhesives^{12, 19} but the differences in bonding capacity to tooth substrates between 4-META and MDP is unclear and not main point of this study. Finally, the last factor that may play role in greater scores of micro leakage for IB than that of CSEB is lack of filler in its

composition which lead to greater polymerization shrinkage of adhesive.

Effect of phosphoric acid:

In this study, pre-etching of enamel margins before CSEB application improved micro leakage results not significantly. It seems that CSEB can effectively seal enamel margins because of the reasons mentioned previously and its good bonding efficacy^{19, 30, 31}. It is considered that is no necessity for pre-etching enamel before using CSEB.

In contrast, separated etching on enamel margins before IB application improved marginal seal significantly. It seems that IB can not seal enamel margins effectively because of previous reasons, importantly insufficient etching ability. In other hand, it is proved, when both etchant and self - etching primer were used, the etching pattern was more distinct than the etchant was used alone¹¹.

We concluded that separated etching on enamel margins with 35% phosphoric acid is necessary to prevent micro leakage.

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