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Effect of Phosphoric Acid Etching of Enamel Margins on the Microleakage of a Simplified All-in-One and a Self-etch Adhesive System

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Clinical Relevance

This study evaluates the effect of phosphoric acid etching of enamel margins on the microleakage of a simplified all-in-one and a self-etching adhesive system in comparison to a conventional total-etch system.

SUMMARY

Sixty buccal Class V cavities with beveled enamel margins were made at the cemento-enamel junction of extracted human premolars and randomly divided into five groups of 12 specimens. Clearfil SE bond was applied to Group 1; Group 2 had 35% phosphoric acid etching of the enamel margins plus Clearfil SE Bond; Group 3 was administered iBond; Group 4 had 35% phosphoric acid etching of the enamel margins plus iBond and Scotchbond Multi-Purpose was used on Group 5. All the groups were restored with a resin composite. After 24 hours of storage in 100% humidity, the samples were thermocycled, immersed in a dye solution and sectioned buccolingually and microleakage of the enamel margins was evaluated on a scale of 0 to 2. Differences among Groups 3 and 4 and Groups 3 and 5 were significant (*p*<0.05); however, no statistically significant differences were found between the other groups (*p*>0.05). The current study shows that simplified all-in-one adhesive systems need

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pre-etching of the enamel margins with phosphoric acid for an effective seal.

INTRODUCTION

New adhesive systems are continually being improved. However, one of the greatest challenges in restorative dentistry is obtaining an effective seal of the tooth/restoration interface.[1] If an effective seal could be achieved, it would be possible to minimize microleakage and its consequences, such as postoperative sensitivity, pulp inflammation and caries recurrence, all of which are known to jeopardize the clinical longevity of the restoration.[2-3] The clinically reliable bonding of resin composites to the enamel surface became possible due to the acid-etching technique introduced by Buonocore in 1955.[4] Phosphoric acid etching creates microporosities in enamel and increases the enamel surface area and wettability and, consequently, the spread and penetration of resin adhesives.[5] Phosphoric acid treatment creates a porous surface that is penetrated by a low-viscosity resin bonding agent. After polymerization of the bonding agent, a durable attachment to the enamel surface is achieved by micromechanical interlocking.[6] The formation of tag-like resin extensions into interprismatic and intercrystallite enamel microporosities has been considered as the predominant mechanism of bonding resin composites to phosphoric acid-etched enamel.[6-7] The bond strength of resin composites to acid-etched enamel is typically in the range of 20-25 Mpa and provides routinely successful retention of resin composites for a variety of clinical applications.[8] Enamel pre-treatment could be simplified by the use of self-etching and all-in-one adhesive systems. These adhesive systems rely on simultaneous etching of dentin and enamel with weaker acids than the traditional 30%-40% phosphoric acid etchants. These alternative acids have not gained popular clinical acceptance, because their effect on enamel is not visibly controllable by the dentist.[9] Scanning electron microscopy (SEM) studies indicate that an enamel etching pattern caused by self-etching adhesives is not as deep and appears to be less retentive compared with the etching pattern resulting from phosphoric acid treatment.[10-12] A shallow, less defined etching pattern is considered to be the result of deficient penetration of the self-etching primer into the enamel surface.[12] The use of phosphoric acid prior to the self-etching adhesive application was considered in order to deepen demineralization in enamel.[5] Some studies showed that self-etching adhesives are not sufficiently strong to decalcify the core of enamel prisms, thus creating a thin layer between the resin composite and thin lamina-like resin extensions, which may adversely affect the durability of enamel adhesion.[13-14] The insufficient etching of self-etching adhesives can be attributed to the diminishing abilities of decalcification due to total inactivation of the acid in contact with the enamel surface.[5] Abo and others[15] suggested that the etching effect is an important factor in the bonding of self-etching adhesives (AD Bond) to enamel. To activate an ideal etching pattern in intact enamel and the dissolution of its surface, it may be useful to etch the enamel margin with an acidic solution before using self-etching adhesives.[16] Several studies have shown that self-etching adhesives could seal enamel margins effectively.[17-18] Some studies reported that self-etching primer systems performed comparably to that of total-etch adhesive systems.[19] However, in other studies, self-etching adhesives showed greater scales of microleakage than adhesives using conventional phosphoric acid as the etchant.[13,16,20-22] There is some concern that the manufacturers are sacrificing enamel bond strength for self-etching primers.[23] One study reported that additional etching of enamel cavity margins resulted in an improved marginal adaptation on the enamel side, but this was not critical for the overall performance of the restorations.[24] Thus, the clinical use of self-etching adhesives to enamel for resin bonding still has to be confirmed by long-term clinical studies.[8]

The purpose of this *in vitro* study was to evaluate the effect of phosphoric acid etching of enamel margins on the microleakage of a self-etching adhesive (Clearfil SE Bond: CSEB) and a simplified all-in-one adhesive system (iBond: IB) in comparison with that of a total-etch adhesive system (Scotchbond Multi-Purpose: SBMP).

METHODS AND MATERIALS

Sixty sound human premolars extracted for orthodontic reasons were selected. The teeth were stored in a 0.1% thymol solution at room temperature for up to four weeks after extraction and were consecutively debrided with slurry of pumice flour and examined to ensure that they were free of defects. In each tooth, a buccal V-shaped cavity (4 mm wide, 3 mm high and 2 mm deep) was made with 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-thirds of the cavity margin in enamel and one-third in root cementum. Preparation depth was determined with a periodontal probe, while an electronic caliper (Mitutoyo, Tokyo, Japan) was used to verify the width and height. An occlusal enamel margin was beveled (1.0 mm wide, 45 degrees) with a flame-shaped diamond bur (D&Z), using a turbine with water coolant. All the 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 a brush, left undisturbed 20 seconds and mildly air-dried. CSEB-bonding was applied with a brush, gently air thinned and light cured for 10 seconds.

Table 1: *Adhesive and Resin-based Composites Used in the Study*

Products	Manufacturer	Composition
Clearfil SE Bond	Kuraray, Tokyo, Japan	Primer: Water; HEMA; Hydrophilicdimethacrylate; dl Camphoquinone; N, N-Diethanol-P-toluidine Bond: MDP; BisGMA; HEMA; Hydrophobic bond: dimethacrylate; dlCamphorquinone; N,N-Diethanol-P-toluidine; silanated colloidal silica
iBond	Heraeus Kulzer, Hanau, Germany	Water; acetone; 4META;Diurethandimethacrylate; 2-Hydroxyethyl methacrylate; 2-(n-Butoxy)ethlyl-4-dimethylamino benzoat; 2,3-Bornan dion butyl-hydroxy-touol; Glutaraldehyde
Scotchbond Multi-purpose	3M ESPE, St Paul, MN, USA	Etchant: 35% phosphoric acid Primer:HEMA; Polyalkenoic aid Copolymer; water Adhesive: Bis-GMA; HEMABis-GMA; TEGDMA;
Filtek Z100 Composite	3M ESPE, St Paul, MN, USA	Silica, zyrconium (66%, 0.01-3.5 μm)

Abbreviations: Bis-GMA: Bisphenol-glycidyl methacrylate; HEMA: 2-hydroxy ethyl methacrylate, 10MDP: 10-methacryloxy decyl dihydrogen phosphate; 4-META: 4-mathacryloxyethyl trimellitate anhydride.

Group 2: The occlusal enamel margin was etched with 35% phosphoric acid (3M ESPE, St Paul, MN, USA) for 15 seconds, rinsed for 15 seconds and dried for two seconds with filtered compressed air. CSEB was then applied, similar to Group 1.

Group 3: IB was applied in three layers (starting with the enamel), left undisturbed for 30 seconds, gently air flowed until no movement, additionally dried until a glossy surface and light cured for 20 seconds.

Group 4: The occlusal enamel margin was etched with 35% phosphoric acid (3M ESPE) for 15 seconds, rinsed for 15 seconds and dried for two seconds with filtered compressed air. IB was then used as in Group 3.

Group 5: Thirty-five percent phosphoric acid was applied onto the enamel and dentin for 15 seconds, rinsed for 15 seconds and dried for two seconds. SBMP-primer was applied with a brush and air thinned for five seconds. SBMP-bonding was then applied with a brush and light cured for 10 seconds.

The materials used in the current study and their characteristics are listed in Table 1. The resin composite (Filtek Z_{100}, 3M ESPE) was placed in three increments, and each increment was polymerized for 40 seconds using a Visilux (3M ESPE) curing-light unit in the same conditions under indirect sunlight. The power density was monitored with a Demetron radiometer (Demetron Research Corporation, Danbury, CT, USA), with the reading being in the range of 450-500 mw/cm^2. After the specimens were stored in 100% humidity for 24 hours, the final finishing and polishing were done with medium and fine Sof-Lex disks (3M ESPE). The specimens were thermocycled for 1000 cycles between 5°C and 55°C water baths and a one-minute dwell time. The specimens were then sealed using a layer of sticky wax, and all surfaces of the teeth were sealed with two layers of varnish, except for 1 mm around the occlusal enamel margins. The specimens were immersed in a 50% silver nitrate solution and kept in complete darkness for six hours. They were then thoroughly rinsed in tap water and immersed for six hours in photographic developing solution under fluorescent light to facilitate reduction of silver ions to metallic silver. The teeth were then rinsed and the nail varnish removed with acetone. The samples were embedded in a cold curing epoxy resin (Epofix, Electron Microscopy Sciences, Hatfield, PA, USA) and sectioned buccolingually through the center of the restoration using diamond discs (Blade XL 12235) at a 250 rpm speed in a Labcut Machine under constant water irrigation, and dye penetration along the occlusal enamel margins was evaluated under a measuring microscope at 50x magnification. The scoring method was:

0 = No dye penetration; 1 = Dye penetration up to half the enamel wall; 2 = Dye penetration of more than half of the enamel wall.

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

RESULTS

Microleakage and mean dye penetration data scores for each group on the occlusal enamel margins are shown in Table 2. Statistical analysis using the Kruskal-Wallis test showed significant differences among the five groups ($p<0.001$). The Mann-Whitney U-test also showed that there were significant differences between specimens in Groups 3 and 4 or between specimens in Groups 3 and 5 ($p<0.05$). The Mann-Whitney U-test revealed no statistically significant differences between Groups 1 and 2, 1 and 5, 2 and 5 or between specimens in Groups 4 and 5 ($p>0.05$).

DISCUSSION

The sealing performance of adhesive resins is affected by cavity configuration (C factor), dimensional changes of the restorative material due to polymerization shrinkage and thermal or hygroscopic expansion, occlusal stresses and the bonding ability and characteristics of resins.[15,25] A microleakage test, combined with thermocycling, is a useful *in vitro* method to assess sealing performance.[15] The *in vitro* use of a large number of

Table 2: *Frequency and Mean Value of Microleakage Scores at Enamel Margins*

Group*	Microleakage Scores**			Mean
	0	1	2	
1	8	1	3	0.5
2	10	2	0	0.16
3	1	7	4	1.25
4	8	3	1	0.41
5	12	0	0	0.00

**Group 1: Clearfil SE Bond; Group 2: Clearfil SE Bond + 35% phosphoric acid; Group 3: iBond; Group 4: iBond + 35% phosphoric acid; Group 5: Scotchbond Multi-Purpose.*
***Score 0: No dye penetration; Score 1: Dye penetration up to half of the enamel wall; Score 2: Dye penetration more than half of the enamel wall.*

thermal cycles can simulate the conditions of restorations in the oral environment in terms of predicting the *in vivo* performance of the restorations, since thermal stresses and water exposure continuously work on the restorations.[15] Barnes and others[26] reported that *in vitro* studies are more prone to dye penetration at the resin composite/tooth interface than *in vivo* studies, therefore, resulting in greater leakage. However, Ferrari and others[27] concluded that *in vitro* and *in vivo* tests provide very similar results. The silver nitrate method of measuring microleakage is an acceptable technique. It is, however, a severe test, because silver ions are smaller than a typical bacteria and thus more penetrative. Therefore, it may be assumed that any system that prevents leakage of the silver ion would also prevent bacteria leakage.[28]

The SBMP system (Group 5) showed the best results on enamel, because of its good bonding efficacy,[23,29-30] sufficient enamel etching ability of 35% phosphoric acid and the presence of polyalkenoic acid copolymers in SBMP-primer that lead to bonding durability in a wet environment.[30-31] Moreover, repeated formation of the calcium–polyalkenoic acid complex causes continuous stress relaxation.[32] Therefore, SBMP has been used in many studies as a reference adhesive for the enamel microleakage test.[17-18,33-34] CSEB (Group 1) also showed acceptable results on enamel margins. The PH–value of CSEB is 1.9.[23] Despite the less distinct enamel etching pattern observed in scanning electron microscopy, there is no separation between adhesive resin and enamel.[30] Furthermore, CSEB contains a functional monomer (MDP) that may also bind to calcium.[15] Yoshida and others[35] reported that MDP tightly adheres to hydroxyapatite and its calcium salt barely dissolves in water. CSEB is proven to effectively bond to enamel.[3,23,36] It also contains silanated colloidal silica nanofillers. The presence of nanofillers in adhesives increases crosslinking and strength of its resin matrix and decreases its polymerization shrinkage, which might be contributing factors to reducing microleakage.[32,37] In contrast, IB cannot prevent microleakage on enamel margins compared with SBMP. The pH–value of IB is 2.1, which might explain why the etching pattern of IB is less distinct than that of CSEB and why its enamel microtensile bond strength is weaker than CSEB.[15] Moreover, IB contains 4-META, which may bond via a chelating reaction to calcium ions in apatite.[38] In several studies, 4-META-containing adhesives showed lower enamel bonding capacity than MDP-containing adhesives;[15,23] however, the differences in bonding capacity to tooth substrates between 4-META and MDP are unclear and are not the main point of the current study. Finally, the last factor that may play a role in the higher scores of microleakage for IB than that of CSEB is a lack of filler in its composition, which leads to greater polymerization shrinkage of this adhesive.

Effect of Phosphoric Acid

Separate etching of enamel margins before CSEB application improved microleakage results, but not significantly. It seems that CSEB can seal enamel margins effectively because of its good bonding efficacy.[23,36,39] It also seems that IB cannot seal enamel margins effectively, which is important due to insufficient etching ability.[12] On the other hand, there is no need for separate etching of enamel margins before using CSEB. But, in contrast, separate etching of enamel margins before IB application significantly improved the marginal seal.

CONCLUSIONS

The current study revealed the following:

1. The pre-etching of beveled enamel margins with phosphoric acid does not seem to significantly reduce microleakage around composite restorations bonded with Clearfil SE Bond (a two component self-etching adhesive system).
2. iBond (a simplified all-in-one adhesive system) needs pre-etching of enamel margins with phosphoric acid for an effective seal.

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