Original Article

Comparative evaluation of the effect of 37% orthophosphoric acid pre-etching on the microtensile enamel bond strength using universal adhesive and two-step self-etch adhesive systems – An *in vitro* study

Krutika Menon, Siddheswaran Vijayaraghavan, N. M. Dhanyakumar

Department of Conservative Dentistry and Endodontics, College of Dental Sciences, Davanagere, Karnataka, India

Abstract

Context: The significance of enamel etching has been well-researched and established. Limited literature is available comparing the enamel bond strengths of the two-step self-etch adhesive system and the newly introduced universal adhesive (UA) system with prior acid etching.

Aims: The aim of the study was to evaluate and compare the microtensile bond strength (μTBS) of composite to enamel with and without 37% orthophosphoric acid pre-etching using a recently introduced UA and a two-step self-etch adhesive.

Subjects and Methods: This *in vitro*, experimental study was conducted on 48 extracted human third molar teeth. Two enamel surface fragments were obtained per tooth, polished using 600-grit silicon carbide abrasive paper, and randomly divided into four groups-CLEARFIL SE BOND with and without acid pre-etching and G-Premio Bond with and without acid pre-etching. The samples were etched using 37% phosphoric acid gel for 30 s, followed by the bonding protocol. Composite resin blocks were built incrementally to a thickness of approximately 6 mm, and the restored samples were stored in distilled water for 24 h at 37°C. They were then sectioned, mounted, and subjected to a μTBS test using a universal testing machine.

Statistical Analysis Used: Statistical analysis of the data was performed using one-way ANOVA and Tukey's *post hoc* test using SPSS software version 22.0.

Results: Samples bonded with the UA subjected to prior acid etching protocol demonstrated the highest enamel μTBS. In contrast, those treated with the two-step self-etch adhesive without prior acid etching demonstrated the lowest enamel μTBS. Pre-etching with phosphoric acid significantly increased the enamel bond strength of both bonding agents, with the universal agent showing better results than the two-step adhesive system.

Conclusions: Enamel etching with phosphoric acid significantly increases the bond strength of self-etch adhesives. UA with prior acid etching showed the maximum enamel μTBS. Two-step self-etch adhesive without prior acid etching yielded enamel μTBSs unacceptable for long-term, durable bonding.

Keywords: Microtensile bond strength; phosphoric acid; pre-etching; self-etch; universal bonding agent

Address for correspondence:
Dr. Krutika Menon,
Room No. 9B, Department of Conservative Dentistry and Endodontics, College of Dental Sciences, Pavillion Road, Davanagere - 577 004, Karnataka, India.
E-mail: krutikamenon@yahoo.co.in

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INTRODUCTION

In this age of adhesive dentistry, continuous efforts are being made to improve the bond strength and durability of composites to dental substrates by making certain modifications to dental adhesives regarding their chemical composition, mechanism of bonding, number of bottles (treatment steps), and application technique.[1] The main challenge is developing a user-friendly adhesive that can provide an equally effective bond to enamel and dentin with distinctly different natures.[2]

Today, the adhesives either follow an "etch-and-rinse" or a "self-etch" (also known as "etch-and-dry") approach. The self-etch systems have greatly simplified the adhesive application process since they do not require rinsing and drying.[3] They can bond effectively to both enamel and dentin and allow for conservative cavity preparations.

The latest generation of simple-to-use, one-step adhesives are complex and intricate mixes of hydrophilic and hydrophobic components. They are known as "Universal adhesives" (UAs) which indicate the manufacturers' claims that they can be applied with different adhesion strategies and with a variety of direct and indirect restorative materials. They can be used with either total-etch, self-etch, or selective enamel etching techniques and, in addition to tooth substrates, can also bond to glass ceramics, zirconia, and metal alloys.[3] The addition of nano-fillers with an average particle size of 12 nm increases the thickness of the hybrid layer and the penetration of resin monomers, which subsequently improves the mechanical properties of this new bonding system.[3]

Bonding to enamel has proven to be durable.[4] While "strong" self-etch adhesives generally perform quite satisfactorily at enamel, bonding of "mild" self-etch adhesives to enamel (especially to unground, aprismatic enamel) remains so far unsatisfactory. At enamel, an etch-and-rinse approach using phosphoric acid remains the preference since it guarantees the most durable bond to enamel and protects the more vulnerable bond to dentin against degradation.[4]

Considering the increasing popularity of UAs, driven by their versatility, the pressing question is whether the UA or two-step self-etch adhesive is superior for enamel bonding.

The benefits of selectively applying phosphoric acid to enamel, to improve the bond strength of the adhesives, before the application of self-etch adhesives (phosphoric acid pre-etching), have been well documented in the literature. A meta-analysis of *in vitro* studies by Rosa *et al*. showed that UAs utilizing the etch and rinse strategy resulted in significantly higher enamel microtensile bond strength (μTBS) values.[5]

No literature is available evaluating the effect of phosphoric acid pre-etching on the enamel μTBS of the recently introduced G-Premio Bond compared with CLEARFIL SE BOND. Thus, it would be valuable to evaluate the effects of phosphoric acid pre-etching on the enamel bond fatigue durability of UAs and two-step self-etch adhesives to assess their endurance in the face of repetitive subcritical loading.

Hence, the aim of this study was to compare the effect of acid pre-etching using 37% orthophosphoric acid on the enamel μTBS following the application of a UA and a two-step self-etch adhesive using a universal testing machine.

The null hypothesis was that there would be no differences in the enamel μTBS using UA and two-step self-etch adhesive, regardless of the presence or absence of phosphoric acid pre-etching.

SUBJECTS AND METHODS

Forty-eight extracted, intact human third molar teeth were collected. Cracked, carious, and fluorosed teeth were discarded. They were cleaned and stored in de-ionized water before use in the experiment. The roots were removed from each tooth, and the crowns were sectioned in the occlusal-cervical direction using a low-speed diamond disc to obtain two enamel surface fragments. The enamel surface of the fragments was polished using a 600-grit silicon carbide abrasive paper under water cooling for 60 s to produce a standardized smear layer.

The specimens of 96 enamel fragments were divided into four groups, with 24 samples in each group.

- Group 1 ($n = 24$ samples): Two-step self-etch adhesive CLEARFIL SE BOND and composite without pre-etching
- Group 2 ($n = 24$ samples): Pre-etching with 37% phosphoric acid, CLEARFIL SE BOND bonding agent, and composite
- Group 3 ($n = 24$ samples): UA G-Premio Bond and composite without pre-etching
- Group 4 ($n = 24$ samples): Pre-etching with 37% phosphoric acid, G-Premio Bond bonding agent, and composite.

In Groups 2 and 4, the enamel surface was etched with 37% phosphoric acid gel (3M ESPE Scotchbond Etchant) for 30 s, followed by 10 s of rinsing with a spray of water and drying with air spray for 5 s. The respective bonding agents were applied and cured as per the manufacturer's instructions [Table 1]. After the bonding procedure, a composite resin block (GC Solare Sculpt) was built up incrementally to a thickness of approximately 6 mm. A 4 mm × 4 mm × 6 mm mold was used to standardize the composite thickness. Each increment of 2 mm was light activated for 20 s. Then the restored samples were stored in distilled water for 24 h at 37°C.

Table 1: Composition and recommended protocol for application of adhesive according to the manufacturer

Adhesive	Manufacturer	Contents	Recommended protocol for application
CLEARFIL SE BOND	Kuraray Medical Inc., Okayama, Japan	Primer: MDP, HEMA, hydrophilic aliphatic dimethacrylate, water, initiator, N, Ndiethanol-p-toluidine Adhesive: MDP, HEMA, BisGMA, hydrophobic aliphatic dimethacrylate, initiator, accelerators, silica, N, N-diethanolptoluidine	Primer was applied to the air-dried tooth surface for 20 s. Medium air pressure was applied to the surface for 5 s. The adhesive was applied to the primed tooth surface and then air-thinned to make a uniform bond film. Primer/adhesive photocured for 10 s
G-Premio Bond	GC Corporation, Tokyo, Japan	MDP, 4-MET, MDTP, dimethacrylate monomer, acetone, water, initiator, silica	The adhesive was applied to the air-dried tooth surface for 10 s and then maximum air pressure was applied to the surface for 5 s. Adhesive photocured for 10 s

MDP: Methacryloxydecyl dihydrogen phosphate, HEMA: Hydroxyethyl methacrylate, MET: Methacryloyloxyethyl trimellitic acid, MDTP: Methacryloyloxydecyl dihydrogen thiophosphate

After 24 h of storage, the restored samples were sectioned into slices measuring 1 mm × 1 mm with 8 mm height, by a low-speed diamond saw in a precision sectioning machine, with a bonding area of 1 mm^2. [Figure 1]

The specimens were attached to a testing jig with cyanoacrylate adhesive and subjected to tension in a universal testing machine at a crosshead speed of 1 mm/min. [Figure 2] The cross-sectional area was calculated. The μTBS was obtained using the following formula:

$$\text{Microtensile bond strength (MPa)} = \frac{\text{Load at failure (N)}}{\text{Enamel} - \text{composite interface area (mm}^2\text{)}}$$

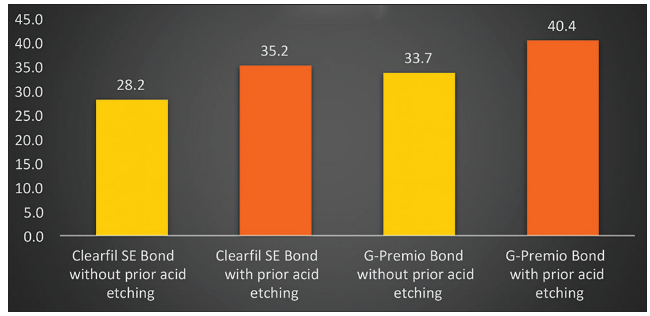
Results were expressed as mean μTBS in MPa for each group [Table 2]. An inter-group comparison was performed using ANOVA and Tukey's *post hoc* HSD using SPSS software version 22.0. (IBM, Chicago, Illinois, USA).

RESULTS

The following results were obtained from the present *in vitro* study:

- There was an increase in enamel μTBS in groups that were subjected to pre-etching with 37% orthophosphoric acid
- Group 4 - (Samples bonded with the UA subjected to prior acid etching protocol) showed the highest enamel μTBS
- Group 1 - (Samples treated with the two-step self-etch adhesive without prior acid etching) demonstrated the lowest enamel μTBS among the experimental groups
- All the groups showed statistically significant differences.

Inter-group analysis by ANOVA and *post hoc* showed a statistically significant difference between all four groups. An increase in enamel μTBS was observed after prior acid etching for both bonding agents. Between the bonding agents, the universal bonding agent showed better enamel μTBS than the two-step self-etch bonding agent. The enamel μTBS was the highest in Group 4, and it was the lowest in Group 1 [Graph 1]. The order was as follows:



Graph 1: Mean enamel microtensile bond strength between different groups. The graph shows the percentage of failures, where the X-axis shows the study groups and the Y-axis shows the percentage of samples

Group 4 > Group 2 > Group 3 > Group 1.

Hence, we reject the null hypothesis and accept the alternate hypothesis that there is a significant difference in the enamel μTBS between samples bonded using UA and two-step self-etch adhesive with prior phosphoric acid etching.

DISCUSSION

The results of the present study showed that the enamel μTBS was significantly higher in the groups subjected to prior acid etching, with the highest strength being recorded in the UA group.

This result of our study was in accordance with several studies evaluating the effect of pre-etching on enamel bond strength,[1,6-12] reiterating the fact that enamel etching with phosphoric acid before the application of bonding agent, significantly increases the bond strength of composite to enamel.

The etch and rinse technique is still the most effective approach to achieve an efficient and stable enamel bond.[13] Acid etching of enamel creates a tremendous increase in surface area available for bonding. It creates pores in the enamel into which the adhesive can flow and polymerize. Evidence shows that resin tags as long as 15–20 μ can be formed at the resin-enamel interface after acid etching. Acid etching has also been shown to create a honeycomb

Table 2: Mean value, standard deviation, standard error, *P* value of enamel microtensile bond strengths of different groups using one-way ANOVA analysis

Groups	*n*	Mean	SD	SE	*P*
Group 1: CLEARFIL SE BOND without prior acid etching	24	28.1500	1.46317	0.29867	0.000
Group 2: CLEARFIL SE BOND with prior acid etching	24	35.2125	1.50890	0.30800	
Group 3: G-Premio Bond without prior acid etching	24	33.6833	2.41097	0.49214	
Group 4: G-Premio Bond with prior acid etching	24	40.4286	2.93192	0.63980	

SE: Standard error, SD: Standard deviation

Figure 1: Preparation of the samples

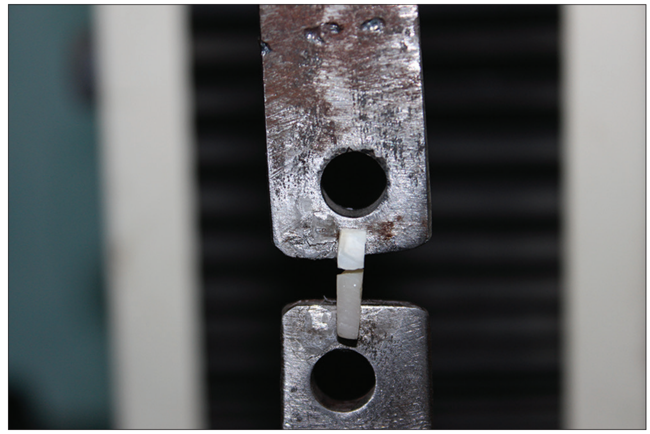


Figure 2: Load at failure recorded

pattern in the enamel surface, resulting in micromechanical retention. It removes approximately 10 µm of enamel from the surface and creates a porous layer approximately 5–50 µm thick.[1]

The UA used in this study has three functional monomers:

i. 4-methacryloyloxyethyl trimellitic anhydride, which is responsible for the bond strength of restoration to dentin and enamel surface
ii. 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) is responsible for bond strength to zirconia, alumina, and nonprecious metals in addition to enamel and dentin
iii. methacryloyloxydecyl dihydrogen thiophosphate which enables acceptable bond strength to precious metals.

In the present study, CLEARFIL SE BOND was selected since it is considered the "gold standard" bonding agent among the self-etch adhesives by some authors as it demonstrates the best results of bond strength to dentin.[14] Studies have reported that the functional monomer 10-MDP in CLEARFIL SE BOND has the highest chemical bonding potential with hydroxyapatite (HAp) and hydrolytic stability among other monomers.[14]

In a study done by Joseph *et al*.[15] comparing the µTBS of CLEARFIL SE BOND two-step self-etch adhesive, Adper Easy One 7th generation bonding agent and Futurabond UA, it was concluded that the UA had the highest bond strength followed by the two-step self-etch bonding agent. This was in agreement with the results of the present study in which the UA with prior etching yielded the highest bond strength.

The present study concluded that between the two bonding agents, the UA demonstrated better enamel µTBS with prior acid etching compared to the two-step self-etch adhesive with prior acid etching. This is in accordance with the study done by Pouyanfar *et al*.,[1] where they compared the enamel µTBS of UA-Scotchbond Universal with and without prior acid etching, Adper Scotchbond Multipurpose, Single Bond, and CLEARFIL SE. They concluded that UA with prior acid etching yielded the highest enamel bond strengths. According to Gwinnett and Matsui[16] if the adhesive material is strong intrinsically, the strength of the mechanical bond would increase with a simultaneous increase in the penetration of the material into the enamel.

In this study, the values of microtensile enamel bond strength of UA used in the self-etch mode were acceptable for durable enamel bonding. This finding was in accordance with the study conducted by Pouyanfar *et al*.,[1] in which the UA Scotchbond Universal without prior etching yielded a bond strength as high as that of two-step self-etch and two-step etch and rinse bonding agents. This could be attributed to the nano-size cross-linkers as well as the HEMA-free formulation of the UA, G-Premio Bond.[17]

Certain differences that might explain the higher µTBS value of the UA are as follows:

- High penetration and wettability results in favorable infiltration into microporosities and dentinal

tubules.[18] Fukegawa *et al*.[19] reported that MDP bonds ionically to HAp and effectively increases the long-term enamel bonding. Although both the adhesives used in this study contain MDP which is capable of bonding to calcium, the purity and quantity might be different, which was found to affect the adhesive performance[20]
- The different pH of the adhesive systems could also have affected the bond strength. The pH value of CLEARFIL SE BOND is 2.0, which is classified as mild, whereas G-Premio Bond has a pH value of 1.5, which is intermediately strong and exhibited a higher bond strength value than CLEARFIL SE BOND[21]
- Another important factor that affects the longevity and clinical success of adhesives is the solvent type. Certain studies have reported that the bond strength of etch and rinse systems is highly dependent on the solvent type.[22] The most frequently used solvents are acetone, ethanol, and water. CLEARFIL SE BOND contains water as a solvent, but G-Premio Bond contains acetone. The higher vapor pressure of acetone than water ensures maximum removal of solvent, which enhances hydrophobic monomer infiltration, reducing phase separation[23]
- In addition, the MDTP monomer which is present in the UA and not in the two-step self-etch adhesive, may have played a role in enhancing the enamel bond strength
- The UA used in this study does not contain HEMA compared to the two-step self-etch adhesive used. Hydrophobic nano-layering formed when functional monomer 10-MDP ionically bonds to HAp is considered to contribute to the long-term durability of the bond to tooth tissue.[17] A study by Yoshida *et al*.[17] concluded that the addition of HEMA inhibited interfacial nano-layering.

In the present study, though significantly lesser than the UA used with prior acid etching, the microtensile values of the two-step self-etch adhesive with prior acid etching were acceptable. However, without prior acid etching, the two-step self-etch adhesive resulted in unsatisfactory enamel μTBSs. This was similar to a study done by Brackett *et al*.,[24] where they compared the enamel μTBS of resin composites to enamel and dentin produced by two adhesives with self-etching primers (SEPs), CLEARFIL SE (Kuraray) and Peak SE (Ultradent), four self-etching adhesives, OptiBond All-In-One (Kerr), CLEARFIL S^3 (Kuraray), Adper Prompt L-Pop (3M ESPE), and iBond (Heraeus Kulzer) and, as a positive control, PQ1 (Ultradent), an etch-and-rinse adhesive. They concluded that milder pH of SEP adhesives is optimal for dentin but may not be sufficiently aggressive for enamel.

The application of self-etch adhesives, though proven extremely efficient for dentin bonding, is associated with some concerns when used on enamel. The thin hybridized complex of resin formed in enamel, produced by the self-etch adhesive, without the usual micrometer size resin tags, could be responsible for the lower μTBS values. The shallower etch pattern of SEPs could also be attributed to the neutralization of the acidic portion of the primer by the calcium and phosphate ions released during decalcification. This, in turn, results in poorer penetration of the SEP into the enamel porosities or interference from mineral precipitates on the enamel surface that mask the etch pattern. Since the acidic primer is not rinsed off, phosphorous and calcium ions that are released during HAp crystal dissolution remain suspended in the primer solution and embedded in the resin after polymerization, thereby causing a cessation of the enamel decalcification process.[25]

Clinical significance

The findings of this study highlight the much better enamel bonding efficacy of UA with phosphoric acid pre-etching in comparison with a gold standard self-etch bonding agent. It also adds to the evidence of the need for phosphoric acid pre-etching while using self-etch bonding agents on enamel.

Limitations and scope of the study

An *in vitro* model, like the one used in this study, does not account for the complexities of an *in-vivo* situation. The outcomes of this study cannot be directly matched to an *in vivo* situation since the bond strength depends on a myriad of intraoral factors, such as masticatory loads, pH alterations, and thermal changes, which need to be simulated for definitive results.[26] To add to this study, SEM analysis of the samples after failure can be done to give a deeper insight into the histological characteristics of the enamel-bonding agent and composite interface.

CONCLUSIONS

Within the limitations of the present *in vitro* study, 37% orthophosphoric acid etching of the enamel before the application of self-etch adhesives, significantly increased the enamel μTBS. UA, with prior acid etching, yielded the enamel μTBS significantly higher than the other groups. Two-step self-etch adhesive without prior acid etching of the enamel yielded μTBSs that were unacceptable for long-term, durable enamel bonding.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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