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Microleakage of a Pit and Fissure Sealant used with Two Brands of Self-etching Adhesives: An *In Vitro* Study

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Abstract:

Background: Self-etching adhesives might be an effective alternative to conventional phosphoric acid etchants in conditioning the enamel surface. The objective of this *in vitro* study was to evaluate and compare microleakage of a fissure sealant applied following the use of two brands of self-etching adhesives.

Materials and Methods: 78 sound maxillary first permanent premolars received enameloplasty and randomly distributed into 3 different groups according to enamel surface conditioning before sealant (Clinpro™) application: (1) Conditioning with 35% phosphoric acid (Scotchbond™ Etchant) (Group 1); (2) conditioning with self-etching adhesive (Xeno®V) (Group 2); and (3) conditioning with self-etching adhesive (Optibond® All-In-One) (Group 3). Samples were: Thermocycled, stained, sectioned, and examined for marginal microleakage.

Results: Marginal leakage was significantly lower with conventional etching than with self-etching adhesives while Xeno®V resulted in resulted in significantly less microleakage than Optibond® All-In-One.

Conclusions: Marginal leakage of the self-etching adhesives (Xeno®V and Optibond® All-In-One) was found to be significantly more compared to that of the conventional phosphoric acid etching even with enamel preparation. Etching enamel with phosphoric acid is still essential for sealing the enamel-sealant interface.

Key Words: Acid etching, fissure sealant, microleakage, self-etch adhesive

Introduction

Pits and fissures are anatomic landmarks on a tooth where the enamel folds inward.[1] The occlusal surfaces of teeth represent 12.5% of all tooth surfaces but are the location of over 50% of all dental caries. Among children, pit and fissure caries represent 90% of all dental caries.[2] Pit and fissure sealants are agents used to seal occlusal surfaces, to prevent caries. Sealants that are applied before a carious lesion develops have been shown to be successful in preventing the development of caries. In fact, the first molar without sealant is 22 times more likely to develop caries than is a molar that is sealed.[3] The technique of occlusal sealing was introduced in the 1960s when a mixture of cyanoacrylate, polymethylmethacrylate, and an inorganic powder was used as a sealing material. Pit and fissure sealants have become the most effective noninvasive treatment to prevent occlusal caries.[4]

A review article has shown the effectiveness of fissure sealants in decreasing caries on occlusal surfaces. However, problems in sealant application can cause leakage, partial, or total loss leading to sealant failure at a rate of 5-10% a year.[5] The efficacy of sealants application depends on many factors such as isolation, use of bonding agent, enameloplasty, and maintenance that may affect sealant retention.[6] Current adhesive systems use either total-etch technique or the self-etch technique. Total-etch adhesives include a phosphoric acid gel that demineralizes dentin and enamel simultaneously, but self-etch adhesive does not need a separate acid-etch step. Self-etch adhesive and bonding, conditions and primes enamel and dentin simultaneously without the need for rinsing. It is based on its ability to partially dissolve hydroxyapatite to cause resin-infiltrated zone with minerals incorporated. Self-etch adhesives include aqueous mixtures of acidic functional monomers, generally phosphoric acid esters or carboxylates, with a pH higher than that of phosphoric acid gels. Self-etch adhesives cause shallow enamel demineralization compared to phosphoric acid due to their higher Ph. Recently, self-etch adhesives have been classified based on the actual interaction depth of the material at dentin, their ability to penetrate the smear layer and pH into four categories; (a) An "ultra-mild" self-etch approach (pH >2.5) with "nano-interaction" of a few hundreds of nanometers, (b) a "mild" self-etch approach (pH~2) with an interaction depth of around 1 μm, (c) an "intermediary strong" self-etch approach (pH between 1 and 2) with an interaction depth between 1 and 2 μm, and (d) a "strong" self-etch approach (pH ≤1) with an interaction of several micrometers deep.[7]

Self-etching primers can be an effective alternative to conventional phosphoric acid etchants in conditioning the

enamel surface to guarantee a constant bonding and marginal seal of composite resin restorations.[8] Self-etching systems are less technique-sensitive than total-etch systems. They seem to eliminate factors such as over-etching, over-drying, and over-wetting.[9] Considering the variety of dentin bonding materials in the market and the announcement of the manufacturers about the related higher efficacy compared to other materials, more *in vitro* and clinical research is needed. Searching literature, there were few studies comparing the efficacy of Xeno®V (Dentsply, Germany) and/or Optibond® All-In-One (Kerr, USA) with Clinpro™ sealant (3M ESPE, USA). Therefore, the aim of this *in vitro* study was to evaluate and compare microleakage of a fissure sealant applied following the use of two brands of self-etching adhesives with conventional phosphoric acid etching. The null hypothesis was that there is no significant difference in the microleakage score of the pit and fissure sealant between conventional etching and the two self-etching adhesives.

Materials and Methods

After obtaining approval from the College of Dentistry Research Centre (CDRC), (Deanship of Scientific Research, King Saud University; No. 2224), 78 freshly extracted sound permanent maxillary first premolars, which were extracted for orthodontic treatment, collected, and used in this study. The teeth were stored not more than 3 weeks in 0.9% physiologic saline at room temperature after scaling and cleaning with water\pumice slurry in rotating bristle brushes to remove calculus and surface-adhered debris. Then, enameloplasty of 1 mm depth was performed on the occlusal surface using tapered diamond bur (#582 S, Dentsply, Germany) in a high-speed handpiece with water. Each bur was used to prepare 4 teeth then discarded, followed by drying for 10 s with oil-free air. The teeth were then subjected to one of the following techniques of enamel surface treatment with 26 teeth in each group:

Group 1: Conventional acid etching. The pits and fissures received traditional acid etching with 35% phosphoric acid (Scotchbond™ Etchant, 3M ESPE, USA) for 30 s followed by rinsing for 10 s and drying for 30 s until the etched surface became matt white.

Group 2: Self-etching adhesive (Xeno®V, Dentsply, Germany). According to the manufacturer instructions, the occlusal surface was gently agitated with 2 layers of Xeno®V using the disposable micro brush applicator for 15 s for each layer. Then, air dried gently for 5 s, followed by light curing for 10 s (Elipar highlight, 3M, ESPE, St. Paul, MN, USA).

Group 3: Self-etching adhesive (Optibond® All-In-One, Kerr, USA). According to the manufacturer instructions, the same steps in Group 2 were followed except for scrubbing it was for 20 s.

The sealant was applied in all the teeth (Clinpro™ Sealant, 3M ESPE, USA) and light cured for 20 s. Then, the sealant margins were checked with sharp explorer. All sealant's margins were intact. All the teeth then stored in distilled water at 37°C for 24 h (Incubator O2, Thermo Scientific, Germany).

The specimens were thermocycled for 3000 cycles in water baths at 5 ± 2°C and 55 ± 2°C with a dwell time of 30 s in each bath and 5 s transit time between baths.[10] The root apices were then sealed with utility wax, and 2 coats of commercial nail varnish were applied on the entire tooth leaving 1 mm window around the sealant The teeth then were immersed in 2% basic fuchsin dye solution for 72 h.[11] Subsequently, teeth were rinsed in distilled water for 15 min. Then, each tooth was embedded in clear acrylic autopolymerizing resin. The mesial and distal sides of each tooth were sectioned longitudinally in bucco-lingual direction using a low speed; water-cooled diamond saw (Isomet 2000, Buehler 41 Waukegan Road, Lake Bluff, IL 60044, USA) and discarded. Then, each tooth was subsequently sectioned longitudinally in a bucco-lingual direction to provide three sections (two lateral sections and one middle section with 1mm thickness for each one) from each tooth for evaluation of microleakage. All the previous steps were performed by one investigator.

The extent of dye and sealant penetration were assessed by a blind examiner subjectively with stereomicroscope at ×60 magnification (Stereoscopic Zoom Microscope SMZ 1000/SMZ 800, Nikon, Japan) only, followed by measuring with software with 3 days in between by the same examiner using a stereomicroscope at x60 magnification with digital camera (Digital Camera DXM 1200F, Nikon, Japan) and Adobe Photoshop CS3 Extended (Version 10.0.1; Adobe Systems Inc., San Jose, California) then analyzed for dye penetration as in Figure 1 and Table 1. Then, depth of dye penetration at cavity walls for both (the subjective and software reading) was assessed according to Grande *et al.*,[12] scoring system using the following score: No dye penetration (Score 0), dye penetration into the occlusal third of the enamel-sealant interface (Score 1), dye penetration into the middle third of the enamel-sealant interface (Score 2), and dye

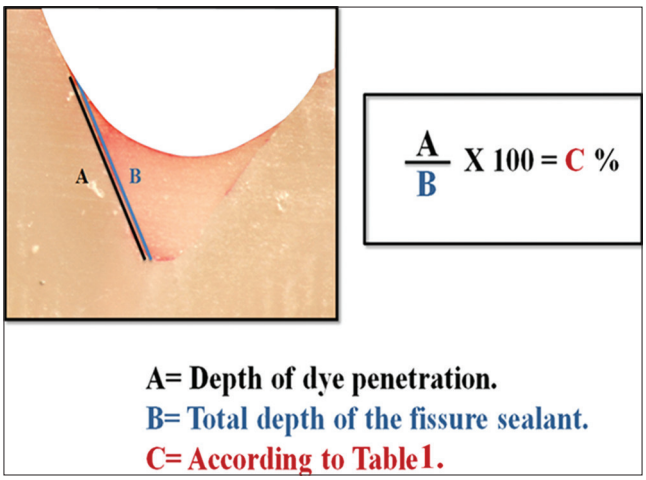

Figure 1: Calculation of the depth of dye penetration.

penetration into the apical third of the enamel-sealant interface (Score 3) (Figures 2-5). The highest score was established as the final score obtained after examining both sides of the section and the buccal and lingual inclined cuspal plans in each side. Four negative and four positive control teeth were selected, and enameloplasty was performed on the occlusal fissures. Termocycled and the apices of the negative control group were covered with utility wax, and the whole tooth was covered with two layers of nail varnish. Then, both groups were immersed in 2% basic fuchsin dye for 72 h, embedded in clear acrylic resin, sectioned and examined under the stereomicroscope.

Table 1: Scoring system according to C-value.

C-value (%)	Grand *et al.*, scoring system
0.1-33.3	1
33.4-66.6	2
66.7-100	3

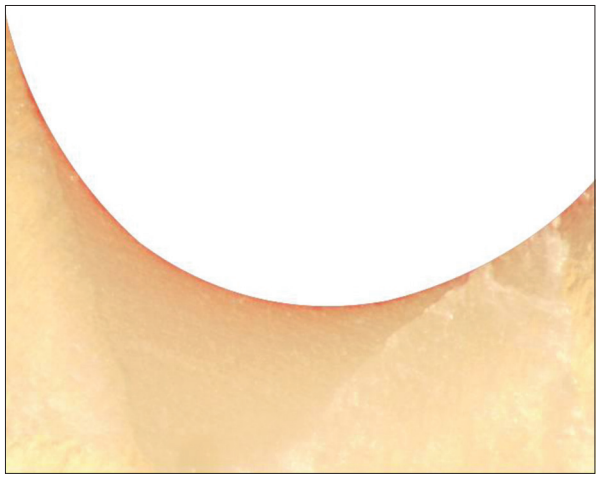

Figure 2: Bucco-lingual tooth section from the conventional etching group under stereomicroscope (×60) showing no dye penetration (Score 0).

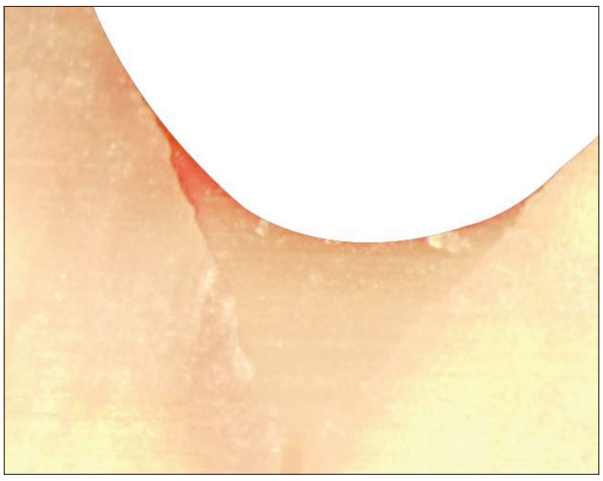

Figure 3: Bucco-lingual tooth section from the Xeno®V group under stereomicroscope (×60) showing dye penetration into the occlusal third of the enamel-sealant interface (Score 1).

The data of microleakage scores were analyzed using SPSS statistical software (version 16; SPSS Inc., Chicago, IL, USA). For the intra-examiner reliability, Cohen Kappa statistic was used. Kolmogorov-Smirnov and Shapiro-Wink were used to test normality; data was not normally distributed. So, a non-parametric Kruskal-Wallis test one-way ANOVA was used for multiple group comparisons, and Mann–Whitney non-parametric *U*-test was used for group-wise comparisons. For all the tests, a $P < 0.05$ was considered as statistically significant.

Results

Results from the subjective reading and software specimen's measurements revealed that the intra-examiner reliability on microleakage - As assessed by Cohen Kappa statistic- showed" almost perfect agreement" (0.97). For the control groups, all specimens in the positive control group showed dye penetration, whereas none of the specimens in the negative

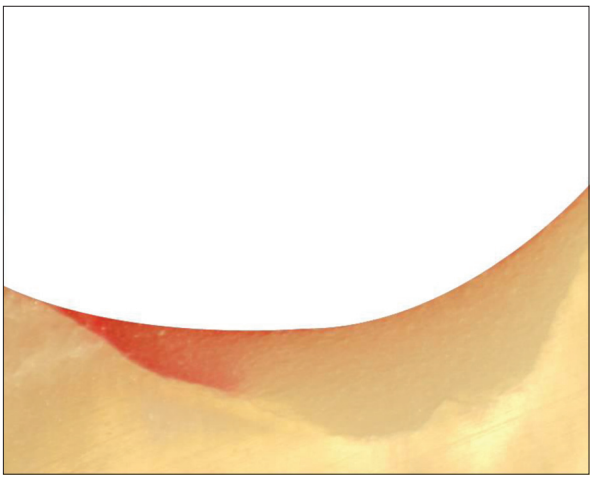

Figure 4: Bucco-lingual tooth section from the Xeno®V group under stereomicroscope (×60) showing dye penetration into the middle third of the enamel-sealant interface (Score 2).

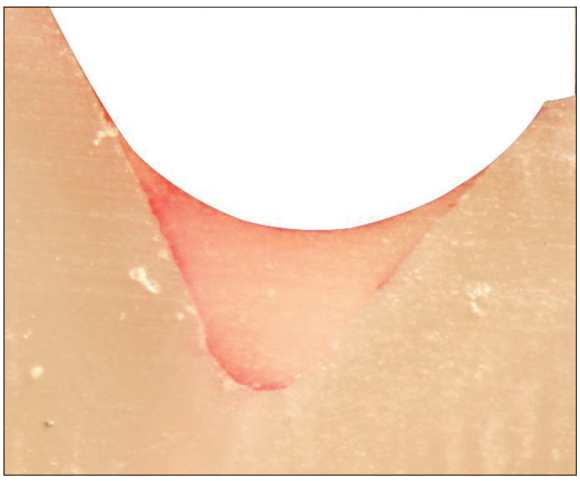

Figure 5: Bucco-lingual tooth section from the Optibond® All-In-One group under stereomicroscope (×60) showing dye penetration into the lower third of the enamel-sealant interface (Score 3).

control group showed dye penetration. Regarding sealant penetration, sealants in all groups penetrated into the various fissures types quite well. None of the sealant was dislodged or displaced at the time of examination. In Group 1 (conventional etching), no dye penetration was observed in 64.1% of the sample (median = 0.00; min-max = [0.00-3.00]). In Group II, no dye penetration was noted in almost 18% of cases (median = 3.00; min-max = [0.00-3.00]). In Group III, all the samples have been penetrated by the dye, and 88.5% of the sample showed penetration up to the lower third (median = 3.00; min-max = [1.00-3.00]). The frequency obtained for the microleakage assessment for each experimental group is presented in Figure 6.

A Kruskal-Wallis one-way ANOVA by ranks test found a high statistical significance difference among the three experimental groups ($P = 0.00$). The Mann-Whitney non-parametric *U*-test for the comparison of independent data samples found Group I to be statistically different from Group II and III. Moreover, Group II is statistically different from Group III (Table 2).

Discussion

The ability of a restoration to minimize the extent of microleakage at the tooth/restoration interface is important in predicting its clinical success. Presence or absence of microleakage can be determined by a variety of different methods. Dye penetration seems to be the simplest and most widely used approach. The penetration of a dye can indicate the lack of a perfect seal. Within its known limitations, it should be viewed as a theoretical level of leakage, which may occur *in vivo* and can be accepted as an aid for developmental purposes.[13] In clinical practice where the sealant is applied without rubber-dam isolation, changing of cotton rolls after rinsing of the etchant is a critical step in which salivary contamination of the etched surface for as short as 1 s results in the formation of surface coating that cannot be removed effectively by rinsing, which in turn will significantly jeopardize the retention and sealing effectiveness of the fissure sealant.[14] With the emergence of seventh-generation adhesive systems, the adhesion procedure has become faster and can reduce or eliminate rinsing and drying procedures for increased operator efficiency.[15] Therefore, the present *in vitro* study was designed to compare the microleakage of two recent "intermediary strong" seventh-generation bonding agents, containing different solvents: Acetone and ethanol (Optibond® All-In-One) and tertiary butanol (Xeno®V), respectively, with conventional phosphoric acid etching (Scotchbond™ Etchant) which were used to bond the same unfilled fluoride releasing resin-based pit and fissure sealant (Clinpro™ Sealant) on sound permanent upper first premolars.

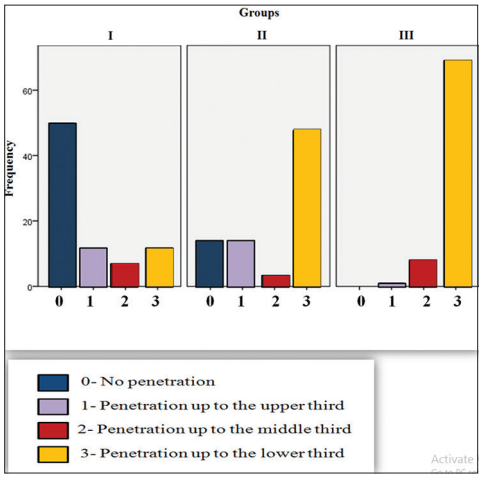

Figure 6: Distribution of microleakage score by groups.

Table 2: Results of the Mann–Whitney test comparing microleakage of the three experimental groups.

Groups	*P* value
I and II	0.000000
I and III	0.000000
II and III	0.000006

It has been claimed by the self-etching adhesives manufacturer that, self-etching adhesives is less effective on unground, unprepared, or uncut enamel. Therefore, in the present study, enameloplasty technique was utilized to remove the outermost layer of prismless enamel. Several studies have shown that enameloplasty provides the cleanest surface[16] and from the microbiological aspect, it is the most rational of the invasive methods.[17] According to Tadokoro *et al.*,[18] the sealant easily penetrates the enlarged fissure and adheres to the walls resulting in better retention. Another explanation for better retention was given by Shapira and Eidelman,[19] who found that mechanical preparation widened and deepened the fissure by eliminating organic material and plaque resulting in better retention. Enameloplasty was carried out using tapered diamond bur which shown to be the best.[17]

Even though, the results of the present study revealed that acid etching (Scotchbond™ Etchant) has significantly lower microleakage score when compared to the self-etching adhesives (Optibond® All-In-One and Xeno®V). This is might be due to the fact that the acidity of self-etch systems, whether the two-step self-etching primer type or the "all-in-one" self-etch adhesive type, is much less than that of phosphoric acid, they are only 10% as acidic as phosphoric acid etchants, with a higher pH.[20] Furthermore, the presence of dissolved calcium phosphates which are not removed by rinsing when using self-etching systems, this might result in a lower resistance to thermo-mechanical stress of the bond, and therefore in the development of marginal openings of the fissure sealing.[21]

Another important aspect is the amount of solvent included in the self-etching solution. Moreover, water is present in all the self-etch systems employed because it is an essential component. Consequently, the thickness of the adhesive layer may be thinner when greater quantities of solvent are used, and incomplete polymerization due to oxygen inhibition may

occur.[22] Thus, partially explains why applying several coats of all-in-one adhesive usually determine less micro-leakage. In our study, we applied two coats of self-etching adhesive systems as recommended by manufacturers, which were than air-streamed, to increase the time for the acidic monomers and primer to prepare the substrate, and then to stimulate the evaporation of the solvent, but despite this more leakage was found after thermocycling with this type of adhesives. The finding of significantly higher microleakage score of Optibond® All-In-One and Xeno®V in comparison with the conventional etching requires rejection of the null hypothesis. The results are in agreement with several *in vivo* and *in vitro* studies as the *in vivo* study of Venker *et al.*,[23] who retrospectively compared a self-etching primer, and phosphoric acid etch in a school-based sealant program over a 1-year period. They found that sealant retention with the use of phosphoric acid was superior to the self-etching primer. Furthermore, Burbridge *et al.*,[24] compared the retention of fissure sealants placed on occlusal surfaces following the use of a self-etching agent and traditional acid etch. The retention rate of the self-etching priming agent was significantly lower, as was the caries preventive effect.

Xu and Ji[25] concluded that self-etching adhesive and flowable resin did not have an excellent clinical effect on caries prevention. Even Perdigão *et al.*,[26] conclude that etching enamel with phosphoric acid is still essential for sealing the enamel-sealant interface, and the self-adhesive sealant may not be indicated for clinical use. As evidenced in this study, the same suggestions arise as the self-etching adhesives are not suitable yet for clinical use with pit and fissure sealant and further research are needed and still conventional acid etching with phosphoric acid is essential.

Contrary to the results of the present study, Asselin *et al.*,[27] found that no significant difference between acid etch and self-etching adhesives in microleakage and bond strength. This contradiction might be attributed to the differences in methodology used in the studies as they thermocycled the specimen for 500 cycles only while in our study we used 3000 cycles. However, we have to consider that thermal changes are frequent in the mouth, which could be a critical factor explaining microleakage around sealants. This is because sealants have one of the highest coefficients of thermal expansion among the dental materials used for restoration. Moreover, because sealants are made of composite materials (BisGMA-based organic resins), they are subject to polymerization shrinkage which could also accentuate the degree of microleakage.[28] Furthermore, they did immersion in the dye for 24 h only, while in the present study the sample was immersed for 72 h, which has been proven by Ansari *et al.*,[11] that immersion for 72 h showed more dye penetration than 24 and 48 h. In their study, they use Adper Prompt L-Pop self-etching adhesive which considered as a strong one with pH 0.9-1. Instead, we use intermediary strong self-etching adhesives with a higher pH. When comparing the microleakage scores in between the two self-etching adhesives the extent of microleakage under sealants bonded with the Optibond® All-In-One adhesive was significantly higher than those achieved with Xeno®V. which can be explained by that the Xeno®V has a lower pH (1.3) where Optibond® All-In-One owns a higher one (1.7).

Concern has also been raised regarding the chemical stability of all-in-one solutions. Some formulations have showed limited shelf-life and refrigerator storage has been recommended to avoid temperature effects.[28,29] To overcome these possible limitations, new formulations of all-in-one adhesives incorporate monomers that are expected to be more stable. For example, refrigeration is no longer required for Xeno®V, due to the inclusion of tertiary butanol. Unlike Xeno®IV with ethanol based solvent which is not stable in high temperature. In future, further research is required to test bond strength along with the microleakage to have a better representation of the oral cavity. Furthermore, since recently opened bottles of adhesives were tested in this study future research is needed to test the effect of temperature changes and repeated bottle opening on the bonding quality of these new adhesives.

Conclusion

Based on the results of the present study, the following conclusions can be drawn:

- Marginal leakage of sealant with self-etching adhesives (Xeno® and Optibond® All-In-One) was found to be significantly more compared to that of the conventional phosphoric acid etching even with enamel preparation. Therefore, conventional etching with 35% phosphoric acid for 30 s before sealant application is still preferable.
- The marginal leakages of sealant with Xeno®V were significantly less than that of the Optibond® All-In-One.

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