

# Scientific Article

## Marginal Microleakage of a Sealant Applied to Permanent Enamel: Evaluation of 3 Application Protocols

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**Abstract:** **Purpose:** The purpose of this *in vitro* study was to investigate and compare the microleakage—occurring after the placement of a light-curing sealant—to unground permanent enamel which had been previously conditioned using 3 different application protocols. **Methods:** Sixty-three molars were randomly distributed in 3 different groups according to the application protocol of the sealant: (1) conditioning of the enamel with 38% phosphoric acid (group 1); (2) conditioning with phosphoric acid and single-bottle dentin bonding agent (group 2); or (3) conditioning with a self-etching adhesive (group 3). Samples were: (1) thermocycled; (2) stained; (3) sectioned; and (4) examined for marginal microleakage. **Results:** No dye penetration was noted for 25%, 59%, and 40% of cases for groups 1, 2, and 3, respectively. **Conclusions:** Placement of a bonding agent layer prior to the sealant allows significantly less microleakage than the traditional conditioning of enamel with phosphoric acid alone. The self-etching adhesive used here seems to be an attractive alternative to the acid-etch and adhesive technique for sealant application in young children, since it would simplify the procedure. Clinical trials should be performed to assess the performance of these products before definitive conclusions can be formulated. (*Pediatr Dent* 2008;30:29-33) Received December 24, 2006 / Last Revision May 8, 2007 / Revision Accepted May 16, 2007.

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Carious lesions are estimated to occur 5 times more frequently in occlusal fissures, and 2½ times more frequently in buccal and lingual fissures than on proximal smooth surfaces.<sup>1</sup> The 2000 US Surgeon General's report, which was based on a national survey of dental health, confirms that overall caries experience, especially smooth surface lesions, is declining.<sup>2</sup> The fissures of the tooth surfaces, however, are relatively inaccessible for plaque control measures and account for nearly 90% of total decayed, missing, and filled surfaces (DMFS) in US schoolchildren. Therefore, forming a barrier between these tooth surfaces and the oral environment by placing pit and fissure sealants has proven to be an effective method for reducing the rate of occlusal caries on permanent posterior teeth.<sup>3,4</sup>

Microleakage is defined as the passage of bacteria, fluids, molecules, and ions between the tooth and the sealing material.<sup>5</sup> *In vitro* microleakage studies make it possible to predict the marginal sealing capacity of the different materials used.<sup>6</sup> Marginal integrity and the capacity of a sealant to prevent microleakage into the fissures are 2 very important factors in

evaluating the clinical success of these agents, since leakage may support a carious process underneath the sealant.<sup>7,8</sup> This is especially important with questionable carious fissures.

As much as for any dental procedure, the success of the sealant depends on many factors, including retention capacity, marginal integrity, and meticulous operator techniques.<sup>9</sup> It was recognized early in the development of the acid-etch technique that isolation is a key to the success of the clinical sealant procedure. Salivary contamination leads to significantly reduced bond strengths of the sealant.<sup>10</sup> Bonding of the sealant to saliva-contaminated enamel leads to massive leakage.<sup>11</sup> Salivary contamination may be difficult to avoid in certain situations, however, since most fissure sealants are placed in young patients who may display less than optimal behavior and have newly erupted teeth that are difficult to isolate.<sup>12,13</sup>

To improve the sealant performance, new techniques or materials have been suggested. Feigal discussed the use of an intermediate bonding layer between enamel and sealants in the reduction of microleakage at the sealant/enamel interface, even in the presence of intentional contamination by saliva.<sup>11,13</sup> This effect could be attributed to the fact that these bonding agents were developed to bond restorations to a continuously wet tissue—the dentin.<sup>8,14</sup> The dentin bonding agents are bifunctional molecules with: (1) a methacrylate group that bonds to the sealant by chemical interaction; and (2) a functional group that bonds to either the organic or inorganic constituents of dentin.<sup>11</sup>

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It has been speculated that the benefit of this additional layer under the sealant is based on a combination of: (1) moisture-chasing effects; (2) increased flow by this less viscous material; and (3) increased flexibility of the adhesive/sealant complex.<sup>15</sup> Unfortunately, the use of a bonding agent prior to the application of the sealant tends to increase the time required and, hence, the cost and the technique sensitivity of the procedure. Thus, the use of a bonding agent should be carefully weighed before adoption.<sup>16</sup>

Bonding chemistry is constantly evolving in dentistry. The all-in-one adhesive system Adper Prompt-L-Pop (3M ESPE, St. Paul, MN) combines etching, priming, and adhesive in one solution, eliminating the rinsing procedure. Therefore, the time required for treatment and the need for patient compliance are reduced. Also, these systems are considered to be less technique sensitive compared to those using separate acid-conditioning and rinsing steps.<sup>17</sup> Considering these elements, self-etching adhesives appear to be an attractive alternative to acid etching with a bonding agent.

Compared to conventional acid-etching techniques, self-etching adhesives achieve similar marginal integrity in dentin.<sup>18</sup> Studies on the effects of these products on enamel are recent, however, and questions have surfaced regarding the reliability of these systems on unground enamel.<sup>19</sup> The volume of literature that currently exists on sealants only includes limited documentation comparing conventional acid-etch systems with nonrinse conditioning acid-etch systems both in vivo and in vitro.<sup>20</sup> Perry and Rueggeberg compared the effect of Prompt-L-Pop and conventional acid etching on the microleakage of a light-cured sealant, and concluded that the self-etch adhesive demonstrated a greater incidence of microleakage and would not be advocated over traditional techniques. These authors state that “there seems to be no definitive information available pertaining to the potential of self-etching bonding systems to adequately etch and adhere resin material to the convoluted occlusal surfaces of posterior teeth, compared with conventional etch technique with bonding.”<sup>21</sup>

Feigal and Quelhas reported equivalent sealant retention, either with the classic etch technique or with Prompt-L-Pop, on permanent molars over a 24-month period.<sup>22</sup> Venkel et al studied the effectiveness over a 12-month period using Prompt-L-Pop, compared to the traditional phosphoric acid etch, on the retention of sealants in a school-based program.<sup>23</sup> In a similar way, Burbridge et al compared the efficacy of another self-etch adhesive, Xeno III, and the acid etch technique with the use of a bonding agent in a randomized controlled trial over a 6-month period.<sup>20</sup> The results of these 2 studies are similar, so further studies are recommended to determine the comparability of the new self-etch adhesives with the more traditional methods.

The purpose of this in vitro study was to compare the microleakage occurring after the placement of a light-curing sealant to unground permanent enamel that has been previously

conditioned with either: (1) phosphoric acid; (2) phosphoric acid and a bonding agent; or (3) a self-etching adhesive.

## Methods

In this institutionally approved study, 60 clinically sound third molars were obtained from oral surgery private clinics. After extraction, all teeth were cleaned of gross debris and stored in a 0.1% thymol solution at  $4\pm 2^\circ\text{C}$  before being treated. The roots of the selected teeth were embedded in resin blocks. Subsequently, the occlusal surfaces were: (1) cleaned using fluoride-free pumice slurry and a white rubber cup; (2) rinsed; and (3) air dried.

The teeth were then randomly selected and assigned to 1 of 3 experimental groups (20 per group):

1. Group 1—Clinpro sealant (3M ESPE, St. Paul, MN) following 38% orthophosphoric acid etching (Scotchbond Etching Gel, 3M ESPE);
2. Group 2—Clinpro sealant following 38% orthophosphoric acid etching and the application of Adper Single Bond Plus adhesive (3M ESPE);
3. Group 3—Clinpro sealant following the application of Adper Prompt L-Pop (3M ESPE) self-etch adhesive.

The materials used were applied to the teeth according to the manufacturers' instructions. All products were light cured with an Optilux 500 light-curing unit (Kerr, Danbury, Conn) whose power density was determined to be 700 mW/cm<sup>2</sup> by an incorporated radiometer. Both the bonding agent and the self-etch adhesive were photocured prior to placement of the sealant. To prevent dye penetration, the dental surfaces—with the exception of the dental surfaces containing the sealants—were coated with a double layer of nail varnish. A 1-mm margin was left between the varnish and the sealant.

All samples were thermocycled (500 cycles lasting 1 minute at 5°C and 55°C) and then immersed in 1% methyl blue for 24 hours. The samples were removed and gently brushed to remove excess dye. The purpose of the thermocycling procedure was to simulate the thermal conditions existing in the oral cavity.<sup>24</sup>

The procedure used to determine marginal leakage was similar to one described by Theodoridou-Pahini and Tolidis.<sup>25</sup> Each tooth was sectioned longitudinally in a mesiodistal direction through the center of the sealant with the diamond wheel of a sectioning machine (Isomet low-speed saw, Buehler, Lake Bluff, Ill). Each section was cleaned, examined, and photographed. The slides obtained were randomly projected twice on a screen, and dye penetration was scored by 2 independent examiners as follows:

- a. 0=no dye penetration;
- b. 1=dye penetration down the mesial or distal wall;
- c. 2=dye penetration down the mesial and distal walls;
- d. 3=dye penetration underneath sealant and down the mesial or distal wall;
- e. 4=dye penetration all around the sealant.

All slides were evaluated twice by 2 examiners. A subsample of 20 teeth was used to determine within- and between-examiner reliability, estimated with Cohen's kappa. Groups were compared with Kruskal-Wallis' 1-way analysis of variance (ANOVA) and the Mann-Whitney test. A *P*-value  $\leq .05$  was considered statistically significant.

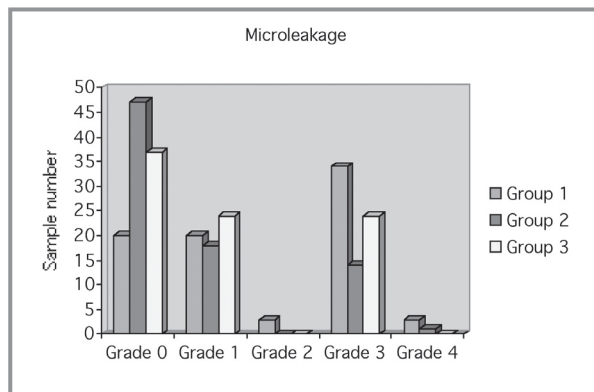


Figure. Distribution of in vitro microleakage scores (0=no dye penetration; 1=dye penetration down the mesial or distal wall; 2=dye penetration down the mesial and distal walls; 3=dye penetration underneath sealant and down the mesial or distal wall; 4=dye penetration all around the sealant) among different experimental groups (group 1=etch; group 2=etch and bonding; group 3=Prompt L-Pop).

### Results

All 60 sealants were present at the time of the examination. The Figure depicts the microleakage values obtained from each experimental group. In Group 1 (etch and sealant), no dye penetration was observed in 25% of the samples (median=1.75; [0.00-4.00]). In Group 2 (sealant with adhesive), no dye penetration was noted in 59% of cases (median=0.25; [0.00-3.00]). In Group 3 (sealant and self-etch adhesive), 40% of the samples showed no infiltration (median=1.00; [0.00-3.00]).

Since excellent agreement existed within and between examiners ( $\kappa > 0.8$ ) the statistical analysis was done using the median of their evaluations. A Kruskal-Wallis 1-way ANOVA by ranks test found statistically significant differences among the 3 groups (*P*=.04). The Mann-Whitney nonparametric test for the comparison of independent data samples found Group 1

to be statistically different from Group 2. No significant differences between Groups 1 and 3 or between Groups 2 and 3 were noted (Table).

### 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. Even though dye penetration seems to be the simplest and most widely used approach, the subjectivity of an assessment must be taken into account, since numerical values are assigned to different degrees of microleakage.<sup>8</sup> Also, the analysis of tooth cross-sections introduces a bias, since microleakage can vary according to the location or angle of sectioning.<sup>26</sup> Most of the dyes used to assess microleakage—referring to the ingress of oral bacteria—however, are many orders of magnitude smaller than the size of oral bacteria. Therefore, dye leakage tends to be an important test of microleakage.<sup>27</sup> It should also be noted that this is an in vitro study in which the sealing procedure was fully controlled, especially regarding moisture control. Therefore, in an in vivo situation, the degree of microleakage around sealants could be expected to be greater, especially if adequate isolation is compromised.<sup>25</sup>

Microleakage at the tooth-sealant interface is, to date, an unavoidable occurrence.<sup>28</sup> In this study, microleakage was found in all 3 experimental groups. 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.<sup>29</sup> 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.<sup>30</sup> Finally, fracture of the material at the interface between the tooth and the sealant by occlusal forces could also lead to microleakage.<sup>31</sup>

In this study, the best results in terms of reducing the microleakage under the sealants were obtained when a bonding agent was added to the conventional conditioning of the enamel with phosphoric acid. These results agree with studies by Borem and Feigal<sup>11</sup> and Pérez-Lajarin et al,<sup>8</sup> in which a decrease in microleakage was achieved when a dentin bonding agent was used, either under noncontaminated or contaminated conditions. Similar results were obtained by Tulunoglu et al using an enamel-dentin bonding agent as an intermediate layer between the primary tooth and fissure sealant, which decreased microleakage.<sup>14</sup> In a 2-year follow-up study, however, Boksman et al reported that the use of a bonding agent under fissure sealants in permanent molars does not affect their clinical effectiveness.<sup>15</sup>

Although it seems logical that retention and microleakage have an inverse relationship, there may only be a focus on the adherent sealant; leakage may still occur around the sealant.<sup>11</sup>

Table. RESULTS OF THE MANN-WHITNEY NONPARAMETRIC TEST COMPARING MICROLEAKAGE OF THE THREE TREATMENT GROUPS (P-VALUES)

	Group 1 (etch)	Group 2 (etch + adhesive)	Group 3 (Prompt L-Pop)
Group 1 (etch)		.01	.19
Group 2 (etch + adhesive)	.01		.18
Group 3 (Prompt L-Pop)	.19	.18	

Adhesion is, therefore, of prime importance in reducing percolation. Interestingly, the number of samples with a score of 2 (dye penetration down the mesial and distal walls) was much lower than the number of samples with a score of 3 (dye penetration underneath sealant and down the mesial or distal wall)—which was true for all 3 experimental groups. Biofilm, food, and bacterial debris accumulate at the base of pits and fissures, and the more difficult access to these areas for the bonding and/or the sealant flow might compromise the adhesion. This would explain the critical importance of marginal seal for the sealant's clinical efficacy. If one accepts this hypothesis, one may then consider that as soon as a marginal leak exists an infiltration under the sealant itself will most likely occur. This explains the difference between scores 2 and 3.

The use of a bonding agent prior to the application of the sealant would tend to increase the clinical time of the procedure and the risk of contamination, since sealants are often placed in uncooperative children on newly erupted teeth that are difficult to isolate. Recent advances in bonding chemistry may bring additional benefits to sealants for young patients. The self-etch approach is probably the most promising from a standpoint of user-friendliness and technique sensitivity. These products do not require a separate etch-and-rinse step because they employ chemically modified acidic monomers that demineralize and penetrate dental hard tissues simultaneously. Such simplifications: (1) minimize the time of treatment (up to 50%)<sup>22</sup>; (2) decrease the need for patient compliance; and (3) minimize potential errors in the application technique.<sup>32</sup> Little is known, however, about the long-term effects of incorporating dissolved hydroxyapatite crystals and residual smear layer remnants within the bonding resin, or about the effects of residual primer/adhesive solvent within the residual structure.<sup>33</sup> Also, since Prompt L-Pop is not equally compatible with all resin materials,<sup>12</sup> other types of sealants should be tested. Finally, sealants should be evaluated with varying types of self-etch adhesives.

To fully justify its clinical use, Prompt L-Pop, when applied prior to the sealant, should reduce microleakage compared to conventional total-etch techniques, if possible to the level or above what is seen when bonding agents are used in conjunction with the acid etch. In this *in vitro* study, Prompt L-Pop exhibited less leakage than the classic etch technique, even though the result was not statistically significant. Gillet et al attributed this effect to the fact that sealants, when combined with self-etching primers, offer better penetration. They recommend self-etching for preventive therapy of pits and fissures.<sup>34</sup> From a statistical standpoint, the performance of Prompt L-Pop—when utilized prior to the application of a sealant—was similar to that of a bonding agent, contrary to previous reports. Interpretation of these results should consider the limitations of an *in vitro* study compared to clinical trials. Since less leakage was found in the bonding agent group, one could still support the application of sealants with a bonding agent as our gold standard.

## Conclusions

Based on this study's results, the following conclusions can be made:

1. The use of an adhesive prior to the application of a pit and fissure sealant significantly reduces microleakage *in vitro* and, therefore, its clinical application is supported.
2. The use of a self-etch adhesive prior to the application of a sealant is an attractive alternative for specific situations in the pediatric population by reducing the chairtime and, therefore, the risk of salivary contamination.
3. Because this is an *in vitro* study, long-term clinical investigations are needed to substantiate these results.

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