Use of a bonding agent to reduce sealant sensitivity to moisture contamination: an in vitro study
Janet C. Hitt, DMD  Robert J. Feigal, DDS, PhD

Abstract

Moisture contamination of etched enamel during application of sealant is the most frequently cited reason for sealant failure. In preliminary studies, a dentin bonding agent (Scotchbond Dual Cure®, 3M, St. Paul, MN) has been shown to bond adequately to etched enamel after salivary contamination. This study investigated bond strength in vitro, when a bonding agent was used beneath sealants under varied conditions of contamination. Five hundred bovine incisor crowns were separated randomly into eight groups. The enamel samples, etched for 60 sec with a 37% phosphoric acid gel, were contaminated with: 1) fresh whole saliva, air dried, 2) fresh whole saliva, left wet, or 3) moisture from a humidity chamber. All contamination conditions were tested for sealant bond strength with and without the bonding agent as an intermediate layer under the sealant. As controls, both sealant and bonding agent under sealant also were applied to clean etched enamel. Bond strength was measured using a universal testing machine. Data were analyzed using a two-way ANOVA. Under conditions of humidity or intact saliva, sealant alone showed significant reduction in bond strength (P < 0.001). Bonding agent under sealant on wet contamination yielded bond strengths equivalent to the bond strength obtained when sealant was bonded directly to clean, etched enamel. Bonding agent used without contamination yielded bond strengths significantly greater than the bond strength obtained when using sealant alone without contamination (P < 0.001). When the saliva was air dried onto the surface, there was no significant difference in bond strengths whether or not a bonding agent was used under the sealant. (Pediatr Dent 14:41-46, 1992)

Introduction

Sealants were introduced 25 years ago as a preventive method for controlling caries. Today, they are the primary method used to control occlusal decay. Numerous clinical studies have documented the efficacy of pit and fissure sealants in caries prevention, with sealant retention being the primary factor in continued efficacy. Most sealants which are lost are discovered at the six-month evaluation, indicating faulty application technique. Saliva contamination of the etched enamel surface before sealant placement is cited as the most common reason for sealant failure. Buonocore stated that saliva contact should be avoided since an acid conditioned enamel surface readily absorbs salivary constituents, reducing surface energy and rendering the surface less favorable for bonding. Other studies concur that surface changes occur which interfere with the bonding mechanism.

Hormati et al. tested the shear bond strength of sealant to etched enamel exposed to saliva contamination. There was a significant difference in bond strength between the group bonded to wet saliva and the other treatment groups, in which the saliva was either air dried or the samples rewashed or re-etched. This study also examined the type of fracture associated with the bond of sealant to enamel. The specimens exposed to saliva contamination generally had an adhesive failure of the bond, fracturing at the enamel-resin interface. The uncontaminated samples most frequently had cohesive bond failures, the failure occurring within the resin itself, leaving the bond intact.

It is generally accepted that an adhesive must initially wet the substrate to form a strong bond with that substrate. Therefore, maximizing the wettability of the tooth surface is critical in achieving a successful bond. Gwinnett et al. examined the fitting surface of sealants which had failed immediately upon placement in vivo. These surfaces tended to be relatively smooth, appearing not to have contacted etched enamel. They concluded that the enamel etching was inadequate or that the sealant had failed to wet and penetrate the enamel. Hormati et al. speculated that the lack of
bond strength found in their study on contaminated specimens could be due to the lack of wettability when a material was applied to a wet contaminated, etched enamel surface. Most of the porosities normally present in etched enamel are plugged when the enamel is wet; the penetration of the resin is impaired, resulting in tags of insufficient number and length.

Current sealant materials are unable to tolerate even minute amounts of moisture. Since complete control of intraoral moisture is difficult to achieve, the development of a hydrophilic resin, insensitive to moisture, may increase successful sealant retention. This would expand the role of sealants in preventive care in patients who present challenges to strict isolation methods such as the younger or handicapped patient.

Scotchbond® (Scotchbond Dual Cure®, 3M, St. Paul, MN), developed as a dentin bonding agent for bonding to the naturally wet dentin surface, has been shown to be an effective adhesive when bonding to enamel. A 1988 study by Baharav et al. concluded that Scotchbond demonstrated better wetting properties on etched enamel compared to Durafill enamel bond, as judged by the amount of tag penetration. Bond strengths up to two times greater were shown in bonding Scotchbond to etched enamel compared to conventional enamel bonding agents (unpublished laboratory data, 3M; 1985).

In 1987, Dorignac published the results of his clinical trial using a filled composite as a sealant, with and without Scotchbond as an intermediate layer under the sealant. Results after 2–1/2 years revealed retention rates of 97.8 and 98.6% in the Scotchbond groups, and 81.2% in the group using sealant alone. Six-month recalls revealed that the group with sealant alone showed a slow but regular loss of sealant, and the Scotchbond groups showed a low initial loss which stabilized by the end of the first year.

The objective of this study was to evaluate Scotchbond bonded to contaminated enamel as an intermediate layer under a sealant material and the effect of this intermediate layer on the bond strength changes caused by moisture contamination.

Materials and Methods

All contamination conditions were tested using the bonding agent (Scotchbond Dual Cure, 3M, St. Paul, MN) as an intermediate layer under the sealant (White Sealant®, 3M, St. Paul, MN). As controls, samples subjected to all conditions had sealant bonded directly to them. Uncontaminated controls, for both the bonding agent-sealant group and for the standard sealant group were run each day.

Enamel for this study was prepared from the crowns of 500 extracted bovine incisors. The roots of the teeth were removed and the pulps extirpated. The crowns were stored until needed (1–3 months) in distilled water. The teeth were potted in polymethylacrylate resin. Enamel surface preparation consisted of grinding facial surfaces of the teeth until an area of enamel was exposed that was at least 3/16 in. in diameter. A lapidary wheel was used for grinding, using running distilled water with successively finer grits of wet/dry sandpaper up to # 600. The teeth were stored at room temperature for no longer than 24 hr prior to continuing the procedure. Any film build-up from storage was removed by rinsing with distilled water and rubbing the polished surface of each plug with a paper towel moistened with distilled water. The area of enamel to be bonded was standardized by using teflon molds with consistent diameter holes. Steps of all procedures were standardized as follows:

1. Etch: 60 sec with a 37% phosphoric acid gel
2. Wash: 45 sec with distilled water
3. Dry: with oil-free compressed air until chalky
4. Contamination:
   a. Saliva air-dried: fresh whole saliva was collected daily from the principle examiner and was syringed onto the etched enamel until a film covered the entire enamel surface. This was left undisturbed for 5 sec prior to drying with oil-free compressed air for 5 sec.
   b. Saliva left intact: Fresh whole saliva was syringed onto the surface and left undisturbed for 5 sec before continuing the procedure.
   c. Humidity: samples were placed in a humidity chamber kept at 96% humidity for 1 min (distilled water humidity).
5. Bonding agent: a thin layer was applied with a sable brush, excess film was blown off with oil-free compressed air. This was light cured for 10 sec using a Visilux II light (3M).
6. Sealant: Sealant buttons for all eight groups were formed by clamping a teflon mold onto the acrylic plug. Sealant was added in two increments and cured for 20 sec each using a Visilux II light. The molds were unclamped 5 min after curing.

Eight groups were run each day of the study. The first two groups were run as a control using no contamination. Group 1 used the standard sealant procedure (sealant cured onto clean, dry, etched enamel). Group 2 had a layer of Scotchbond applied and cured before sealant application.

Groups 3 and 4 tested the contamination condition of saliva contact dried onto the enamel after etching.
After 5 sec of contact the saliva was dried on the enamel. In Group 3 sealant was added directly onto the dried, contaminated enamel. Group 4 had a layer of Scotchbond applied before adding the sealant. Groups 5 and 6 tested “unnoticed” contamination; contact with saliva which was left on the enamel. In Group 5 the sealant button was applied onto the wet film of saliva. In Group 6, Scotchbond was brushed into the film of saliva, excess material was blown off, then cured. Sealant was added to the cured Scotchbond. The final two groups tested the effects of humidity-induced moisture on the bond. After being etched and dried, groups 7 and 8 were placed in a humidity chamber for 1 min at 96% humidity. Group 7 samples then were removed and sealant was added to the film of moisture present. Group 8 samples were removed and Scotchbond was brushed into the moisture layer present, the excess blown off and then cured. Sealant was added to the cured Scotchbond. An excess number of enamel samples was ground each day to allow for sample rejection because of inadequate area of enamel. After the samples for each group were prepared, any extra samples were assigned to experimental groups. Only half the number of samples were prepared for Group 5 (sealant to wet contaminated enamel), since all the tested samples except for one had a value of 0.00. Minor variations in sample numbers are due to sample rejection because of inadequate enamel area.

The specimens were stored after preparation in distilled water at 37°C for 18-24 hr prior to testing adhesive strength. The samples were loaded in random order on the universal testing machine (Instron #1123®, Instron Corp., Canton, MA), and oriented so the direction of pull was always towards the cervical margin of the tooth. The testing machine was calibrated to normal sensitivity at the beginning of each testing session and was connected to an autographic machine set at a crosshead speed of 2 mm per min and 50 kg full scale on the chart paper. The sealant buttons were sheared off and the strength of each adhesion was calculated by dividing the shearing force by the area of the button (kg/cm²).

A 2 x 4 factorial design was used to analyze the effects of the two treatment methods on sealant bond strength under four conditions of contamination. Data were analyzed using a two-way analysis of variance. Bonferroni t-tests were used to compare groups.19

Results

The summary data for all groups are reported in Table 1. Table 2 reports the ANOVA results. There was a significant difference in overall bond strength between sealant and bonding agent under sealant (P < .005) and in bond strength between contamination conditions. However, there also was a significant interaction between the use of the bonding agent and the condition of contamination. The pattern of differences in bond strength without the bonding agent among the conditions of contamination does not match the pattern of differences found when the bonding agent is used.

<table>
<thead>
<tr>
<th>Materials</th>
<th>No Contamination</th>
<th>Saliva Contact Dried Off</th>
<th>Saliva Contact Left Undisturbed</th>
<th>Humidity Chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sealant</td>
<td>Group 1</td>
<td>Group 3</td>
<td>Group 5</td>
<td>Group 7</td>
</tr>
<tr>
<td>mean = 167.0</td>
<td>mean = 127.6</td>
<td>mean = 0.005</td>
<td>mean = 2.7</td>
<td></td>
</tr>
<tr>
<td>SD = 33.3</td>
<td>SD = 41.7</td>
<td>SD = 0.03</td>
<td>SD = 9.5</td>
<td></td>
</tr>
<tr>
<td>N = 52</td>
<td>N = 69</td>
<td>N = 38</td>
<td>N = 70</td>
<td></td>
</tr>
<tr>
<td>Scotchbond + sealant</td>
<td>Group 2</td>
<td>Group 4</td>
<td>Group 6</td>
<td>Group 8</td>
</tr>
<tr>
<td>mean = 205.0</td>
<td>mean = 128.7</td>
<td>mean = 178.0</td>
<td>mean = 172.3</td>
<td></td>
</tr>
<tr>
<td>SD = 45.7</td>
<td>SD = 46.6</td>
<td>SD = 41.3</td>
<td>SD = 60.7</td>
<td></td>
</tr>
<tr>
<td>N = 53</td>
<td>N = 70</td>
<td>N = 78</td>
<td>N = 70</td>
<td></td>
</tr>
</tbody>
</table>

* Means are reported as kg/cm².

Shear strength for all groups measured as the force required to shear the sealant button from the enamel surface.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean Square</th>
<th>F Ratio</th>
<th>Tail Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scotchbond</td>
<td>1</td>
<td>1.11623E+6</td>
<td>666.57</td>
<td>0.0000</td>
</tr>
<tr>
<td>Contamination</td>
<td>3</td>
<td>238013.6462</td>
<td>142.13</td>
<td>0.0000</td>
</tr>
<tr>
<td>Interaction</td>
<td>3</td>
<td>255375.7163</td>
<td>152.5</td>
<td>0.0000</td>
</tr>
<tr>
<td>Error</td>
<td>493</td>
<td>1674.5742</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA Table for Bond Strength Data. Probability values indicate significant differences due to presence of Scotchbond, presence of contamination and a significant interaction.
Group variances were unequal by Levene's test for equality of variances. This technically violates the assumptions of ANOVA. However, the Brown-Forsythe test, which does not require equal variances gave results equivalent to standard ANOVA. Results thus can be considered valid.

The pattern of means seen in Table 1 suggests that the use of the bonding agent under sealant yields significantly greater bond strength than using sealant alone under normal and contaminated conditions. To compare specific groups, Bonferroni t-tests were used (Figure). This t-test incorporated an adjustment into the alpha level because more than two groups were being compared. Since group variances were unequal, a version of the Bonferroni t-test was used which allows differences in variances.

When there was no contamination, using a bonding agent under a sealant resulted in a bond strength of 205.0 ± 45.7 that was significantly greater than the bond strength obtained when using sealant alone (167.0 ± 33.3), P < 0.001. Data show that substantial differences in bond strength occurred in the presence of contamination. The most marked effect occurred in the wet contaminated groups. The bond strength between enamel and sealant suffered greatly in the presence of saliva (0.005 ± 0.03) or humidity (2.7 ± 9.5). Yet when the bonding agent was used under the sealant, the bond strength was uncompromised (wet saliva group: 178.0 ± 41.3; humidity group: 172.3 ± 60.7). Both of the dried saliva groups showed a significant decrease in bond strength compared to sealant on uncontaminated enamel with no significant difference in bond strength between sealant on dried saliva (127.6 ± 41.7) and bonding agent under sealant on dried saliva (128.7 ± 46.6).

**Discussion**

In this study, using a bonding agent under sealant as an intermediate layer significantly increased bond strength on wet contaminated enamel. When there was no contamination, as in ideal operating conditions, using the bonding agent under the sealant yielded a significantly stronger bond.

Bovine teeth have served as a test substrate in many in vitro experiments. The large, relatively flat area of the labial surface of the incisors and the availability of bovine teeth make them a useful alternative to human teeth. Nakamichi et al. compared the bond strength of five dental cements and two composite resins to human and bovine enamel. No statistically significant difference was found in adhesive strength between the two types of enamel.

Fresh, whole human saliva is an acceptable material in testing saliva contamination. Koulourides et al. conducted a study in which the saliva from different individuals was tested to determine its capacity to reharden softened enamel. Synthetic saliva solutions also were tested. Their results indicated that the ability to reharden softened enamel surfaces varies between persons but tends to be consistent for the same individual. Adding various ions to saliva or using a synthetic solution seemed to improve the initial hardness recovery values, but final hardness values were not greater than for the unaltered human saliva.

Bond strength measurements have been evaluated as to tensile bond strength (force at right angle to tooth/resin interface) and as to shear bond strength (force parallel to tooth/resin interface). A problem associated with tensile testing of the bond is that of increased cohesive failures because the force is transmitted through the bulk of the adhesive, obscuring the true bond strength values. Testing bond strength by the shear method places the maximum force at the tooth/resin interface with a more reproducible interfacial fracture observed. This results in far fewer cohesive failures.

**Technique Sensitivity**

Gwinnett suggests that the resin-enamel interface is the weakest area of the bonding mechanism. This area is dependent upon the clinical technique and proficiency of the operator.
Rock and Bradnock\(^3\) found a significant difference in the retention rates of sealants when placed by different operators. All factors were controlled between the two operators, with the only variable in the study being each operator's application technique. Simonsen's\(^4\) three-year clinical results reflected this same difference in retention rates within operators, with retention rates increasing in patients treated later in the study. This can be attributed to the increased proficiency of the operator when performing the procedure. These and other studies argue that technique is an important factor in determining successful sealant retention.

The aspect of technique sensitivity most often cited as a reason for sealant failure is moisture contamination of the newly etched enamel surface. Our study suggests that this important technique-sensitive factor may be counteracted by modifying the technique.

**Contamination**

Even when stringent moisture control procedures are attempted during sealant application, contamination can occur. Minute contamination also occurs in areas where total isolation is not possible, such as buccal or lingual grooves which originate subgingivally. These contamination conditions are a likely cause of sealant failure.

It is significant that the use of Scotch bond on wet contaminated enamel permits bond strength equivalent to the bonding obtained in noncontaminated situations. Scotch bond as used in this study was applied in the presence of excessive amounts of contamination, yet it bonded to the enamel with the same bond strength as sealant to uncontaminated enamel. The amount of contamination encountered in a clinical situation would likely be less excessive and may result in greater bond strength.

The pattern of differences in bond strength seen in the ANOVA when sealant without Scotch bond is used in contaminated conditions does not match the pattern of differences found when Scotch bond is used under the sealant. Clearly, the interaction is due to almost total failure of Groups 5 (sealant on wet intact saliva) and 7 (sealant on humidity).

Hormati et al.\(^{13}\) speculated that the lack of bond strength achieved in their study of composite to saliva-contaminated enamel could be due to the lack of wetting ability of a wet etched enamel surface. Most of the porosities normally present are plugged with moisture when the enamel is wet. This results in a lack of penetration of the resin which results in tags of insufficient number and length to give adequate retention of the resin to enamel.

We can only speculate as to why Scotch bond is able to bond to wet contaminated enamel. Scotch bond appears to have a more hydrophilic nature than current sealant materials, and may somehow displace the saliva from the enamel surface, permitting the penetration of the Scotch bond into the enamel porosities. The decreased bond strength found for both sealant alone and Scotch bond under sealant in the dried saliva groups can be explained by the dried saliva blocking some of the enamel porosities, therefore decreasing the number of enamel pores available for material penetration.

**No Contamination**

Simonsen\(^{29}\) advised the use of an unfilled resin as an intermediate layer under a filled sealant when placing a small preventive resin restoration. He thought that using an unfilled resin improved the clinical handling of the material, ensuring optimal tag penetration. Dorignac\(^{18}\) concluded that using a halogenated bis-GMA resin such as Scotch bond results in reliably increased retention when used as an intermediate layer beneath a sealant. The use of a bonding agent under a sealant in this study resulted in a significantly stronger bond than of sealant alone when operating under the ideal condition of no contamination. Adding this additional step to the sealant application procedure might be justified.

**Summary**

While this study shows beneficial effects of a bonding agent on the sealant to enamel bond strength, more stringent tests must be conducted to prove clinical effectiveness in the face of moisture contamination. Studies of leakage between sealant and enamel as well as long-term clinical retention studies are now in progress.

This study in no way suggests that improper technique for sealant placement can be advocated. Bond strength is improved measurably for the Scotch bond under sealant when there is no contamination. In addition, this study tested contamination at only one step of the sealant procedure. Contamination with moisture at other parts of the procedure, as one would expect using inadequate clinical isolation, may result in negative effects on sealant retention which are beyond the benefits of a bonding agent.

**Conclusions**

1. When there is no moisture contamination, using Scotch bond under sealant results in a significantly stronger bond strength of sealant to enamel than when using sealant alone.
2. When there is moisture contamination, using Scotch bond under sealant results in bond strength equivalent to the bond strength of sealant on uncontaminated enamel.
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Dr. Hitt is in private practice, and also is clinical assistant professor, and Dr. Feigal is associate professor and chairman, in the Division of Pediatric Dentistry, University of Minnesota School of Dentistry, Minneapolis, MN. Reprint requests should be sent to Dr. Robert J. Feigal, 6-150 Moos Tower, University of Minnesota, Minneapolis, MN 55455.