Shear bond strength of adhesive systems to saliva-contaminated dentin

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Abstract
This study evaluated the shear bond strength of two adhesive systems: Scotchbond Multi-Purpose (SBMP) and Clearfil Liner Bond 2V (CLB2V) on different dentin surface conditions. Bovine incisors were collected and prepared according to the ISO’s (TR 11405) directions. The samples were divided into nine groups (n=10): 1- SBMP control group (without contamination); 2- SBMP: saliva contamination after priming; 3- SBMP: saliva contamination after bond application; 4- SBMP: saliva contamination after acid etching; 5- SBMP: as group 4 with re-etching after saliva contamination; 6- CLB2V control group (without contamination); 7- CLB2V: saliva contamination after priming; 8- CLB2V: saliva contamination after bond application; and, 9- CLB2V: saliva contamination before priming. After the storage period in water at 37°C, the samples were tested with an Instron testing machine, at crosshead speed of 0.5 mm/min. The results were submitted to ANOVA (one way) and Tukey’s test at 5% significance level. For SBMP groups, the mean shear bond strengths were: 1 - 4.8 MPa, 2 - 5.0 MPa, 3 - 5.2 MPa, 4 - 6.6 MPa and 5 - 4.2 MPa. There was no statistical difference among groups tested. For CLB2V groups 6, 8, 9, the mean shear bond strengths were 8.0 MPa, 6.9 MPa and 8.8 MPa respectively and demonstrated no statistical differences. Group 7 (4.7 MPa) differed from all other groups except 8. Slight salivary contamination does not influence the shear bond strength of SBMP during any stage and slight salivary contamination influences the shear bond strength of CLB2V after priming step.

Key Words:
dentin-bonding agents, saliva, contamination, shear bond strength, resin composite.

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Introduction

The search for improved adhesive materials has been object of considerable research in recent years. Buonocore was the first to demonstrate that the adhesion of acrylic restorative resin to enamel is possible using acidic etching with 85% phosphoric acid on the enamel surface for one minute. Today, it is known that bonding efficiency is necessary to oppose and withstand contraction stress during curing of restorative composite and insure retention and marginal sealing of restoration. If stress generated during curing is greater than the bond strength of the adhesive system, clinical failure may occur.

The chemical composition of the bonding systems and the condition of the dental structure may affect the bond strength. For years, textbooks of operative dentistry and adhesive manufacturers’ instructions have recommended the use of a rubber dam for best results. However, recently published studies show that rubber dam is applied in fewer than 10% of restorative treatments. In these treatments, contamination of the tooth surface by saliva, blood, and excessive moisture is likely to occur and bond strength is decreased. Some studies have reported that saliva-contaminated and un-washed surfaces provide significantly lower bond strengths than non-contaminated surfaces. Blood-contaminated surfaces also decrease the bond strength more than the other adverse conditions. When salivary contamination occurs, it is clinically accepted that conditioned enamel and dentin must be re-etched prior to procedure with the adhesive technique to acquire sufficient bond strength.

The purpose of this study was to evaluate the effect of saliva contamination upon different stages of bonding procedure.

Material and Methods

Two different adhesive systems were used, Scotchbond Multi-Purpose (3M ESPE, Dental Products, St Paul, MN, USA) and Clearfil Liner Bond 2V (Kuraray, Co. Ltda, Kurashiki, Japan)

For the specimen preparation, 90 freshly extracted permanent bovine incisors were collected and prepared according to the ISO’s (TR 11504) directions. After root removal, crowns were cleaned and embedded with buccal surfaces on the outside of a PVC cylinder using chemically cured acrylic resin (Clássico Artigos Odontológicos Ltda, Campo Limpo Paulista, SP, BRASIL). The buccal surfaces of teeth were ground with a mechanical grinder (APL-4, Arotec, Cotia, SP, BRASIL) to expose superficial dentin, and subsequently polished with wet 80-, 180-, 400- and 600-grit silicon carbide abrasive paper. Specimens were randomly divided into nine groups of ten specimens each. Scotchbond Multi-Purpose was applied in five groups and Clearfil Liner Bond 2V, in the other groups.

Group 1: Scotchbond Multi-Purpose (SBMP) control group. The manufacturer’s instructions were followed for adhesive application. The dentin surfaces were etched for 15 seconds with 37% phosphoric acid, rinsed with air-water spray and gently air dried, not desiccated. The primer was applied to the etched surfaces with a disposable brush tip and gently air dried for 5 seconds. The bond agent was applied and light-cured for 20 seconds using a XL3000 curing light (3M ESPE, Dental Products, Germany). A restorative composite, Z100 (3M ESPE, Dental Products, St Paul, MN, USA), was inserted into a 4 mm diameter x 5 mm height mould filled by 3 increments, each light cured for 40 seconds.

Group 2 (SBMP): As group 1, except that the etched and primed surfaces were contaminated with fresh whole saliva, which was left undisturbed for 20 seconds. The excess of saliva was removed using a gentle air spray and the bond agent was applied and light-cured for 20 seconds.

Group 3 (SBMP): As group 1, except that the salivary contamination occurred after applying the bonding agent. The surface was gently air dried before the insertion of restorative composite.

Group 4 (SBMP): As group 1, except that salivary contamination occurred after acid etching. The surface was gently air dried before the priming step.

Group 5 (SBMP): As group 4, except that after 20 seconds, the saliva was rinsed, dried with air-water spray for 15 seconds and surface was re-etched for 10 seconds.

Group 6: Clearfil Liner Bond 2V (CLB2V) control group. All the manufacturer’s directions were followed. After cleaning the dentin surface with pumice slurry, a mixture of primers A and B in equal proportions was applied to the dentin surface with circular movements. Bonding agent was applied on the surface, air dried and light-cured for 20 seconds. The restorative composite was applied in the same manner as described for Scotchbond Multi-Purpose groups;

Group 7 (CLB2V): As group 6, except that, after applying the primer, the surface was contaminated with whole fresh saliva for 20 seconds. The excess of saliva was removed gently using an air spray and the adhesive applied and light-cured for 20 seconds.

Group 8 (CLB2V): As group 6, except that salivary contamination occurred after application of bonding agent. The surface was gently air dried before the insertion of restorative composite.

Group 9 (CLB2V): As group 6, except salivary contamination occurred before all steps of bonding procedure. This study was performed in controlled temperature and humidity conditions of 23 ± 1°C and 50± 10% relative humidity. The specimens were stored in distilled water for 24 hours at 37°C. After this period, they were tested on an Instron testing machine. Shear load was applied parallel to the resin composite/dentin interface using a stainless steel tape of 5 mm width and 10 cm length, forming a loop involving the composite cylinder. The test was performed at a crosshead...
speed of 0.5mm/minute and the shear bond strength was calculated in MPa.
After debonding, the specimens were examined under a light optical stereomicroscope (Carl-Zeiss, Oberkochen, Germany) at 40x magnification to verify failure type. The failure type was classified as adhesive (failure at adhesive layer), cohesive (failure at dentin or resin composite) and mixture (failure between adhesive layer and substrat).

Results
The results were submitted to one-way analysis of variance (ANOVA) followed by Tukey’s test at a 5% significance level.
The results of the Scotchbond Multi-Purpose groups are shown in Table 1 and Figure 1. According to the multiple comparison tests, there were no statistical differences among groups 1, 2, 3, 4 and 5. The salivary contamination had no significant influence on the mean shear bond strength among tested groups.
The results of Clearfil Liner Bond 2V groups are shown in Table 2 and Figure 2. There were no statistical difference among groups 6, 8 and 9, although group 7 demonstrated a higher mean. Group 8 was not statistically different from group 7.

Table 1 - Mean shear bond strengths for Scotchbond Multi Purpose groups (MPa).

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>MEANS (SD)</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.890 (1.26)</td>
<td>a</td>
</tr>
<tr>
<td>2</td>
<td>5.018 (2.85)</td>
<td>a</td>
</tr>
<tr>
<td>3</td>
<td>5.283 (1.74)</td>
<td>a</td>
</tr>
<tr>
<td>4</td>
<td>6.602 (2.12)</td>
<td>a</td>
</tr>
<tr>
<td>5</td>
<td>4.243 (0.98)</td>
<td>a</td>
</tr>
</tbody>
</table>

Means designated with the same letter are not significantly different (p>0.05).
SD = Standard deviation.

Table 2 - Mean shear bond strengths for Clearfil Liner Bond 2V groups (MPa).

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>MEAN (SD)</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>8.014 (1.42)</td>
<td>a</td>
</tr>
<tr>
<td>7</td>
<td>4.792 (2.17)</td>
<td>b</td>
</tr>
<tr>
<td>8</td>
<td>6.982 (2.09)</td>
<td>ab</td>
</tr>
<tr>
<td>9</td>
<td>8.823 (3.06)</td>
<td>a</td>
</tr>
</tbody>
</table>

Means designated with the same letter are not significantly different (p>0.05).
SD = Standard deviation.

Discussion
An adequate bond strength is obtained after a complex process that involves substrate conditions and adhesive chemical composition amongst other factors. Most cavity preparations extend beyond enamel and dentin. It is well established that an enamel bond is more predictable than a dentin bond, since its chemical composition depends on the depth of dentin and individual patient factors. A significant fraction of the total water content in dentin is confined to dentin tubules and the density of tubules varies with dentinal depth. The water content is lower in superficial than in deep dentin.

In this study, two different adhesive systems were used. The manufacturer of Scotchbond Multi-Purpose recommends total smear layer removal by acid etching for 15 seconds. On enamel, the acid-etching step increases the surface free energy improving the bonding system infiltration. Conversely, on dentin the acid etching step decreases the surface free energy due to loss of hydroxyapatite crystallites and exposure of collagen fibrils. The demineralized dentinal matrix must first be infiltrated by a primer that has hydrophilic properties and then by a bonding agent with bifunctional HEMA molecules and hydrophobic monomers. However, this procedure has some disadvantages, such as, the dentinal matrix may be etched for a long period causing overwetting or overdrying. In addition, the three-steps procedure is time consuming and the surface may be contaminated with saliva.
or blood during the restorative procedure. The second bonding system used was Clearfil Liner Bond 2V, an example of a “self-etching primer”. Watanabe et al. in 1990, hypothesized that a bonding system could be developed based on the use of non-rinse acidic monomers that simultaneously etch and prime dentin and enamel. The advantage of this self-etching primer system is a simplified technique, which does not require rinsing and drying steps, avoiding overwetting or overdrying of demineralized dentin. Finally, the risk of incomplete resin infiltration is decreased by simultaneous infiltration of the acidic monomers.

The Clearfil Liner Bond 2V system is also based on hybridization of hard dental tissues, demonstrating some differences compared to the acid-etch step adhesive systems. Submicron hybrid layers are formed and resin-tag formation is less pronounced within such submicron hybrid layers, collagen fibrils are not completely deprived from hydroxyapatite. This residual hydroxyapatite may serve as a receptor for additional intermolecular interactions with groups of functional monomers, resulting in higher bond strengths with more resistance to the hydrolytic degradation process and thus may aid by sealing the restoration margins for longer periods.

During the bonding procedure, one of the major clinical problems is the cavity contamination by saliva or blood. Salivary contamination is always found in cavities below the gingival margins or during incorrect use of a rubber dam. Pashley et al. reported that blood or salivary contamination promotes physical obstacles by depositing of macromolecules of these contaminants into dentinal tubules. Benderli et al. concluded that saliva contamination might be a risk factor to the bonding success.

Our findings demonstrate, however, that a light salivary contamination does not interfere on the bond strength values, as observed in prior studies. The groups treated with Scotchbond Multi-Purpose showed no statistical difference among groups, showing tolerance of the bonding system at different steps of salivary contamination in this study. In contrast, all factors that prevent, or making difficult, the penetration of salivary proteins into the dentin tubules is favorable to bonding success after contamination. As previously reported by El-Kalla, it may be postulated that the hydrophilic nature of adhesives allows them to work well in the presence of saliva in all groups. The lower the protein concentration of saliva (0.2-1.0 g%) in the film formed by salivary proteins, in group 3, did not prevent the chemical bond between the composite resin Z100 and the bonding agent.

The manufacturer of Scotchbond Multi-Purpose and Xie et al. assert that a re-etching step with phosphoric acid after salivary contamination is the only way to recover the bond strength decrease during salivary contamination. According to the presented data, bond strength values following re-etching of a contaminated surface (group 5) were the same as those of the control group. It should be emphasized, however, that a risk of deep and excessive dentin demineralization with incomplete infiltration of bonding system exists. This fact may decrease the mean bond strength and promote nanoleakage.

Groups 8 and 9 were treated with Clearfil Liner Bond 2V and showed no statistical difference in mean shear bond strength compared to Group 6 (control). The salivary contamination after applying the bonding agent (group 8) did not prevent bonding between the agent and composite resin, caused by the lower protein concentration of saliva. Like the control group, self-etching primer of this system was able to promote bond strength on surfaces without prophylaxis with pumice slurry (group 9), probably due to the capacity of acidic monomers to infiltrate beyond the organic barrier formed by salivary proteins and the smear layer.

The mean bond strengths decreased more, however, when the salivary contamination occurred after priming step (group 7). This reduction in bond strength is probably caused by water present in the saliva, which diluted the acidic monomer concentration in primers A and B. Consequently, the reduction in acidity decreases the demineralization procedure at the substrate.

Salivary contamination can occurs during restorative procedures at deep cervical lesions or due to incorrect use of the rubber dam. This situation often occurs in pediatric dentistry caused by obvious difficulties. Unnoticed contamination during any clinical procedure should also be considered. Thus, salivary contamination during the bonding procedure has a significant effect on bond strength depending on the composition and bonding mechanism of adhesive system.

Conclusions
From this study we can conclude that slight salivary contamination does not influence the shear bond strength of Scotchbond Multi-Purpose during any stage; but slight salivary contamination influences the shear bond strength of Clearfil Liner Bond 2V after the priming step.

References