Effect of Different Saliva Decontamination Procedures on Bond Strength to Dentin in Single Bottle Systems

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Statement of Problem: Following the increasing use of composites in restoring anterior and posterior teeth, problems due to its technique sensitivity have become a major concern. One of these problems is the possibility of contamination of dentin with saliva, blood and/or gingival fluid in different stages of bonding procedure, even with application of different methods of isolation. However, by introduction of Single-bottle dentin adhesives, the contamination possibility reduced to two stages. Scientific documents show that saliva contamination reduces bond strength of composites to dentin. Application of simple and efficient methods for reducing or eliminating saliva contamination enables clinicians to carry out dental treatment without any concern about deterioration of clinical longevity of restoration.

Purpose: This study was designed to compare the effect of different decontamination methods on the shear bond strength of composite to dentin using a “Single-bottle” adhesive.

Materials and Methods: Seventy-two extracted sound human molars and premolars were selected. Enamel of buccal surface was ground flat to expose dentin. The teeth were divided into 9 groups of 8 each. In control group (1) the adhesive “Excite” was used according to the manufacturer, without any contamination. Conditioned and saliva contaminated dentin was (2) rinsed and blot dried, (3) rinsed, dried and re-etched. In groups 4, 5, 6 uncured adhesive was saliva contaminated and then: (4) only blot dried (5) rinsed, blot dried with adhesive reapplication and (6) resurfaced with bur, rinsed, dried and followed by repeating the whole process. In groups 7, 8, 9 cured adhesive was contaminated with saliva and then: (7) rinsed and dried (8) rinsed, blot dried with adhesive reapplication (9) same as group (6). Then “Tetric Ceram” composite cylinders were bonded to dentin surfaces. Samples were thermo cycled in 5°C and 55°C water, 30 seconds in each bath with a dowel time of 10 seconds for 500 cycles. Finally, samples were sheared using Instron testing machine and shear bond strength data were subjected to one way ANOVA analysis, and Tukey HSD PostHoc. Mode of failure of samples was examined under Stereomicroscope (x40) and using Log-rank survival data analysis.

Results: No statistically difference between mean shear bond strength of groups 1,2,3 was observed (P=0.543). Comparison of groups 4,5,6 with group 1 showed that shear bond strength of group 4 was significantly lower (P<0.001). Mean shear bond strength of groups 1,7,8,9 were not significantly different (P=0.150). The major mode of failure was cohesive
either in composite or dentin bonding.

**Conclusion:** Blot-drying of saliva-contaminated uncured Single-bottle adhesive significantly decreased shear bond strength of composite to dentin. In other contamination protocols, the effect of treatments applied did not differ.

**Key words:** Saliva contamination; Dentin bonding agents; Single-bottle dentin bonding system; Shear bond strength

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Composite resins, though acceptable in many areas, are technique sensitive. Isolation of the operating field is a prerequisite for success in esthetic treatments with these materials. (1) In a clinical study, all the composite restorations inserted without rubber dam, showed marginal leakage. (2)

Unfortunately, it is not possible to use rubber dam in all clinical cases, and when using cotton rolls or packs during the bonding procedure, some kind of contamination may happen. The effect of saliva contamination on the bond strength of composite to tooth structure has been studied. The results vary in different researches due to the difference in bonding system or tooth substrate (enamel or dentin).

Although newer bonding systems are more resistant to the effect of salivary contamination, it is generally accepted that contamination with saliva decreases the bond strength of composite to dentin. (2,3,4,5,6) A definite approach to limit the effects of saliva contamination has not been proposed yet.

The aim of this study was to compare the effect of different treatments of contaminated dentin, in different steps of single bottle adhesive usage, on the shear bond strength of composite to dentin.

**Materials and Methods**

In an interventional in-vitro study, seventy-two extracted sound molar and premolar teeth were selected.

The teeth were cleaned from tissue remnants and stored in distilled water with thymol for a week and in distilled water until they were used (<6 month from extraction).

Then the teeth were embedded in self-cure acrylic resin in metal cylindrical molds, in a manner that anatomic crown was kept out.

The teeth were randomly divided into nine groups each of which had eight teeth.

The buccal surface of teeth was ground until a flat dentinal surface was exposed at the same distance from DEJ in all samples.

The materials used consisted of Total Etch (37% phosphoric acid), Excite (single bottle dentin adhesive), Tetric Ceram (micro hybrid composite). All the products were manufactured by Vivadent.

The tested groups were prepared as follows:

**Group 1:** No contamination = Control

Dentin was etched for 15 seconds, washed vigorously with water, then the surface was blot dried and bonding agent applied as the manufacturer recommended. Then the composite was inserted on its surface by a cylindrical mold of 2mm height, 2.5mm diameter. The composite was inserted in 2 increments. Each increment was cured for 40 seconds.

**Group 2:** The etched dentin got contaminated by fresh, healthy saliva for 10 seconds. The samples were washed for 5 seconds, blot dried, and then bonding agent and composite were applied as in group 1.

**Group 3:** The etched dentin got contaminated with saliva for 10 seconds, washed for 5 seconds and dried. The etching was reapplied for 10 seconds, and the bonding procedure was repeated as in group 1.

**Group 4:** In this group saliva contamination was applied after etching and bonding application, but before curing the bonding. The contaminated surface was blot dried, bonding was cured and composite was inserted.
Group 5: In this group, after etching and bonding application and before curing, the contamination occurred. Then the surface was washed for 5 seconds. The bonding was reapplied, and composite insertion was done as in group 1.

Group 6: The contaminated uncured bonded surface was cut using a bur, and all the bonding process was repeated as in group 1.

Group 7: In these specimens, saliva contamination occurred after curing the bonding agent. Then the surface was washed for 5 seconds and again a layer of bonding agent was applied to the surface and cured, finally the composite was inserted.

Group 8: Saliva contamination occurred after curing the bonding agent. Then the surface was washed for 5 seconds then blot dried and again a layer of bonding was applied to the surface and cured. Then composite was placed.

Group 9: The contaminated cured bonded surface was cut by a bur as in group 6 and the bonding process was repeated as in the control group.

After the bonding procedures, the samples were thermocycled for 500 cycles in distilled water at 5°C and 55°C (30 seconds in each bath) and 10 seconds between each bath.

Then the samples were tested for shear bond strength. An Instron universal testing machine with a crosshead speed of 0.5 mm/min was used to apply shear stress to the bonding interface.

Shear bond strengths data were subjected to one-way ANOVA and Tukey HSD Post Hoc tests.

The mode of failure of surfaces was then examined by stereomicroscope with a magnification of ×40. Then, Log-Rank survival data analysis applied for comparing bond strength between groups.

**Results**

Table I shows the mean shear bond strength of the samples. The difference of shear bond strength between groups 1,2,3 was not significant (P=0.543) (Fig 1).

Comparing the groups 4, 5, 6 and the control, the results showed that shear bond strength of group 4 was significantly lower (P<0.001), but groups 5 and 6, were neither significantly different from each other, nor from the control group (Fig 2). Shear bond strength between groups 7,8,9 and the control was not significantly different (P=0.15) (Fig 3).

Microscopic observations revealed that the failures were mostly cohesive either in composite or dentin bonding (Table II).

Survival analysis showed no significant difference between shear bond strength of the groups 1, 2, 3 but the bond strength of group 1, was significantly different from groups 4, 5, 6 and from groups 7, 8, 9.

**Table I- Mean shear bond strength of the groups 1-9 (MPa)**

<table>
<thead>
<tr>
<th>Group No</th>
<th>Shear Bond Strength (Mean± SD) MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.64 ± 3.75</td>
</tr>
<tr>
<td>2</td>
<td>17.27 ± 2.60</td>
</tr>
<tr>
<td>3</td>
<td>15.24 ± 6.56</td>
</tr>
<tr>
<td>4</td>
<td>4.72 ± 3.91</td>
</tr>
<tr>
<td>5</td>
<td>19.96 ± 7.60</td>
</tr>
<tr>
<td>6</td>
<td>15.84 ± 8.78</td>
</tr>
<tr>
<td>7</td>
<td>17.37 ± 2.84</td>
</tr>
<tr>
<td>8</td>
<td>19.45 ± 8.22</td>
</tr>
<tr>
<td>9</td>
<td>21.30 ± 4.66</td>
</tr>
</tbody>
</table>

**Table II- Failure mode of samples after stress applying**

<table>
<thead>
<tr>
<th>Group No</th>
<th>Mode of Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA  M  CC  CD</td>
</tr>
<tr>
<td>1</td>
<td>5  1  1  1</td>
</tr>
<tr>
<td>2</td>
<td>5  2  1  1</td>
</tr>
<tr>
<td>3</td>
<td>6  2  2  2</td>
</tr>
<tr>
<td>4</td>
<td>6  1  1  1</td>
</tr>
<tr>
<td>5</td>
<td>7  1  1  1</td>
</tr>
<tr>
<td>6</td>
<td>4  1  2  2</td>
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<tr>
<td>7</td>
<td>5  2  1  1</td>
</tr>
<tr>
<td>8</td>
<td>5  3  1  1</td>
</tr>
<tr>
<td>9</td>
<td>2  3  1  1</td>
</tr>
</tbody>
</table>

CA=Cohesive in Adhesive  M = Mixed
CC=Cohesive in Composite  CD=Cohesive in Dentin
Fig 1- Comparison of shear bond strength (mean and 95% confidence interval) in the control group 1 and groups 2 and 3

Fig 2- Comparison of shear bond strength in the control group (1) and groups 4,5,6

Fig 3- Comparison of the shear bond strength in the control group (1) and groups 7,8,9

Discussion
Salivary contamination of operating field is a frequent problem in restorative procedures, especially when rubber dam isolation is difficult or impossible, eg, in deep cervical lesions, or when an indirect esthetic restoration is seated,(7) or even in patients having problem in opening their mouth.

Effect of salivary contamination is a matter of great controversy. Some studies reported that saliva-contaminated and unwashed enamel provided significantly lower bond strengths of resin composite to enamel.\(^{(1,8)}\) Others reported that the use of dentin bonding agents under fissure sealants reduced their sensitivity to saliva contamination\(^{(9-10)}\) and provided high bond strengths.

Pashley believes that bonding systems are sensitive to excess moisture, artificial saliva and plasma\(^{(11)}\) and has reported “The absorption of macromolecules into the dentin” as the major mechanism.\(^{(12)}\)

It seems that the hydrophilic nature of the newer dentin adhesives may bring about some concern about their behavior in presence of saliva contamination.\(^{(6)}\)

In the present study, natural saliva was chosen as the contaminant, because artificial saliva may confound the results. In addition, many studies have accepted whole healthy human saliva as an acceptable contaminating medium.\(^{(8,13,14,15)}\) An in-vitro model to mimic clinical conditions, proved saliva and plasma to be detrimental to hybrid layer formation.\(^{(16)}\)

Shear bond strength is a common method to evaluate efficiency of dentin bonding.\(^{(6)}\) It has been reported that if shear bond strength of composite to dentin and enamel ranges between 15-35 MPa, the system is clinically acceptable.\(^{(17)}\)

In the present study, no significant difference was seen when the contaminated dentin was either washed only, or washed and re-etched. In previous bonding systems, after contamination, it was necessary to etch the dentin again, but in one bottle systems, washing and blot drying of the contaminated dentin seems to be enough.

Powers et al (1995) reported the same results.\(^{(4)}\)
Also, Fritz et al (1998) showed that re-etching is not necessary when contamination with saliva happens.\(^7\)

EL-Kalla and Godoy, believed that when saliva-contamination happens after etching the dentin, blot-drying surfaces achieve bond strengths equal to uncontaminated groups.\(^{18}\) The manufacturers recommend to etch the contaminated etched dentin, and some researches have reported reduced bond strengths following saliva contamination and have insisted on removing the surface of the dentin and repeating the whole bonding process.\(^{19}\)

The following explanations are proposed to clear the results:

a) In wet bonding, residual moisture following acid washing prevents the salivary proteins from entering dentinal tubules and exposed collagen.\(^{20}\)

b) Hydrophilicity of adhesive makes it able to chase with water and saliva. Ethanol in the Excite system, also gives water. Chasing ability to the bonding agent.\(^{18}\)

c) Water in the saliva may hydrate the dentin surface and improve the bonding.\(^{21}\)

Comparison of samples contaminated after application of adhesive and before curing, with the control group, showed that bond strength in group 4 was significantly lower than other three groups.

In this group, the samples were not washed, so the salivary proteins prevented the composite from intimate contact with adhesive. Also the residues of saliva after blot-drying may inhibit complete polymerization of the adhesive.

Blot-drying seems to remove adhesive, therefore reduction in bond occurs. Microscopic observations (very thin bonding layer, even destroyed in some places), can justify this statement. Observing no difference in groups 5, 6 and 1, may prove that only blot-drying of the contaminated uncured bonding is detrimental and “washing and reapplying the adhesive” or “resurfacing and repeating the process” are both acceptable. Fritz et al, have reported similar results.\(^7\)

When comparing different treatments, in samples, which became contaminated after curing the adhesive, no significant difference was observed.

Fritz states that cured adhesive, when contaminated must be removed because it cannot be wetted with the composite material. The glycoproteins are reported to be the barrier. He also believes that during washing and drying, the oxygen-inhibited layer is removed. As a result, copolymerization with the next layer is impaired.\(^7\)

It should be noted that the salivary proteins are removed during washing the contaminated surface, so it is doubtful if it can act as a barrier in composite bonding.

Finger et al have proved that composite bonding to cured adhesive is the same, when the oxygen inhibited layer is removed or not.\(^{22}\)

In his research, Fritz, washed the contaminated cured adhesive, air-dried and applied another adhesive layer.\(^7\)

We didn’t air dry the surface, but blot-dried it and the second adhesive layer was then applied. May be that’s why we came to opposite results. Because we used wet bonding, undiffused collagens have not collapsed and the new adhesive layer penetrated the porosities, so the bond strength did not weaken.

All the samples failed either by mixed or cohesive (in adhesive, composite, or dentin) type. This means that an intimate bond existed between dentin and composite.

It must be mentioned that when stress is applied to the interface, microcracks initiate, and by their progression, the final fail happens.

It is not possible to determine the exact point a microcrack begins, when observing the failure mode.\(^{17}\)

It has been stated that shear bond strength of 17-20 MPa, can match the polymerization shrinkage, so all the treatments proposed have given a reliable bond, except group 4.
References: