Comparative evaluation of shear bond strength of different bonding system with effect of saliva contamination and decontamination: An in vitro study

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Abstract
Introduction: This study aims for the bond strength measurement of two new self etch bonding agent and effect of salivary contamination and decontamination on the bonding agent strength.

Materials and Methods: Premolar teeth, 60, removed during orthodontic treatment were collected and the buccal surfaces of the tooth were made to a plane dentinal surface. The randomly divided samples were distributed into three subgroups for Optibond All in one kerr (BSA) and three subgroup for Single Bond Universal 3M (BSB) of 10 each. For optibond all in one (BSA), BSA-I is the control group (self-etch bonding agent applied to flat dentine), BSA-II is the contamination group (self-etch bonding agent applied, followed by saliva application and then dried with air), BSA-III is the decontamination group (application of self-etch bonding agent, followed by contamination of saliva, dried with air and then reaplication of self-etch bonding agent).

For Single Bond Universal 3M (BSB), BSB-I - The control group (self-etch bonding agent applied to flat dentine), BSB-II - The contamination group (self-etch bonding agent applied, followed by saliva application and then dried with air), BSB-III - The decontamination group (application of self-etch bonding agent, followed by contamination of saliva, dried with air and then reaplication of self-etch bonding agent).

Followed by the bonding procedure, a 5mm composite block with bulkfill (ivoclar vivadent) was built on the flat dentine surface. Instron universal testing machine (USA) with a 1 mm per minute cross head speed was used to test the bonding strength. Data Obtained were subjected to statistical analysis by one way ANOVA test, and tukey’s multiple comparison and unpaired t-test for the inter group comparison.

Result: In the Kerr group (BSA), the shear bond strength of the contamination sub-group (BSA-II) decreased to 5.57 ± 1.77 MPa, as opposed to 13.87 ± 2.12 MPa of control group (BSA-I) and 14.85 ± 2.25 MPa of BSA-III group, which was significant statistically. The bond strength of BSA-I (control group) and BSA-III (decontamination group) showed no significant difference.

In 3M Universal group (BSB) bond strength significantly decreased in BSB-II (contamination group) 3.72 ± 1.29 MPa when compared to BSB-I (control group) 5.62 ± 0.53 MPa and BSB-III (decontamination group) 9.86 ± 1.09 MPa, which was significant statistically. The bond strength of BSB-III, wherein 3M universal bond was reapplied after contamination of saliva was found to be significant statistically than BSB-I and BSB-II.

Conclusion: Both the self etch system, Optibond All in one kerr and Single Bond Universal 3M showed reduction in dentine bond strength when Contamination by saliva was done during restorative procedure. Reapplication of the bonding agent for the kerr Optibond All in one and 3M Single Bond Universal can recover the bond strength after air drying off the saliva over the dentine surface. In 3M Single Bond Universal group, added application of bonding agent improved bond strength significantly after saliva decontamination.

Keywords: Shear bond strength, Self-etch bonding agent system, Salivary contamination, Salivary decontamination.

Introduction
Dentine bonding has been a topic of appreciable interest as a result of it’s a more heterogeneous component with higher water and organic constituents than enamel.1 Bond strength has been found to be dependent on Tooth structure and Chemical composition.2 Hence, improving the strength of bonding agent in restorative materials has been the target of analysis within the recent years.

In dentistry enamel and dentine bonding have been an important part. The sturdiness of the bonding agent bond between composite resin and tooth is of great importance for the longevity of bonding agent and composite restorations.3 Saliva contamination during restorative procedure could be a frequent downside in clinical procedures, particularly in cases like deep cervical lesions, sub gingival dental caries wherever rubber dam isolation is troublesome or not possible. In modern restorative dental medicine bonding effectualness and salivary contamination has been a debatable topic.4,5

There has been a dramatic progression within the restorative and bonding procedure to enamel and dentin in last forty years since Buonocore introduced the technique of etching with phosphoric acid to enhance adhesion to enamel.6 Over the years because the demand for aesthetic restorations have inflated, technique simplification has become the goal of researchers and makers totally different of various bonding agent system resulting in different generations in dental bonding agent.7 Most current enamel etchants contain 30-40% phosphoric acid and the bonding strength which can be produced by them can be 20 Mpa.8,9 Bond strengths in this range give routinely productive retention and protection of resins for a range of clinical applications.
Factors which can effect the bond and retention of composite restorative materials can be wetness of gingival fluid, saliva, hand-piece oil, blood\textsuperscript{10} and will have an effect on the quality and retention of the restoration, which may at the tooth restoration interface show micro-leakage. This could lead to the fracture of restoration, recurrent caries, tooth sensitivity and tooth discoloration.\textsuperscript{11} So the bonding procedure should be done without contamination and with good isolation. However, often caries which require proper bonding are found in the troublesome to isolate areas, particularly in which the location is close to or at the margin of gingiva where saliva can contaminate the operating field.\textsuperscript{12}

Silverstone et al.\textsuperscript{13} have reported that bond strength decrease between the composite and enamel surface takes place when contamination by saliva over etched dentine is present. Bond strength is reduced since monomers are prevented from penetrating the pores in etched enamel by salivary proteins.\textsuperscript{10} Microscopic examination revealed that organic pellicle which is formed on saliva contaminated acid etched enamel that might not be removed with water.\textsuperscript{13} The organic pellicle coating impaired mechanical adhesion and reduced resin accessibility.

However, the contaminated enamel may be reconditioned by a further ten seconds of acid etching.\textsuperscript{14} Dentine bonding is complex in comparison to enamel bonding. So, the results of several studies associated with the bonding effectiveness of dentine bonding agents under saliva contamination has varied.\textsuperscript{1}

Developed recently, bonding systems like the self-etch bonding agents have shown to be resilient to salivary Contamination.\textsuperscript{11,19} Self-etch systems can limit the steps that simplifies the bonding procedure.\textsuperscript{20-22} These dentine bonding agents have a fewer components and fewer application steps which reduces the chance of saliva incorporation in restorative procedure.\textsuperscript{23}

Hence, to evaluate the influence of saliva when incorporated in the restorative procedures, and its effect on bond strength, and to find out the impact of decontamination on bond strength during restorative procedure using two self etch bonding agents, this study was conducted.

Composition of 3M universal bonding agent:
1. MDP Phosphate Monomer
2. Dimethacrylate resins
3. HEMA
4. Vitrebond\textsuperscript{TM} Copolymer
5. Filler
6. Ethanol
7. Water
8. Initiators
9. Silane

Composition of Kerr Optibond All in one bonding agent
1. Monomers:
   a. Glycerol phosphate dimethacrylate – self-etching bonding agent monomer
   b. Co-monomers including mono- and di-functional methacrylate monomers
2. Solvents: water, acetone and ethanol
3. Photo-initiator: camphorquinone based
4. Fillers: three nano-sized fillers
5. Fluoride-releasing fillers: sodium hexafluorosilicate and ytterbium fluoride

Materials and Methods
Two dentine bonding agents were tested in the study: OptibondAll in one kerr (BSA), Single Bond Universal 3M (BSB). Bulfill (ivoclar vivdent) was used in both the groups. Sixty premolars teeth which were removed during orthodontic treatment were obtained for the test. The teeth were then divided randomly into two groups KERR (BSA) group and 3M (BSB) group of 30 samples each.

For each bonding agent, the specimens were divided into non-contaminated (control group), contaminated and decontaminated sub-groups (experimental groups). Ten specimens were made for each procedure. In the experimental groups, saliva which was freshly collected was applied to the bonded dentinal surface of the sample with a disposable applicator tip for 5 seconds, followed by the decontaminant treatment. Details of the bonding procedure for each bonding agents are mentioned below. After the bonding procedure, a composite block of 5 mm was built on the flat dentinal surface using a round plastic tube having an internal diameter of 4.9 mm by progressively adding 2 mm thick increments. For proper polymerization of each added layer of composite resin, the light tip was positioned as close to the tooth as possible.

Instron Universal testing machine (USA) was used to measure Shear bond strength. Shear testing apparatus was loaded with each specimen and a shearing rod with a chisel shaped end with a cross-head speed of 1 mm per minute was used to load the specimens at the dentine-composite interface. The data obtained for shear bond strength was then subjected to statistical analysis by One way ANOVA test and Tukey multiple comparison & Unpaired t-test for the intergroup comparison.
BSA - Kerr Optibond all in one

20 Seconds - Apply bonding agent to tooth surface by scrubbing action.
20 seconds - re dip in bonding agent and reapply bonding agent to tooth surface by scrubbing action.
5 seconds - Dry the bonding agent
10 Seconds - Light cure

BSB - 3M Single Bond Universal

20 Seconds - Apply bonding agent to tooth surface by scrubbing action
5 Seconds - Dry the bonding agent
10 Seconds - Light cure

Result

The shear bond strength test results of Kerr Optibond (BSA) and 3M Universal (BSB) are depicted in Fig. 1 & Tables 2 and 3. On performing one-way ANOVA test, a significant difference at $P <0.05$ was revealed. Groups BSA and BSB are compared in Tables 4 and 5.

In the Kerr group (BSA), the subgroups - control (BSA-I) and contamination (BSA-II) showed a very highly significant difference ($P = 0.00015$). The shear bond strength witnessed a fall in value to $5.57 \pm 1.77$ MPa as opposed to $13.87 \pm 2.12$ MPa of the control group. Nonetheless, on reapplication of self-etch bonding agent after the salivary contamination (BSA-III), the bond strength showed an increase to $14.85 \pm 2.25$ MPa ($P = 0.00009$). Whereas the bond strength between the control and the decontamination subgroups ($P = 0.50$) showed no significant difference.

In 3M Universal group (BSB), the shear bond strength of (BSB-I) was $5.62 \pm 0.53$ MPa when dentine was not contaminated. In (BSB-II) where salivary contamination occurred after the curing of the bonding agent, the bond strength decreased to $3.72 \pm 1.29$ MPa, which was significant ($P = 0.035$). On reapplication of 3M Universal bonding agent after saliva contamination. The bond strength of BSB-III was $9.86 \pm 1.09$ MPa. This statistically showed a very high significance when compared to the control group and the group where salivary contamination was done ($P = 0.00035$).

Unpaired t-test was used to do the Intergroup comparisons between BSA and BSB. The comparison of bond strength when the specimens were not contaminated with saliva is shown in Table 6. Statistically very highly significant difference was revealed between the mean bond strengths of subgroups BSA-I and BSB-I ($P=0.00014$). On comparison of the control groups Kerr Optibond group showed higher bond strength than that of 3M Universal group.

Graph 1: Inter-group comparison of BSA and BSB

Table 2: Mean bond strength values (MPa) of Kerr Optibond group (BSA)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sub group</th>
<th>N</th>
<th>Mean SBS ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>Non-Contaminated Group BSA-I</td>
<td>10</td>
<td>13.88 ± 2.12</td>
</tr>
<tr>
<td></td>
<td>Contaminated Group BSA-II</td>
<td>10</td>
<td>5.57 ± 1.77</td>
</tr>
<tr>
<td></td>
<td>Decontamination Group BSA-III</td>
<td>10</td>
<td>14.85 ± 2.25</td>
</tr>
</tbody>
</table>

Table 3: Mean bond strength values (MPa) of 3M universal Group (BSB)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sub Group</th>
<th>N</th>
<th>Mean SBS ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSB</td>
<td>Non-Contaminated Group BSB-I</td>
<td>10</td>
<td>5.62 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>Contaminated Group BSB-II</td>
<td>10</td>
<td>3.72 ± 1.29</td>
</tr>
<tr>
<td></td>
<td>Decontamination Group BSB-III</td>
<td>10</td>
<td>9.86 ± 1.09</td>
</tr>
</tbody>
</table>
Table 4: Comparison among BSA group

<table>
<thead>
<tr>
<th>Group</th>
<th>(A) Subgroup</th>
<th>(B) Subgroup</th>
<th>Mean Difference A-B</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>Non-Contaminated group (BSA-I)</td>
<td>Contaminated group (BSA-II)</td>
<td>8.30</td>
<td>0.00015 vhs</td>
</tr>
<tr>
<td></td>
<td>Non-Contaminated group (BSA-I)</td>
<td>De-contaminated group (BSA-III)</td>
<td>-0.98</td>
<td>0.5026 ns</td>
</tr>
<tr>
<td></td>
<td>Contaminated group (BSA-II)</td>
<td>De-contaminated group (BSA-III)</td>
<td>-9.28</td>
<td>0.00009 vhs</td>
</tr>
</tbody>
</table>

*vhs- very highly significant, ns- non significant.

Table 5: Comparison among BSB group

<table>
<thead>
<tr>
<th>Group</th>
<th>(A) Sub Group</th>
<th>(B) Sub Group</th>
<th>Mean Difference A-B</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSB</td>
<td>Non-Contaminated group (BSB-I)</td>
<td>Contaminated group (BSB-II)</td>
<td>1.9</td>
<td>0.0349 sig</td>
</tr>
<tr>
<td></td>
<td>Non-Contaminated group (BSB-I)</td>
<td>De-contaminated group (BSB-III)</td>
<td>-4.24</td>
<td>0.0004 vhs</td>
</tr>
<tr>
<td></td>
<td>Contaminated group (BSB-II)</td>
<td>De-contaminated group (BSB-III)</td>
<td>-6.14</td>
<td>0.00035 vhs</td>
</tr>
</tbody>
</table>

*vhs- very highly significant, sig- significant.

Table 6: Comparisons of mean bond strengths between BSA-I (control) and BSB-I (control)

<table>
<thead>
<tr>
<th>Sub Group</th>
<th>Group</th>
<th>N</th>
<th>Mean ± S.D</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>BSA-I</td>
<td>10</td>
<td>13.87 ± 2.12</td>
<td>0.00014 vhs</td>
</tr>
<tr>
<td></td>
<td>BSB-I</td>
<td>10</td>
<td>5.62 ± 0.53</td>
<td></td>
</tr>
</tbody>
</table>

*vhs- very highly significant.

Discussion

In restorative dentistry dentine bonding strength is of prime importance. Composite cylinder is bonded over the dentine surface with the bonding agent in study. Shear force using universal testing machine employing a knife-edge probe is applied at the composite–dentine interface. For determining the relative efficacy of bonding agent, shear bond strength evaluation should not be the only tool.

Saliva contamination in the restorative field is a frequent drawback in restorative procedures, particularly where deep cervical lesions present or to place an indirect restoration, where rubber dam isolation is troublesome or not possible, such as or even in patient’s limited mouth opening. Within the present study, natural saliva was chosen as the. Whole healthy human saliva is accepted as a suitable contaminating medium. An in vitro model to mimic clinical conditions verified that hybrid layer formation can be disrupted by saliva and plasma.

Saliva incorporation during restorative procedures can be quite conflicting. Some have reported that effect of saliva incorporation in restorative procedure had no effect in one-bottle bonding agent systems. Others have shown that the saliva in restorative procedures can effect bonding significantly. The factors that may cause for reduction in the bond strength:

1. Glycoprotein present in saliva can act as a barrier over the bonding agent layer, which prevents complete attachment of composite over it, which results in faulty curing of composite
2. Monomer fails to reach the collagen network of dentine because of salivary protein
3. Bonding agent could be diluted by the saliva and weakens the hybrid layer formation.

Fritz et al showed that after contamination with the saliva happens re-etching of the surface does not provide any sufficient strength. EL-Kalla and Godoy believed that drying the surface after salivary contamination over etched dentine can regain the bond strength. Further Studies have conjointly shown that bonding efficacy can be improved after reaplication of bonding agent after salivary contamination.

The deliquescent nature of the newer bonding agents allows them to perform some degree within the presence of saliva by displacing or diffusing through it and then infiltrate and polymerize among the exposed collagen bundles of demineralised superficial dentine.

Within the Kerr Optibond group, when the saliva contamination was done after the application of bonding agent, bond strength reduced considerably. However, the bond strength can be regained after the bonding agent was reapplied.
In 3M Universal group, when salivary contamination occurred after curing the bonding agent, there was a significant decrease in bond strength.

World Health Organization has shown that cured bonding agent layer of one-bottle bonding agent system when incorporates saliva, has a negative effect on bonding strength. Studies have shown that dentine bond strengths of all-in-one bonding agents can be hampered by saliva in corporation and to restore the bond strength additional application of bonding agent is required after debriding and washing the dentine surface.

An interesting finding in 3M Universal subgroup where the bonding agent was reapplied after saliva contamination was that, the bond strength obtained in the sub-group was higher than the control group. This increase in bond strength was very highly significant (P= 0.0004). The increased bond strength can be due multiple coatings of bonding agent. Under multiple applications, the increased dentine composite bond strength can be due to several reasons. The concentration of the comonomers that exists after each coating will increase as the solvent is evaporated. Hybrid layer is improved and the ratio of the polymerized vs. unpolymerized bonding agent layer due to oxygen inhibition. Hashimoto and others have stated that bond strength can be improved by applying multiple coats of hydrophilic agent, since it can displace or diffuse through the biofilm to reach the underlying layer.

Intergroup comparison of BSA-I (control) and BSB-I (control) showed that Kerr Optibond has higher bond strength to that of 3M Universal. This difference in the bond strength was very highly significant (P= 0.00014). To prove these results further studies, need to be conducted.

Conclusion
The following conclusions can be drawn within the limitations of this in vitro study:

1. The selfetch primer [Kerr Optibond (BSA-I)] showed better bond strength than self-etch bonding agent [3M Universal (BSB-I)] when salivary contamination is not there.
2. Restorative procedure contaminated by saliva, reduces the dentine bond strength of both the self-etch primer (BSA-II) and bonding agents (BSB-II).
3. Re-application of the bonding agent for the Kerr Optibond group (BSA-III) and re-application of the bonding agent for the 3M Universal group (BSB-III), bonding agent can be recovered once the saliva is dried by air.
4. In the 3M Universal group, the added application of bonding agents to decontaminate (BSB-III), not only the bond strength was recovered, but also improving it, can be a result of many bonding agent coatings.

References
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