Evaluation of Effect of Different Cavity Disinfectants on Shear Bond Strength of Composite Resin to Dentin using Two-Step Self-Etch and One-Step Self-Etch Bonding Systems: A Comparative in vitro Study

M Surya Chaitanya Reddy, MC Mahesh, Shreetha Bhandary, J Pramod, Ashwija Shetty

ABSTRACT

Aim and objectives: To evaluate the effect of 2% chlorhexidine, 2% sodium hypochlorite, 3% hydrogen peroxide on shear bond strength of composite resin to dentin using two-step and one-step self-etch bonding systems and to study the mode of failure of specimens under stereomicroscope.

Materials and methods: Eighty extracted sound human posterior teeth were used. The occlusal surfaces were ground to expose the dentin and were then randomly divided into 4 main groups. In group I, no cavity disinfectant was used and served as control. In groups II, III, IV, 2% chlorhexidine, 2% sodium hypochlorite and 3% hydrogen peroxide were used as cavity disinfectants respectively. Each group was then divided into two subgroups of 10 teeth each according to the bonding agent used, two-step self-etch (Adper SE Plus) and one-step self-etch (Adper Easy One) respectively. A transparent cylindrical plastic tube was loaded with microhybrid composite and placed over the dentin and light cured for 40 seconds. The specimens were subjected to shear stress in the universal testing machine.

Results: Pretreatment with 2% chlorhexidine, 2% sodium hypochlorite and 3% hydrogen peroxide, had a negative effect on the shear bond strength of self-etching bonding systems.

Conclusion: The highest bond strength was found in 2% chlorhexidine group followed by 2% sodium hypochlorite group and the lowest bond strength was found in 3% hydrogen peroxide group.

Clinical significance: All three cavity disinfectants used in this study reduced the shear bond strength and hence should be used with caution.

Keywords: Cavity disinfectant, Self-etching adhesive, Shear bond strength.

Source of support: Nil
Conflict of interest: None declared

INTRODUCTION

Residual bacteria on the dentin surface due to incomplete removal of caries are a potential problem in restorative dentistry. According to Brannstrom, possible sources of infection in a prepared cavity are bacterial microleakage, bacteria present in the smear layer, in dentinal tubules, at the dentino-enamel junction and bacteria recontaminating the prepared cavity prior to placing a restoration.

Tissue fluids from the pulp and the organic content of dentin provide sufficient substrate for microbial growth under restorations. Growth of microorganisms under a restoration may result in tooth hypersensitivity, weakening of bond strength, development of secondary caries, pulpal inflammation and necrosis of the pulp.

Recently self-etch adhesives are gaining popularity because of simplified bonding procedures and reduced technique sensitivity. Self-etch adhesives are able to demineralize the smear layer and underlying dentin while simultaneously allowing resin monomers to penetrate into the demineralized zone, which results in the creation of a hybrid layer. However, incorporation of smear layer particles into the hybrid layer is one potential disadvantage of self-etching systems. The microbial content of the smear layer may further lead to pulpal infection.

To reduce the potential risk of pulpal inflammation resulting from bacterial activity, the use of antibacterial materials during restorative procedures has been recommended. Many chemicals have been tested as cavity disinfectants including chlorhexidine digluconate, disodium...
ethylene diamine tetra acetic acid dihydrate (EDTA), sodium hypochlorite, silver diamine fluoride, hydrogen peroxide, iodine, tubulicid red, ozone and cetyl pyridinium chloride.

A potential problem with the use of disinfectants is the possibility of an alteration in the bond strength of restorative material to dentin. It has been reported that 2% chlorhexidine does not influence the micro-tensile bond strength of total etch and self-etch systems. The same results have been reported with etch and rinse adhesive systems. Application of 5.25% sodium hypochlorite for 60 seconds increased bond strength of two-step self-etch but had no effect on etch and rinse systems. Pretreatment with sodium hypochlorite, hydrogen peroxide or chlorhexidine solution had a negative effect on shear bond strength of self-etch bonding systems.

Hence, the present study was carried to evaluate the effect of 2% chlorhexidine, 2% sodium hypochlorite, and 3% hydrogen peroxide on shear bond strength of composite to dentin using two-step self-etch and one-step self-etch adhesives.

MATERIALS AND METHODS

Extracted human posterior teeth were collected from the Department of Oral and Maxillofacial Surgery, AECS Maaruti College of Dental Sciences and Research Centre, Bengaluru.

Eighty posterior teeth without caries were selected for the study. Teeth selection criteria included posterior teeth removed for orthodontic or periodontal reasons, teeth with intact clinical crown, teeth with sufficiently wide occlusal surface and teeth removed intact while extracting.

Carious teeth which fractured while extracting, teeth which were discolored, teeth which were restored, teeth which were badly attrited, teeth with developmental anomalies and teeth with deep cervical abrasions were excluded from the study.

Scalers and no. 11 Bard Parker blade were used for removal of remnants of calculus and periodontal ligament. The teeth were sectioned using a diamond disk with a water coolant, parallel to the occlusal surface to expose dentin. Prepared teeth were then embedded in autopolymerizing resin to form a square of 2 × 2 cm dimension. Flattened dentin surfaces were polished using emery paper. Teeth were randomly divided into four main groups of 20 teeth each.

In group I, prepared dentin surfaces were not treated with any cavity disinfectant and served as control group. In group II, III, IV prepared dentin surfaces were treated with 2% chlorhexidine, 2% sodium hypochlorite and 3% hydrogen peroxide respectively for 20 seconds and air dried for 10 seconds. Each group was then divided into two subgroups according to the bonding agent used, either Adper SE Plus or Adper Easy One. A plastic tube with 2 mm height, 5 mm internal diameter loaded with microhybrid composite resin (Filtek Z-250, 3M ESPE, USA) was placed over the cured adhesive and light cured for 40 seconds. Specimens were stored in an incubator at 37°C and 100% humidity for 24 hours. They were then subjected to shear stress in a universal testing machine (LR-5K, Lloyd Instrument, England) with a cross head speed of 1 mm/min until failure occurred. Peak failure load was converted to shear bond strength by dividing failure load with the bonding area.

After the testing procedure, fractured surfaces were observed under a stereomicroscope at 20× magnification to determine failure modes.

RESULTS

The obtained data were statistically analyzed by one-way ANOVA. Tests showed that when dentin bonding agents were used according to manufacturer instructions, two-step self-etch system exhibited higher shear bond strength than one-step self-etch system and the difference was statistically significant (Table 1). When 2% chlorhexidine was used as cavity disinfectant two-step self-etch adhesive system exhibited statistically significant higher bond strength than one-step self-etch adhesive system (Table 2). When 2% sodium hypochlorite and 3% hydrogen peroxide were used as cavity disinfectants no statistical difference in bond strength existed between two-step self-etch and one-step self-etch adhesive systems (Tables 3 and 4) (Graph 1).

Results showed, that when Adper SE Plus was used, statistically significant difference in bond strength existed between two-step self-etch and one-step self-etch adhesive systems (Tables 3 and 4) (Graph 1).

Table 1: Subgroup 1 vs subgroup 2

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgroup 1</td>
<td>10</td>
<td>19.89</td>
<td>1.36</td>
<td>18.16</td>
<td>22.09</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Subgroup 2</td>
<td>10</td>
<td>15.75</td>
<td>1.46</td>
<td>13.31</td>
<td>17.55</td>
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</table>

Table 2: Subgroup 3 vs subgroup 4

<table>
<thead>
<tr>
<th>Group</th>
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<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>t-value</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgroup 3</td>
<td>10</td>
<td>15.87</td>
<td>1.27</td>
<td>14.03</td>
<td>18.06</td>
<td></td>
<td>&lt;0.001</td>
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<tr>
<td>Subgroup 4</td>
<td>10</td>
<td>13.41</td>
<td>1.08</td>
<td>12.04</td>
<td>15.25</td>
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When Adper Easy One was used, the bond strength of the control group was higher than the experimental groups and this difference was statistically significant. However, even though there was a difference in shear bond strength between the experimental groups it was not statistically significant (Table 6 and Graph 3).

Table 4: Subgroup 7 vs subgroup 8

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hypochlorite group</td>
<td>10</td>
<td>13.275</td>
<td>0.628</td>
<td>11.68</td>
<td>13.81</td>
<td>0.059</td>
<td>0.811</td>
</tr>
<tr>
<td>Subgroup 7</td>
<td>10</td>
<td>13.127</td>
<td>1.826</td>
<td>10.60</td>
<td>16.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subgroup 8</td>
<td>10</td>
<td>13.275</td>
<td>0.628</td>
<td>11.68</td>
<td>13.81</td>
<td>0.059</td>
<td>0.811</td>
</tr>
</tbody>
</table>

Table 5: Tukey test performed to find out mean difference between Adper SE Plus subgroups

<table>
<thead>
<tr>
<th>Subgroup (I)</th>
<th>Subgroup (J)</th>
<th>Mean difference (I-J)</th>
<th>p-value</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
<td>Subgroup 1</td>
<td>Subgroup 3</td>
<td>4.018</td>
<td>&lt;0.001</td>
<td>2.620</td>
</tr>
<tr>
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<td>Subgroup 7</td>
<td>0.212</td>
<td>&lt;0.001</td>
<td>4.671</td>
</tr>
<tr>
<td>Subgroup 3</td>
<td>Subgroup 7</td>
<td>0.212</td>
<td>&lt;0.001</td>
<td>5.219</td>
</tr>
<tr>
<td>Subgroup 5</td>
<td>Subgroup 7</td>
<td>0.212</td>
<td>&lt;0.001</td>
<td>6.653</td>
</tr>
</tbody>
</table>

Table 6: Tukey test performed to find out mean difference between Adper Easy One subgroups

<table>
<thead>
<tr>
<th>Subgroup (I)</th>
<th>Subgroup (J)</th>
<th>Mean difference (I-J)</th>
<th>p-value</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
<td>Subgroup 4</td>
<td>Subgroup 6</td>
<td>2.338</td>
<td>0.015</td>
<td>0.367</td>
</tr>
<tr>
<td>Subgroup 6</td>
<td>Subgroup 8</td>
<td>2.265</td>
<td>0.019</td>
<td>0.294</td>
</tr>
<tr>
<td>Subgroup 8</td>
<td>Subgroup 10</td>
<td>0.073</td>
<td>1.000</td>
<td>0.057</td>
</tr>
<tr>
<td>Subgroup 2</td>
<td>Subgroup 4</td>
<td>2.338</td>
<td>0.015</td>
<td>0.367</td>
</tr>
<tr>
<td>Subgroup 6</td>
<td>Subgroup 8</td>
<td>0.290</td>
<td>0.979</td>
<td>–1.681</td>
</tr>
<tr>
<td>Subgroup 8</td>
<td>Subgroup 10</td>
<td>0.363</td>
<td>0.959</td>
<td>–1.608</td>
</tr>
</tbody>
</table>

Graph 1: Comparison of mean shear bond strength between two-step self-etch and one-step self-etch adhesive system within the study groups.

Graph 2: Comparison between Adper SE Plus groups.
Specimens in control group showed mostly cohesive and mixed failures whereas specimens in experimental groups showed mostly adhesive failures (Table 7).

**DISCUSSION**

Cavity preparation is a surgical procedure that attempts to remove all infected dentin prior to placing a restorative material. Bacterial activity below a restoration may result in increased pulp sensitivity, pulpal inflammation and secondary caries. Histological and bacteriological examinations have shown that most teeth harbor microorganisms even after caries excavation. These bacteria remaining in a prepared cavity could survive for longer than a year. Digital and optical assessment criteria for detecting carious dentin have shown caries remaining in 59% of teeth even after the teeth were judged to be caries free. Based upon these evidences, the use of an antibacterial cavity cleanser has been recommended for disinfecting the prepared cavity.

Presently many chemical disinfectants such as sodium hypochlorite, hydrogen peroxide, ozone, EDTA, etc. are used for this purpose. Chlorhexidine, 2% sodium hypochlorite, 3% hydrogen peroxide are the commonly used cavity disinfectants and several studies have recommended their use. Even though, the antibacterial ability of these cavity disinfectants is well established, use of these chemicals before the application of adhesive systems could potentially interfere with the ability of the adhesives to micromechanically bond to the dentin. Consequently use of these cavity disinfectants may reduce the bond strength. The present study compares the effect of three commonly used cavity disinfectants—2% chlorhexidine, 2% sodium hypochlorite, 3% hydrogen peroxide on the shear bond strength of two-step self-etch adhesive (Adper SE Plus) and one-step self-etch adhesive (Adper Easy One).

Evaluation of shear bond strength, tests a combination of tensile and compressive forces between a material and tooth surface, as well as within the material. In vitro bond strength tests are useful and essential for predicting the performance of adhesive systems and possible correlation with clinical issues. Shear bond strength tests have been widely used, mainly because of ease of specimen preparation and simple test protocol. In the present study shear bond strength testing was done to evaluate the effect of cavity disinfectants on the shear bond strength of composite to dentin.

Chlorhexidine is a broad spectrum antiseptic with pronounced antimicrobial effects and is shown to be effective in reducing cariogenic bacteria. Chlorhexidine solution is active against a wide range of microorganisms, because it is bacteriostatic at low concentrations and bactericidal at higher concentrations.

Sodium hypochlorite is another alternative as a cleansing agent due to its tissue dissolving properties. The antimicrobial effectiveness of sodium hypochlorite is based on its high pH (hydroxyl ions action). The high pH of sodium hypochlorite interferes in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism and phospholipid degradation observed in lipidic peroxidation.

H₂O₂ is active against viruses, bacteria, yeasts, and even bacterial spores. Production of catalase or superoxide dismutase by several bacteria can afford those species some protection against H₂O₂. H₂O₂ produces hydroxyl free radicals (OH), which attack several cell components such as proteins and DNA.

In the present study, when dentin bonding agents were used according to manufacturer instructions, two-step self-etch adhesive (Adper SE Plus) exhibited a mean shear bond strength value of 19.89 MPa and one-step self-etch adhesive (Adper Easy One) exhibited mean shear bond strength value of 15.75 MPa. This result is in accordance to previous

### Table 7: Fracture modes observed in various groups

<table>
<thead>
<tr>
<th></th>
<th>Adhesive failure</th>
<th>Cohesive failure</th>
<th>Mixed failure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adper SE Plus</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Adper Easy one</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Chlorhexidine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adper SE Plus</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Adper Easy one</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Sodium hypochlorite</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adper SE Plus</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Adper Easy one</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Hydrogen peroxide</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adper SE Plus</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Adper Easy one</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>
studies in which two-step self-etch adhesive produced higher bond strength than one-step self-etch adhesives.\textsuperscript{21,22}

In our study, pretreatment of dentin surfaces with 2% chlorhexidine resulted in reduction of bond strength both for two-step and one-step self-etch adhesive. Reduction in bond strength of one-step self-etch adhesive was more than that of two-step self-etch adhesive and this difference was statistically significant. Fractured specimens in the groups treated with chlorhexidine presented mostly adhesive failures.

The presence of chlorhexidine may have decreased wettability of dentin and this may have resulted in lower bond strength values.\textsuperscript{23} Results of this study are in accordance with a study by Herenio et al\textsuperscript{23} in which application of 2% chlorhexidine resulted in lower bond strength for two-step self-etch adhesive. Another study by Edson Alves de Campos et al\textsuperscript{24} reported reduction of bond strength values for two-step self-etch adhesive and one-step self-etch adhesive when 2% chlorhexidine was applied to dentin.

Results of this study with regard to application of 2% chlorhexidine are in contrast to the results of the study by Fabricio Lusccino Alves de Castro et al\textsuperscript{8} and Edson Alves Campos et al.\textsuperscript{25} Activation of matrix metalloproteinase by self-etching adhesives may cause degradation of collagen fibrils in hybrid layer. Since chlorhexidine inhibits MMPs, normal structural integrity of collagen network of hybrid layer was observed.

In the present study, pretreatment of dentin surfaces with 2% sodium hypochlorite reduced the mean shear bond strength of both two-step self-etch adhesive and one-step self-etch adhesives. However, this difference was statistically not significant. Fractured specimens in the sodium hypochlorite pretreatment groups showed mostly adhesive failures.

Compromised bonding is thought to arise because the reactive residual free radicals generated by the oxidizing effect of NaOCl compete with the propagating vinyl free-radicals generated during light activation of the adhesive, leading to incomplete polymerization by premature chain termination. These free radicals are also entrapped in the porous structure of mineralized dentin. This result is in agreement with studies conducted by Gen Taniguchi et al\textsuperscript{26} and Taweesak Prasansuttiporn et al\textsuperscript{27} which also found a decrease in the bond strength. However, this is in contrast to a study by Doglas Cecchin et al\textsuperscript{27} in which use of 1% NaOCl resulted in higher bond strength with a self-etching adhesive system.

Pretreatment of dentin surfaces with 3% hydrogen peroxide resulted in reduction of bond strength both for two-step and one-step self-etch adhesive. However, the difference was not statistically significant. Fractured specimens in the hydrogen peroxide pretreatment groups showed mostly adhesive failures.

Reduction in bond strength in hydrogen peroxide treated dentin could be caused by residual solution in the collagen matrix and dentinal tubules that eventually broke down into oxygen and water. Liberation of oxygen could either interfere with resin infiltration into etched dentin or inhibit polymerization of resins that cure via a free-radical mechanism.\textsuperscript{24} A previous study by Ertugrul Ercan et al\textsuperscript{1} reported reduction in dentin bond strength of total etch and self-etch adhesives when 3% hydrogen peroxide was applied. Another study by CJ Soares et al\textsuperscript{28} reported reduced bond strength when dentin surfaces were treated with 3% hydrogen peroxide before using total etch adhesive.

None of the previous studies have reported increase in bond strength after application of hydrogen peroxide.

A clinically desirable goal would be the complete elimination of all bacteria from the cavity before the placement of the restoration. But all studies till date have reported a reduction in bond strength when cavity disinfectants have been used prior to bonding. On balance, it would be wise to select a disinfectant which has the least effect on the bond strength of the selected bonding agent.

Within the limitations of this study, 2% chlorhexidine would appear to be the most appropriate disinfectant for two-step self-etch adhesives.

CONCLUSION

The following conclusions can be drawn from the present study.

1. All tested disinfectants, i.e. 2% chlorhexidine, 2% sodium hypochlorite and 3% hydrogen peroxide, significantly reduced the bond strength of two-step and one-step self-etch adhesives.
2. Application of 2% chlorhexidine affected the bonding of one-step self-etch adhesives more than two-step self-etch adhesives.
3. Also, 2% chlorhexidine affected the bond strengths of both tested bonding agents to a lesser extent than 2% sodium hypochlorite and 3% hydrogen peroxide.
4. Also, 2% sodium hypochlorite and 3% hydrogen peroxide reduced the bond strength of both one-step and two-step self-etch adhesives to the same extent.

CLINICAL SIGNIFICANCE

Use of 2% chlorhexidine, 2% sodium hypochlorite, 3% hydrogen peroxide reduced the bond strength of two-step self-etch and one-step self-etch adhesives. However, 2% chlorhexidine can be used as cavity disinfectant along with
two-step self-etch adhesive system as the reduction in bond strength was less significant.

REFERENCES


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