Effect of Three Different Contamination Removal Methods on Bond Strength of a Self-etching Adhesive to Dentin Contaminated with an Aluminum Chloride Hemostatic Agent

Amir Ahmad Ajami, Mehdi Abed Kahnamoii, Soodabeh Kimyai, Siavash Savadi Oskoei, Fatemeh Pournaghi-Azar, Mahmoud Bahari, Maryam Firouzmandi

ABSTRACT

Aim: This study evaluated the effect of three different contamination removal methods on bond strength of one-step self-etching adhesive to dentin contaminated with an aluminum chloride hemostatic agent.

Materials and methods: One hundred noncarious adult molars were used in this study; 2-mm-thick dentin disks were prepared and randomly assigned to 5 groups (n = 20). Group I: control; group II: hemostatic agent-contaminated; group III: hemostatic agent-contaminated and rinsing with water; group IV: hemostatic agent-contaminated and ethylene diamine tetraacetic acid (EDTA) application; and group V: hemostatic agent-contaminated and phosphoric acid application. Clearfil S3 Bond was used to bond composite to dentin surfaces. Subsequent to adding composite cylinders the shear bond strength test was performed. Data were analyzed by one-way ANOVA and Tukey test. Two additional specimens from each group were prepared and evaluated under scanning electron microscope (SEM).

Results: There were statistically significant differences in bond strength among the groups (p < 0.001). In two-by-two comparisons statistically significant differences were observed in bond strength values between all the groups (p < 0.001) except for groups I and IV (p = 0.933).

Conclusion: Aluminum chloride hemostatic agent adversely affected the bond strength of self-etch adhesive to dentin. However, application of EDTA increased the bond strength to the level of normal dentin.

Clinical significance: EDTA and phosphoric acid removed aluminum chloride hemostatic agent contamination of dentin surfaces. However, unlike EDTA phosphoric acid failed to increase the bond strength of self-etch adhesive to dentin to the level of the control group.

Keywords: Contamination, EDTA, Hemostatic agent, Self-etch adhesive, Shear bond strength.

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Conflict of interest: None

INTRODUCTION

It is important to control hemorrhage and decrease gingival crevicular fluid seepage during bonded restoration placement because contamination with moisture and blood have a detrimental effect on the bond strength of bonded restorative materials and tooth structures. In some oral cavity locations, such as the gingival area, blood and sulcular fluid flow as a result of gingival trauma from tooth preparation procedures or gingival inflammation. Hemostatic agents, commonly referred to as astringents, have been in clinical use in dentistry for many years to control hemorrhage and gingival crevicular fluid flow, the most common of which contain alum (aluminum potassium sulfate or aluminum ammonium sulfate), aluminum chloride, ferric sulfate and racemic epinephrine. All these chemicals give rise to a combination of tissue shrinkage and/or hemostasis, enabling the dental practitioner to achieve a clean and dry field for bonding procedures. However, these agents have some drawbacks, too. Aluminum chloride, with a concentration of 20 to 25%, is a common hemostatic agent. It has been reported that aluminum chloride-containing hemostatic agents interfere with adhesive techniques. One reason for a decrease in bond strength might be the remaining aluminum on the surface of dentin.
contaminated with the hemostatic agent.\textsuperscript{6} It has been reported that enamel treated with AlCl\textsubscript{3} solution for 20 minutes has the potential to take up aluminum from the solution, especially within the first 20 \textmu m.\textsuperscript{8} In addition, this AlCl\textsubscript{3}-treated enamel inhibits demineralization of hydroxyapatite (HAP), exposed to a demineralizing agent.\textsuperscript{9,10} This mechanism has been explained by replacement of calcium in the HAP by aluminum, resulting in the formation of Al(OH)\textsubscript{2}H\textsubscript{2}PO\textsubscript{4}, which is insoluble.\textsuperscript{11} Since HAP is also the most important constituent of dentin-like enamel, the influence of AlCl\textsubscript{3} on dentin might be similar to that on enamel.\textsuperscript{6}

Advances in enamel/dentin bonding during the past decade have resulted in improvements in two distinctly different adhesion techniques: The etch-and-rinse and the self-etch approaches.\textsuperscript{12} A disadvantage of etch-and-rinse adhesive technique, however, is that there is often discrepancy between dentin demineralization and monomer penetration depths.\textsuperscript{13-15} It has been postulated that unprotected collagen fibrils are prone to hydrolytic degradation in the long-term, which results in deterioration of the resin-dentin interface, reducing bond strength.\textsuperscript{16,17} Self-etch adhesives were introduced in an attempt to offset some of the disadvantages of etch-and-rinse adhesives. They contain nonrinsing, acidic monomers capable of simultaneous etching and priming. Collapse of the collagen network and incomplete resin infiltration of demineralized dentin should theoretically be prevented through concomitant demineralization and infiltration of monomer.\textsuperscript{18,19} Since no rinsing is necessary, the use of this adhesive is less technique-sensitive and less time-consuming. Furthermore, there is less postoperative sensitivity as a result of removal of the smear layer and smear plugs when nonrinsing adhesives are used for dentin bonding procedures.\textsuperscript{12} However, the acidic monomers in the self-etch systems are weak acids; therefore, they do not remove the smear layer or clean the surface as well as phosphoric acid. As a result, contamination might be a more serious issue in comparison with the total-etch systems.\textsuperscript{20} These adhesives appear to undergo more decreases in bond strength after application of hemostatic agents; therefore, it is even more important to rinse and clean dentin.\textsuperscript{7}

It has been reported that commercially available cavity cleaners (Prepquick and Consepsis), rinsing with water and doubling of primer application time in two-step self-etching adhesives have the potential to restore bond strength to dentin contaminated with hemostatic agent to some degrees.\textsuperscript{6,7} An attempt was made in the present study to evaluate the effect of three different contamination removal methods on bond strength of one-step self-etching adhesive to dentin contaminated with an aluminum chloride hemostatic agent. The null hypotheses tested were: (1) There are no differences in bond strength of self-etching adhesives to contaminated dentin after application of three contamination removal methods. (2) There are no differences in bond strengths between the control group and groups with different contamination removal methods.

**MATERIALS AND METHODS**

A total of 110 noncarious adult molars were selected for the purpose of this \textit{in vitro} study (100 teeth for bond strength test and 10 teeth for SEM evaluations). The teeth were stored in 0.4\% chloramine T solution at 4\degree C and used within three months after extraction.\textsuperscript{21}

The teeth were cleaned with a scalpel and all the attached soft tissues were removed. A 2-mm-thick dentin disks were prepared by perpendicular sections to the long axis of the crown using a diamond saw (Ortho Technology, The Hague, Netherlands) at slow speed under water spray. The dentin surfaces were wet-ground using 600-grit silicon carbide papers to produce a clinically relevant smear layer.

**Specimen Preparation for Shear Bond Strength Test**

One hundred dentin disks were embedded in acrylic resin blocks and randomly divided into 5 groups of 20:

- **Group I (control):** Dentin surface was air-dried to remove excess moisture.
- **Group II:** Hemostatic agent containing aluminum chloride was applied on dentin surface for 2 minutes and then air-dried.\textsuperscript{6}
- **Group III:** Hemostatic agent containing aluminum chloride was applied on dentin surface for 2 minutes, rinsed with high pressure water spray for 5 minutes and then air-dried to remove excess moisture.\textsuperscript{22}
- **Group IV:** Hemostatic agent containing aluminum chloride was applied on dentin surface for 2 minutes, 10\% EDTA solution (70\degree C; pH = 4.7) was administered for 60 seconds, rinsed for 30 seconds and then air-dried to remove excess moisture.
- **Group V:** Hemostatic agent containing aluminum chloride was applied on dentin surface for 2 minutes; then, 35\% phosphoric acid was administered for 15 seconds, rinsed for 30 seconds and air-dried to remove excess moisture.\textsuperscript{21}

The compositions of all the materials used are presented in Table 1.

**Bonding Procedures**

Clearfil S3 Bond was used on the dentin surface according to manufacturer’s instructions. Plastic molds, with an internal diameter and height of approximately 1 and 1.5 mm,
respectively, were placed at two positions on the primed dentin surface at a distance of 1 mm from the dentinoenamel junction (DEJ). A2 shade of composite (Filtek Z250; 3M ESPE, St Paul, MN, USA) was used to fill the mold and then light-cured for 40 seconds using a halogen light-curing unit (Astralis 7; Ivoclar Vivadent, Liechtenstein, Austria). The bonding interface was covered with liquid glycerine gel (Oxyguard II, Kuraray Medical Inc., Okayama, Japan) for 3 minutes to enable optimal anaerobic polymerization; then the gel was thoroughly rinsed.\(^{23}\) The bonded specimens were left at room temperature (25°C) for 1 hour before removal of the plastic mold by longitudinal cutting with a blade.\(^{23}\)

### Shear Bond Test

After 24 hours, the composite cylinders were checked under a stereomicroscope (Nikon SMZ1000, Tokyo, Japan) at ×30 for bonding defects. The cylinders, exhibiting interfacial gap formation and/or bubble inclusion, were excluded and replaced. The shear bond test was performed with universal testing machine (Hounsfield Test Equipment, Model H5K-S, Tinius Olsen Ltd., Surrey, England). A shear force was applied to each specimen at a cross-head speed of 1 mm/min. After debonding, bond strengths were recorded in MPa and fracture mode of each specimen was classified as follows: Adhesive failure, mixed failure, cohesive failure in dentin, and cohesive failure in composite.\(^{24}\)

### Statistical Analysis

The shear bond strength data were analyzed by one-way ANOVA. Two-by-two comparisons were made using Tukey test at a significance level of \(p < 0.05\). Failure mode data were descriptively reported.

### SEM Observation of the Dentin Surfaces in the Study Groups

Two specimens from each group were prepared for SEM imaging. Subsequent to different surface treatments in the study groups and application of the adhesive resin the surfaces were rinsed with acetone and water to remove resin. Then, the specimens were air-dried and sputter-coated with gold and observed in high-vacuum conditions under SEM (CamScan MV2300, Czech Republic). In addition, amounts of the aluminum on the dentin surfaces of the specimens in the groups were determined using an energy dispersive X-ray analyzer (Röntec GmbH, Berlin, Germany) connected to the SEM.

### RESULTS

#### Bond Strength Evaluation

‘The mean and standard deviations of bond strengths (in MPa) for each group are presented in Table 2.’ One-way ANOVA revealed statistically significant differences in bond strength values among the groups, with the bond strengths under the influence of surface treatments (\(F_{2,81} = 134.95, p < 0.001\)). The shear bond strength values of the control group (group I) were significantly higher than those in the other groups (\(p < 0.001\)), except for group IV (\(p = 0.933\)). ‘Failure modes are listed in Table 3.’ The majority of the specimens (68%) exhibited adhesive failures; 29% showed mixed failures; and 2% failed cohesively in composite and 1% in dentin.

#### Scanning Electron Microscopy

Figures 1A to J show scanning electron micrographs of the dentin surfaces of the five groups under study. In the control

### Table 1: Composition and manufacturer of materials used in the study

<table>
<thead>
<tr>
<th>Material</th>
<th>Composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemostatic agent</td>
<td>Aluminum chloride, alcohol ethylv, hydroxyquinoline sulfate</td>
<td>Produced by author</td>
</tr>
<tr>
<td>Conditioners</td>
<td>Ethylene diamine tetraacetic acid; MDP: 10-methacryloyloxide decyl diphosphate; Bis-GMA: Bisphenol A diglycidyl methacrylate</td>
<td></td>
</tr>
<tr>
<td>Adhesive system</td>
<td>Clearfil S3 Bond MDP (monomer), Bis-GMA, HEMA, dicanphorquinone, ethyl alcohol, water, silanated colloidal silica</td>
<td></td>
</tr>
<tr>
<td>Resin composite</td>
<td>Microhybrid composite filled with zirconia/silica</td>
<td>3M ESPE, St Paul MN, USA</td>
</tr>
</tbody>
</table>

EDTA: Ethylene diamine tetraacetic acid; MDP: 10-methacryloyloxide decyl diphosphate; Bis-GMA: Bisphenol A diglycidyl methacrylate; HEMA: Hydroxyethyl methacrylate

### Table 2: Mean and standard deviations (SD) of shear bond strengths (in MPa) in the study groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No treatment (control)</td>
<td>20.17 ± 1.61(^a)</td>
</tr>
<tr>
<td>II</td>
<td>Hemostatic agent</td>
<td>10.27 ± 0.88(^b)</td>
</tr>
<tr>
<td>III</td>
<td>Hemostatic agent + water</td>
<td>12.72 ± 1.21(^c)</td>
</tr>
<tr>
<td>IV</td>
<td>Hemostatic agent + EDTA</td>
<td>19.87 ± 1.20(^a)</td>
</tr>
<tr>
<td>V</td>
<td>Hemostatic agent + phosphoric acid</td>
<td>15.92 ± 0.98(^d)</td>
</tr>
</tbody>
</table>

Bond strength values with the same letter are not significantly different by Tukey test.
Effect of Three Different Contamination Removal Methods on Bond Strength of a Self-etching Adhesive

Figs 1A to J: Scanning electron micrographs of dentin surfaces in the study groups [Mag ×1000 (left side micrographs) and Mag ×5000 (right side micrographs)]. (A) and (B) show SEM micrographs of dentin surfaces in the control group. Smear layer has covered dentin surfaces. (C) and (D) show SEM micrographs of contaminated dentin surfaces. Hemostatic agent contamination is visible on dentin surfaces. Smear layer has covered dentin surfaces. (E) and (F) show SEM micrographs of contaminated dentin surfaces rinsed with water. Hemostatic agent contamination has been removed from the surfaces to some extent. Smear layer has covered dentin surfaces but the tubules orifices were more distinct. (G) and (H) show SEM micrographs of contaminated dentin surfaces rinsed with EDTA solution. Hemostatic agent contamination has been removed from the surfaces. Smear layer has been removed and the dentinal tubules are open; smear plugs in tubule orifices can be seen in some areas. (I) and (J) show SEM micrographs of contaminated dentin surfaces rinsed with phosphoric acid. Hemostatic agent contamination has been removed from the surfaces. Smear layer has been removed and the dentinal tubules are patent without peritubular dentin.
group most of the dentin tubules were occluded by the smear layer. In group II, hemostatic agent contamination was visible on the surface of dentin and tubules were completely occluded. Group III specimens showed contaminants on the surface less than that in group II and the tubule orifices were more distinct. EDTA and acid phosphoric conditioning in groups IV and V, respectively, had removed most of the contaminants. Dentin subjected to acid conditioning exhibited clearly visible patent dentinal tubules lacking the peritubular dentin whereas the dentin subjected to EDTA conditioning was clear, without the smear layer, and open dentinal tubules retained peritubular dentin. In a few tubules the remaining smear plugs still occluded the tubule opening. ‘Aluminum contents of the dentin surfaces of the specimens in the groups measured by energy dispersive X-ray analyzer are shown in Table 4.’ As it can be seen EDTA and acid phosphoric conditioning have removed all the aluminum from the surfaces.

**DISCUSSION**

It has been reported that different dentinal substrate conditions, including occlusogingival depth and distance from DEJ, will affect the results of bond strength tests. In the present study, all the dentin disks were prepared from the middle third of the crown and the composite cylinders were placed at the same distance from the DEJ in order to control the bonding area.23

In the present study, the self-etch adhesive exhibited a decrease in bond strength to dentin when the dentin substrate was contaminated with a hemostatic agent containing AlCl3 before the bonding procedure, which was consistent with the results of previous studies by Kuphsuk et al and O’keefe et al.6,7 Although hemostatic agents are acidic solutions with pH values of 0.7 to 3, replacement of calcium in hydroxyapatite by aluminum results in the formation of Al(OH)2H2PO4, which might increase resistance dentin surface to acid attack.6 Since, Clearfil S3 Bond is a mild self-etching adhesive with pH values of 2.3 to 2.7 and its bonding ability depends on forming short resin tags and a relatively thin submicron hybrid layer,25 it may not readily etch a more acid resistant dentin surface contaminated with hemostatic agent as seen in SEM photomicrographs (Figs 1C and D).

Decrease in bond strength may also be partly attributed to deposition of aluminum in the form of unbound minerals on the dentin surface and to formation of a layer of residue which the self-etching monomer may not able to sufficiently remove; this phenomenon might decrease monomer infiltration into dentin. The same phenomenon might also explain the increase in adhesive failures when self-etching adhesives are used on contaminated dentin surface without prior conditioning.

According to the results of the present study, rinsing or conditioning of contaminated dentin surfaces prior to the bonding procedure resulted in improved bond strength; however, there was still a significant difference with shear bond strengths of the control group. Only in the EDTA group the bond strength was not significantly different from that in the control group.

Five-minute water rinse with high pressure resulted in an increase in bond strength when compared to nonrinse nonconditioned contaminated group. Water rinse might have physically removed the unbound residue of the contaminant and the smear layer to some extent [as seen in SEM photomicrographs (Figs 1E and F)] and the monomer infiltration might have improved; however, the bond strength was still much lower than that in the control group. In addition, the duration of water rinsing (5 minutes) is clinically unacceptable.

It is probable that phosphoric acid has interacted with the Al(OH)3H2PO4 compound and aluminum residues, removing aluminum by forming AlPO426 and improving demineralization and infiltration ability of the self-etch adhesive. This phenomenon might explain increased bond strength after phosphoric acid conditioning when compared to the contaminated group. On the other hand, many studies have shown that etching with phosphoric acid etching prior to the application of self-etching adhesives decreases bond strength to dentin.27-31 Incomplete infiltration of bonding agent into the demineralized collagen network might be the reason for decreased bond strength.29,30,32 This phenomenon was confirmed by the results of the present study through observation of significantly lower bond strength in the phosphoric acid-conditioned group in comparison with the control group despite the fact that phosphoric acid removed

**Table 3: Failure modes of the specimens in study groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Adhesive failures</th>
<th>Cohesive failures in dentin</th>
<th>Cohesive failures in composite</th>
<th>Mixed failures</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>11 (55%)</td>
<td>0</td>
<td>1 (5%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>II</td>
<td>17 (85%)</td>
<td>0</td>
<td>0</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>III</td>
<td>18 (90%)</td>
<td>0</td>
<td>0</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>IV</td>
<td>10 (50%)</td>
<td>0</td>
<td>1 (5%)</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>V</td>
<td>12 (60%)</td>
<td>1 (5%)</td>
<td>0</td>
<td>7 (35%)</td>
</tr>
</tbody>
</table>

**Table 4: Aluminum contents of the dentin surfaces of the specimens in the groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (%)</th>
<th>Weight (%)</th>
<th>Sigma</th>
<th>Atomic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>1.43</td>
<td>0.08</td>
<td>2.32</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>0.69</td>
<td>0.07</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
most of the contaminants as evidenced by SEM observations (Figs 1 I and J). Since, Clearfil S3 bond was applied in a dry bonding procedure collapse and shrinkage of the collagen network might have occurred. Any collapse, even partial, might hinder resin infiltration. Failure mode analysis revealed a case of cohesive failure in dentin, which was attributed to an extensively demineralized dentin into which the bonding resin could not penetrate properly, contributing to a formation of a prone-to-break weak zone.33

Clearfil S3 Bond depends on its 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) monomer to decalcify, penetrate and create a bond with calcium ions and hydroxyapatite, simultaneously paving the way for the two-fold micromechanical and chemical bonding mechanism.25 As it is seen in SEM photomicrographs phosphoric acid conditioning has removed peritubular dentin and smear plugs, reducing the amount of calcium available for chemical interaction with 10-MDP.

EDTA is a hexaprotic system which can be designated as $H_6Y^2+$. Its degree of protonation depends on the pH of the solution, and its ability to coordinate metal ions depends on the pH of the solution and the nature of the metal ions.34 The conventional methods for determination of aluminum are based on complexometry using EDTA.35 Considering the pH value of EDTA (4.7) used in this study, it is assumed that at this pH value EDTA acts as a mild demineralizing agent in addition to its aluminum chelating activity. The EDTA solution was heated up to 70ºC since it seems that at high temperatures the binding of $Al^{3+}$ tends to increase the dissociation of $Al(OH)_2H_2PO_4$ and chelating ability of EDTA. Chelating ability of EDTA rapidly rose to a higher level within 1 minute and further exposure to EDTA only doubled this effect in 15 minutes.36,37 Therefore, it seems that 1-minute administration of EDTA is sufficient to remove aluminum contaminant. It appears application of EDTA and rinsing of dentin surfaces contaminated with hemostatic agent removed any residues and aluminum content present on the surface, producing a clean surface for bonding. In addition to chelating the aluminum contaminant EDTA has been widely used to dissolve mineral phase of dentin without denaturing dentin proteins, thereby avoiding major alterations of the native fibrillar structure of dentin.38 Since, the unaltered collagen fibrils are believed to preserve most of their intrafibrillar minerals they are more stable and less affected by dehydration, which subsequently improves the infiltration of the adhesive resin.22,39-43 In addition, the remaining hydroxyapatite serves as a substrate to chemically interact with 10-MDP monomer. Given what was mentioned above, the increase in bond strength of EDTA-conditioned contaminated dentin to near that in the control group is justifiable.

Currently, few studies have reported the effect of hemostatic agents on tooth structures. The differences in the chemical compositions of hemostatic agents might affect tooth structures differently. Therefore, further studies using different hemostatic agents are necessary. Sealing ability and bond durability of hemostatic agent-contaminated dentin rinsed with EDTA should also be investigated.

CONCLUSION

When an aluminum chloride hemostatic agent was used bond strength of self-etch adhesive was lower compared to normal dentin. Application of EDTA restored the bond strength; however, acid phosphoric and water failed to increase the bond strength to the level of that in the control group.

CLINICAL SIGNIFICANCE

EDTA and phosphoric acid removed aluminum chloride hemostatic agent contamination in dentin surfaces. However, unlike EDTA, phosphoric acid failed to increase the bond strength of self-etch adhesive to dentin to the level of the control group.

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