

## Influence of Ankaferd Blood Stopper on shear bond strength of bonding systems

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This study investigated the effect of Ankaferd Blood Stopper (ABS) contamination on bond strength of total- and self-etching systems. Seventy mandibular third molars sectioned in a mesio-distal direction were mounted in acrylic resin, and flat dentin surfaces were exposed. The specimens were randomly assigned to seven groups ( $n=20$ ), according to the surface treatment: Group I, ABS contamination+37% phosphoric acid+Solobond M; Group II, ABS contamination+Clearfil SE Bond; Group III, ABS contamination+All Bond SE; Group VI, 37% phosphoric acid+ABS contamination+Solobond M; Group V, Solobond M; Group VI, Clearfil SE Bond; and Group VII, All Bond SE. Next, a resin composite (Grandio) was built up using a plastic apparatus and polymerized. The specimens were tested in shear mode at a crosshead speed of 1 mm/min. There were significant differences in bond strengths between the control and ABS-contaminated samples. These findings suggest that ABS contamination reduced bond strength of total- and self-etching adhesives.

**Keywords:** Ankaferd Blood Stopper, Shear bond strength, Self-etching adhesive, Total-etching adhesive

### INTRODUCTION

Prevention of moisture contamination is of paramount importance when using dentin bonding agents. However, many carious lesions are found in areas in which it is difficult to obtain appropriate isolations, especially when the site is near or at the gingival margin, where blood contamination is more likely to occur<sup>1-3</sup>.

Blood contamination reduces resin/dentin bond strength significantly more so than salivary contamination<sup>4</sup>. The influence of blood contamination on bond strength can be attributed to its high protein content which, along with macromolecules such as fibrinogen and platelets, can form a film on the dentine surface, obstructing the penetration of the adhesive system into dentine tubules<sup>1,5</sup>. Clinical and *in vivo* studies have been reported discussing various haemostatic agents used for the management of hemorrhage in clinical dentistry, such as hydrogen peroxide ( $H_2O_2$ ), ferric sulfate, aluminum chloride, trichloroacetic acid and Ankaferd Blood Stopper (ABS) (Ankaferd Drug INC, Istanbul, Turkey)<sup>6-10</sup>. A previous study showed that  $H_2O_2$  had negative effects on bond strength<sup>6</sup>. The bond strength of a self-etching adhesive to dentin contaminated with ferric sulfate or aluminum chloride dramatically decreases compared to the normal dentin group<sup>8,9</sup>. On the other hand, trichloroacetic acid had a positive effect on bond strength between enamel and resin composite<sup>7</sup>. However, there is little evidence about the effects of ABS on shear bond strength of resin composite<sup>10</sup>.

ABS is a standardized mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica*, each of which has some effect on the endothelium, blood cells, angiogenesis,

cellular proliferation, vascular dynamics, and/or cell mediators<sup>11-15</sup>. ABS is a unique folkloric combined medicinal plant extract that has been approved in Turkey for the management of post surgery dental bleedings and external hemorrhage<sup>10,16</sup>. ABS can be used as a spray, solution, and tampon. Goker *et al.*<sup>16</sup> showed the haemostatic effects of ABS and reported its therapeutic potential to be used for the management of hemorrhage. ABS quickly forms a network inside plasma or serum (in a matter of seconds). The ABS-induced network formation is related to the functions of blood proteins and red blood cells. General haemostatic and biochemical tests have shown that the network is formed *via* the interaction between ABS and proteins in blood, primarily via the product's interaction with fibrinogen.

Because individual clotting factors (coagulation factors II, V, VII, VIII, IX, X, XI, and XII) were not affected by the network formation, the anti-haemorrhagic process was possibly driven by protein agglutination. Blood cells (erythrocytes and platelets) also aggregated and participated in the network formation, with the erythrocytes forming a mass. These observations suggest that the ABS-induced formation of the protein network affected the entire physiological haemostatic process without unequally affecting any individual clotting factor. Therefore, ABS is not only effective on patients with normal haemostatic values, but also on those with primary and secondary hemostasis<sup>16,17</sup>.

ABS can be employed as a product to aid in clinical procedures to stop intensive dental hemorrhage<sup>18</sup>. In a clinical experience, ABS was found to be an effective haemostatic agent for the treatment of excessive bleeding following dental surgery in 4 patients with hemorrhagic diathesis<sup>19</sup>.

The aim of this study was to evaluate the effect of

ABS contamination on bond strength of total- and self-etching adhesives systems. The null hypotheses tested was that Ankaferd Blood Stopper contamination would not affect the bond strength of total- and self-etching adhesives systems.

### MATERIALS AND METHODS

Seventy caries-free mandibular third molars were stored in 0.5% chloramin solution at 4°C and used within one month after extraction. The teeth were sectioned in a mesio-distal direction with a low-speed diamond disk saw (Buehler, Lake Bluff, IL, USA) under a water coolant. The sectioned teeth were embedded in autopolymerizing acrylic resin, and a flat dentin surface was exposed. The exposed dentin surfaces were further flattened on wet with a 600-grit Si-C paper for 60 s to standardize the smear layer. The teeth were then rinsed with distilled water to remove any debris. The prepared teeth were randomly divided into 7 groups ( $n=20$ ) according to the following factors: ABS contamination protocols (before phosphoric acid or after, before primer/bonding resin) and the adhesive systems being used as control groups.

Group I: One drop of ABS solution was applied directly to the dentin surface and air-dried for 10 s. The standardized vials of the ABS (ABS patent number 2009-906002) used in the experiments were supplied by

Ankaferd Drug INC, Istanbul, Turkey. The dentin surfaces of all teeth were etched with 37% phosphoric acid for 15 s; this was followed by thorough washing and air-drying. Then a total-etching bonding system (Solobond M, Voco, Cuxhaven, Germany) was applied as recommended by the manufacturer (Table 1).

Group II: One drop of ABS solution was applied directly to the dentin surface and air-dried for 10 s. Then a two-step self-etch bonding system (Clearfil SE Bond, Kuraray, Tokyo, Japan) was applied as recommended by the manufacturer (Table 1).

Group III: One drop of ABS solution was applied directly to the dentin surface and air-dried for 10 s. Then a one-step self-etch bonding system (All Bond SE, Bisco, IL, USA) was applied as recommended by the manufacturer (Table 1).

Group IV: The dentin surfaces of all teeth were etched with 37% phosphoric acid for 15 s; this was followed by thorough washing and air-drying. Then one drop of ABS solution was applied directly to the dentin surface and air-dried for 10 s. Solobond M was applied as described at Table 1.

Group V: The dentin surfaces of all teeth were etched with 37% phosphoric acid for 15 s; this was followed by thorough washing and air-drying. Solobond M was applied as described in Table 1. This group served as a control group.

Group VI: Clearfil SE Bond was applied as described

Table 1 Composition and application mode of adhesive systems used in this study

| Materials        | Composition  | Manufacturer<br>(Batch no)  | Mode of application  |
|------------------|--|---|--|
| Solobond M       | Bis-GMA, HEMA, BHT, acetone, organic acids   | Voco, Cuxhaven, Germany<br>(1005234)  | Apply etchant (37% phosphoric acid) for 15 s, rinse and air-dry, apply adhesive to the prepared surface with a brush, let act for 30 s, disperse adhesive with a faint air jet, light cure for 20 s. |
| Clearfil SE Bond | <i>Primer:</i> MDP, HEMA, hydrophilic dimethacrylate, dl-camphorquinone, N,N-diethanol-p-toluidine, water<br><br><i>Bond:</i> MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-camphorquinone, N,N-diethanol-p-toluidine, silanated colloidal silica | Kuraray, Osaka, Japan<br><br>( <i>Primer:</i> 00972A,<br><i>Bond:</i> 01443A) | Apply primer with a brush for 20 s, gently air dry, apply adhesive with a brush, air flow gently, light cure for 10 s.   |
| All Bond SE      | <i>Part I:</i> Ethanol, sodium benzene sulfinate<br><br><i>Part II:</i> HEMA, bis (glyceryl 1,3 dimethacrylate) phosphates, biphenyl dimethacrylate  | Bisco, IL, USA<br><br>Part I : 1000009517<br>Part II: 1000009518              | Mix Part I and Part II until uniformly pink, apply 1–2 coats, gently air dry starting from 5 cm for 5 s until there is no visible movement of the material, light cure for 10 s.                     |

Abbreviations: Bis-GMA; bisphenol-A-glycidyl methacrylate, HEMA; 2-hydroxyethyl methacrylate, BHT; butylated hydroxyl toluene, MDP; 10-methacryloyloxydecyl dihydrogen phosphate

in Table 1 and used as control.

Group VII: All Bond SE was applied as described in Table 1 and used as control.

After adhesive application, a resin composite (Grandio, Voco, Cuxhaven, Germany) was built up on the dentin of each specimen by packing the material into a cylindrically shaped plastic apparatus with an internal diameter of 2.34 mm and a height of 3 mm (Ultradent Products, South Jordan, UT). Excess composite was carefully removed with an explorer, and the specimens were light-cured with an LED (Valo, Ultradent Products Inc, South Jordan, USA) (1,000 mW/cm<sup>2</sup>) for 20 s. All specimens were stored in a moisture medium at 37°C for 24 h. Shear bond strength (SBS) was tested using a Universal Testing Machine (Instron, USA) with a notched blade attached to a compression load traveling at a crosshead-speed of 1 mm/min. Maximum loads at bond failure were recorded in Newtons (N), and bond strengths were calculated in megapascals (MPa).

Fracture surfaces were examined under a dissecting microscope (SZ-TP Olympus, Japan) at a magnification of 20×, and failure modes were classified as either adhesive fracture (*i.e.*, failure at the dentin-resin interface); cohesive fracture (*i.e.*, failure within the resin composite or dentin); or mixed fracture (*i.e.*, a combination of adhesive and cohesive fracture).

Following the debonding procedure, specimens of each sub-group was overlaid with gold and observed

under a Scanning Electron Microscope (SEM) (LEO 440, Oxford, England) at 2,000× magnification.

Shapiro-Wilk test was used for continuous variables. One-way analysis of variance (ANOVA) and Tukey HSD post hoc test was used for the comparison of the data obtained from Group I, IV and V. Student's *t*-test was used for the comparison of Group II with VI and Group III with VII. Failure mode distributions were compared using the chi-square test. Significance for all statistical tests was predetermined at  $p < 0.05$ .

## RESULTS

Mean shear bond strength values of the experimental groups are listed in Table 2, 3. There were statistically significant differences between the bond strength values of Group I and Group V, Group I and Group IV, Group IV and Group V, Group II and Group VI, and Group III and Group VII ( $p < 0.05$ ).

In all groups, adhesive type failure mode was the most common ( $p < 0.05$ ) (Table 2, 3). Among the groups, there was no significant difference for the failure modes ( $p > 0.05$ ).

Figures 1, 2, 3 show SEM imaginations of fractured specimens from each group. There were both resin tags and resin remnants at Groups V and VI when compared to the other groups. Closed dentinal tubules were seen in Groups I, II, and III. The resin tags and opened dentinal

Table 2 Shear bond strengths to dentin in MPa and failure mode distributions of groups

| Groups  | Shear bond Strength<br>(Mean)±Standard<br>Deviation (SD) | Failure mode |          |     |
|---|--|--------------|----------|-----|
|   |  | Adhesive     | Cohesive | Mix |
| Group I<br>(Ankaferd+Acid etching+Solobond M)       | 7.15±(4.73) <sup>a</sup>                                 | 17           | –        | 3   |
| Group IV<br>(Acid etching+Ankaferd+Solobond M)      | 12.23±(3.64) <sup>b</sup>                                | 16           | –        | 4   |
| Group V<br>(Acid etching+Solobond M, Control Group) | 16.06±(5.76) <sup>c</sup>                                | 16           | –        | 4   |

Means followed by superscript with same letter indicate no statistical differences at  $p < 0.05$ .

Table 3 Shear bond strengths to dentin in MPa and failure mode distributions of groups

| Groups  | Shear bond Strength<br>(Mean)±Standard<br>Deviation (SD) | Failure mode |          |     |
|---|--|--------------|----------|-----|
|   |  | Adhesive     | Cohesive | Mix |
| Group II<br>(Ankaferd+Clearfil SE Bond)       | 9.12±(4.53)  | 15           | –        | 5   |
| Group III<br>(Ankaferd+All Bond SE)           | 9.50±(3.19)  | 18           | –        | 2   |
| Group VI<br>(Clearfil SE Bond, Control Group) | 16.89±(3.91)   | 16           | –        | 4   |
| Group VII<br>(All Bond SE, Control Group)     | 14.75±(4.60)   | 18           | –        | 2   |

Means followed by superscript with same letter indicate no statistical differences at  $p < 0.05$ .

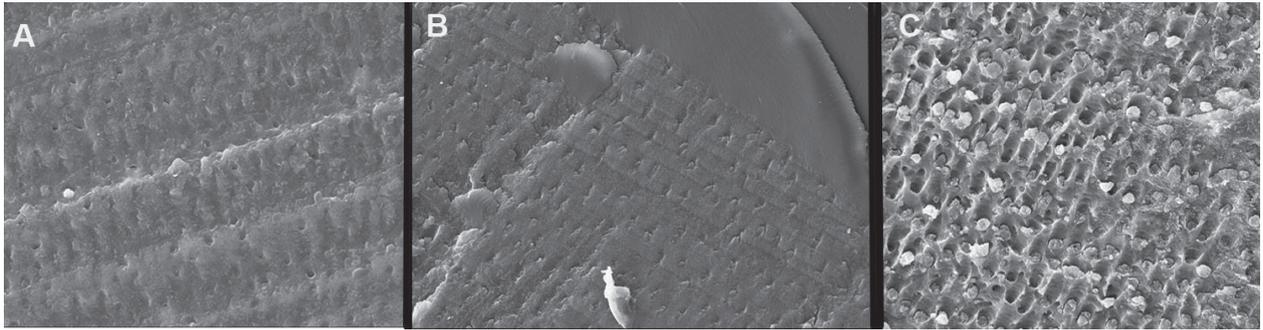


Fig. 1 Morphological aspects of fractured surface of Group I, IV and V at 2,000× magnification (A: ABS+Acid etching+Solobond M, B: Solobond M, Control Group, C: Acid etching+ABS+Solobond M).

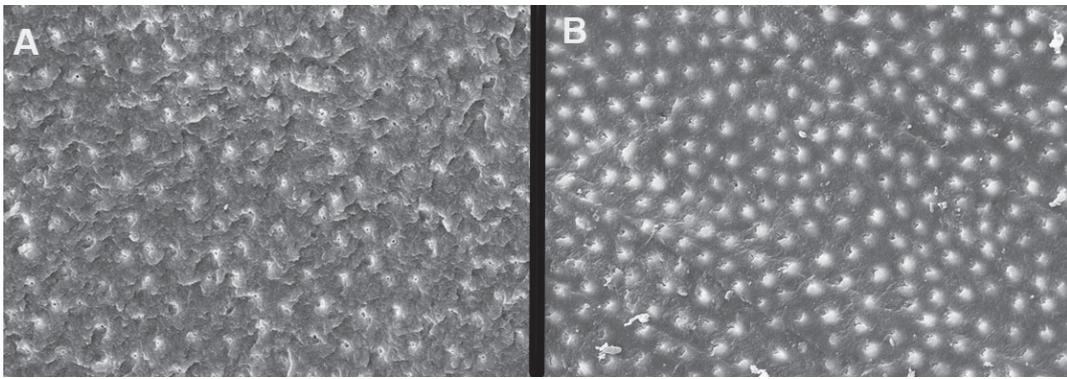


Fig. 2 Morphological aspects of fractured surface of Group II and VI at 2,000× magnification (A: Clearfil SE Bond, Control Group, B: ABS+Clearfil SE Bond).

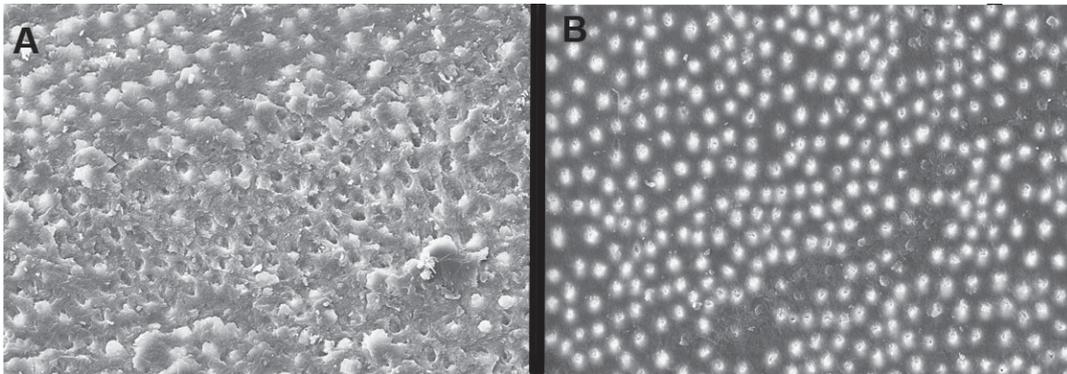


Fig. 3 Morphological aspects of fractured surface of Group III and VII at 2,000× magnification (A: All Bond SE, Control Group, B: ABS+All Bond SE).

tubules were observed in Groups IV and VII.

#### DISCUSSION

In this study, the effect of ABS contamination on bond strength of total- and self-etching adhesives systems to dentin was evaluated. ABS contamination reduced bond strength of both total- and self-etching adhesives.

When rubber dam isolations are not used, contamination of the operating field with saliva or blood is a frequent problem<sup>20</sup>. Saliva and blood contamination may also create a thin film on the dentin surface that hinders adhesive penetration into the dentinal tubules<sup>21</sup>. Previous studies have indicated that contamination with blood during restorative procedures leads to a significant decrease in bond strength of resin-based materials to

teeth<sup>22-24</sup>). However, sometimes using a rubber dam is impossible when the cavity margin was under the gingival. However, in this situation, gingival bleeding may occur. In these situations, the clinicians had rinsed thoroughly after the blood and use of haemostatic agents. The present study used both total- and self-etching adhesives. Self-etching adhesives systems provide a faster application due to a reduced number of components and application steps<sup>25</sup>). Although a short application time reduces the risk of blood contamination in the field of operation, it may sometimes be impossible to maintain a dry operative field. In the total-etching system, gingival bleeding can occur after rinsing off the phosphoric acid gel, coincidentally coming into contact with cavosurface margins<sup>1,2,20</sup>).

*In vitro* shear bond strength testing is commonly used to quantitatively analyze and rank the performance of adhesive systems on enamel and dentin<sup>26,27</sup>). The SBS test is a simple procedure for the experimental evaluation of adhesives system<sup>28</sup>). Shear bond testing simulates vertical forces similar to biting forces. It has been proven adequate and effective for evaluation and comparison of different adhesive systems and restorative materials. Therefore, we used the shear bond strength test in this study.

Bond strength of bonding systems to dentin is negatively influenced by blood contamination<sup>10,29,30</sup>). The manufacturer produced many haemostatic agents to stop the bleeding. However, there is limited data about the effect of ABS on bond strength. The present study investigated the effect of ABS used for the management of hemorrhage before adhesive application on the bond strength of a two-step total-etching adhesive and a two-step and a one-step self-etching adhesives. Based on the results of the current study, ABS application reduced the shear bond strength of all three adhesives to dentin. In this study, ABS appeared to be a physical barrier that impairs the mechanical retention of adhesive resins. In the two-step total-etching adhesive system, Group I had a lower SBS value than Group IV. In Group IV, acid applied before ABS contamination may have promoted the increase of the permeability and the demineralization of dentin so that monomers could better infiltrate into the exposed collagen fiber mesh.

Except from Trakyali and Oztoprak's<sup>10</sup>) study, there is no published data about comprehensive observations or intraoral applications concerning the ABS effect on bond strength. The results of that study are similar to the findings of the present study. However, according to Trakyali and Oztoprak<sup>10</sup>), although a statistically significant difference was observed between SBS values of Ankaferd-contaminated group (9.58±0.95 MPa) and the control group (19.56±1.84 MPa), the SBS values of the Ankaferd-contaminated group were between 6 and 10 MPa. It was clinically acceptable.

The fracture analysis findings of this study showed a predominance of adhesive failure. Cohesive failures point out high bond strength, but adhesive failures point out low bond strength<sup>31,32</sup>).

The SEM analysis showed that fewer resin tags

were observed at the ABS applied groups due to closed dentinal tubules. This condition supported our claim that the ABS appeared to be a physical barrier that limits the affinity of the resin bonding materials to the tooth structure. In addition, acid application before ABS contamination opened the dentinal tubules and had limited the negative effect of ABS. Consequently, this procedure may have promoted the increase of the permeability and the demineralization of dentin so that a bonding resin could better penetrate to dentin.

## CONCLUSIONS

Even considering the limitations of this *in vitro* study, ABS contamination reduced the bond strength of both total- and self-etching adhesives. When clinicians decide to use an ABS solution as a haemostatic agent, they should prefer the total-etching adhesive system. We recommend further *in vivo* or *in vitro* studies to evaluate the effect of the removal of ABS with various agents shortly before dental adhesive application.

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