

# Using Hemostatic Agents During Orthodontic Bonding: An *In Vitro* Study

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## ABSTRACT

**Objective:** The aim of this *in vitro* study was to compare the effect of a traditional hemostatic agent on the shear bond strength (SBS) of conventional and self-etching bonding systems to find an efficient procedure for orthodontic bonding.

**Material and Methods:** Extracted human premolars (n=108) were divided into 6 equal groups. Conventional light cure primer was used in groups 1, 2, and 3. Acid etching and primer were applied directly in group 1. Enamel surfaces were covered with a Ankaferd Blood Stopper (ABS), which was used as a hemostatic agent in groups 2 and 3. Orthophosphoric acid was applied on ABS-covered enamel in group 2. Before the acid was applied, the enamel surface was cleaned with wet surgical gauze in group 3. A self-etching primer was used in groups 4, 5, and 6. Similar to the first 3 groups, primer was applied on the enamel surface in group 4. Samples were covered with ABS before the primer was applied in groups 4 and 5. The ABS residue was cleaned with gauze before primer application in group 6. The samples were debonded using a universal testing machine. In addition, SBS and residual adhesive were evaluated.

**Results:** Samples contaminated with hemostatic agent and bonded with the self-etching primer without cleaning the hemostatic agent showed significantly lower SBS ( $p<0.05$ ). Cleaning the ABS on the enamel surfaces increased the bonding strength of self-etching primers. Groups 5 and 6 showed significantly lower ARI scores ( $p<0.05$ ).

**Conclusion:** Before bonding orthodontic attachments with conventional and light-cure primers, ABS can be safely used. However, using self-etch primers directly on the ABS-applied enamel surface should be avoided. (*Turkish J Orthod* 2015;28:38–43)

**KEY WORDS:** Bonding, Hemorrhage, Impacted teeth, Shear bond strength

## INTRODUCTION

Traditional composite resin bonding materials require a dry tooth surface for optimum bonding strength.<sup>1</sup> In some clinical situations, however, such as bonding the molar tubes near the gingival border or bonding attachments on surgically exposed impacted teeth, it is difficult to isolate the field and obtain a dry tooth surface.<sup>2</sup> Bleeding is one of the most common complications during bonding of the attachments in a surgical intervention. The blood flow into the bonding area may make a standard bonding procedure impossible.<sup>3</sup> Blood and saliva contamination decrease bonding strength regard-

less of the bonding system used.<sup>4,5</sup> Hemostatic agents are widely used to prevent blood flow into the bonding site. The effect of these agents on bond strength is questionable. Several hemostatic agents have been tested for their effect on bonding strength in earlier studies. Contamination of aluminum chloride ( $AlCl_3$ ) was reported to decrease the bonding strength of self-etching primers.<sup>6</sup> The effect of calcium sulfate (CaS) on bond strength is still in question when the agent is used as a hemostatic agent.<sup>7</sup>

Ankaferd Blood Stopper (ABS; Ankaferd Drug Inc, Istanbul, Turkey) is a plant extract used in Turkish

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traditional medicine as a hemostatic agent; it can also be used to obtain a blood-free tooth surface during the bonding procedure.<sup>8</sup> ABS induces very rapid (<1 second) formation of a protein network in plasma and serum samples.<sup>9</sup> The rapid activity can be useful in surgical operations or clinical situations when it is difficult to isolate the bonding area. Previous studies tested ABS against a decrease in the shear bond strength (SBS) of orthodontic brackets. Traklyali and Oztoprak<sup>8</sup> asserted that ABS could be safely used during orthodontic bonding of surgically exposed teeth, although the SBS with ABS application was nearly half the bonding strength in the control group.

Although self-etching primers have lower bond strength than traditional bonding systems, self-etching primers are useful in areas where isolation is difficult.<sup>10,11</sup> A self-etching primer reduces the number of steps in the bonding procedure, and application is simpler than in the etch-and-rinse systems, which reduces the risk of contamination.<sup>12</sup> When self-etching primers are used in bonding attachments on impacted teeth during surgical intervention, the time saved can also be beneficial in reducing the total time of the surgery.

The aim of this *in vitro* study was to compare the effect of a relatively new hemostatic agent (ABS) on the SBS of conventional and self-etching bonding systems and their bond failure interfaces to demonstrate an efficient and effective method of bonding when a hemostatic agent is used during bonding attachments with surgical intervention.

## MATERIALS AND METHODS

One hundred eight human premolars extracted for orthodontic purposes from patients between 14 and 17 years old were collected for this study. The teeth were cleaned of soft tissue and stored in distilled water. Samples were examined under a light microscope with  $\times 20$  magnification for any visible defects. A criterion for tooth selection was no visible defect and/or restoration on the buccal enamel. The sample size of this *in vitro* study was determined using the data from the study of Özer *et al.*<sup>13</sup> based on a significance level of 0.05 and a power of 95% to detect a meaningful difference between the SBS of the groups. The power analysis indicated that 16 teeth were needed per group. Because of possible dropouts, 18 teeth per group were included in the study.

The extracted teeth were stored in distilled water, and the water was changed weekly to avoid bacterial accumulation. The teeth were mounted in casts with the long axis of the tooth parallel to the bottom of the mold and the plastic molding cup filled with dental stone. Tooth samples were randomly divided into 6 groups after cleaning, pumicing, and polishing. Mesh-based stainless steel upper premolar orthodontic brackets (Gemini, 3M-Unitek, Monrovia, CA, USA), with a 0.022-inch slot and a surface area of 9.08 mm<sup>2</sup>, were bonded to all the teeth by the same operator. A conventional etch-and-rinse system and a light-cure primer (Transbond XT primer, 3M Unitek, Monrovia, CA, USA) were used in the first 3 groups. The last 3 groups were bonded with a 1-step self-etching primer (Transbond plus self-etching primer, 3M Unitek, Monrovia, CA, USA). All the samples were bonded with the same light-cure adhesive (Transbond XT, 3M Unitek, Monrovia, CA, USA) according to the manufacturer's instructions.

The bonding procedures were performed as follows:

- *Group 1:* The enamel surfaces were etched with 37% phosphoric acid for 15 seconds, rinsed with atomized water, and dried thoroughly afterward. A thin coat of Transbond XT primer was applied to the enamel surfaces and light cured for 15 seconds. The brackets were bonded directly with Transbond XT light-cure adhesive resin. The adhesive was light-cured for 20 seconds with a light-emitting diode light source.
- *Group 2:* The enamel surfaces were covered directly with ABS and dried with air spray at least for 30 seconds until there was no visible movement of the material. After the ABS was thoroughly dried, enamel was etched with 37% orthophosphoric acid. Transbond XT light-cure primer and the same curing times for group 1 were used for bonding.
- *Group 3:* Teeth were also covered with ABS in group 3. The ABS-contaminated bonding surfaces were lightly swiped with wet surgical gauze to clean the ABS residue before the acid was applied. The bonding and acid-etching protocols were the same as for groups 1 and 2.
- *Group 4:* A 1-step self-etch bonding system was used in group 4. The bonding system was applied according to the manufacturer's instructions.

**Table 1.** Descriptive statistics of the shear bond strengths

	n	Mean	SD	Minimum	Maximum	p <sup>a</sup>
Group 1 (Transbond XT)	18	10.909	3.610	4.370	19.459	A
Group 2 (ABS + Transbond XT)	18	11.371	5.272	4.162	21.332	A
Group 3 (ABS + cleaning + Transbond XT)	18	9.255	3.458	4.995	17.378	A
Group 4 (Transbond plus)	18	10.995	3.512	6.035	19.251	A
Group 5 (ABS + Transbond plus)	18	4.942	2.327	0.000	9.469	B
Group 6 (ABS + cleaning + Transbond plus)	18	10.350	3.477	5.931	19.667	A

<sup>a</sup> Different letters show statistical difference ( $p < 0.05$ ).

- *Group 5:* Teeth were covered with ABS, and the surfaces were dried before the same self-etching system was applied.
- *Group 6:* The same procedures for the group 5 were applied for group 6 with one exception: the ABS on the bonding surface was lightly cleaned with wet surgical gaze before the bonding primer was applied.

The same adhesive resin (Transbond XT) and light-curing times were used in all groups.

After the bonding procedure was performed, the samples were stored in distilled water at 37°C for 24 hours for maximum bond strength. A universal test machine (Lloyd Instruments Plc, Fareham, UK) with a crosshead speed of 0.5 mm/min was used to measure the SBS. The testing apparatus was modified to measure SBS. Measurements were made in Newtons and converted into megapascals with the following equation: Shear force (MPa) = Debonding force (N)/(w/l) (mm<sup>2</sup>), where w = width of the bracket base, l = height of the bracket base, and 1 MPa = 1 N/mm<sup>2</sup>.

After the bond strength tests, the enamel surfaces and bracket bases were illuminated with a cold-light source (Photonic PL2000 Photonic Optische Geräte GmbH & Co KG, Vienna, Austria) under a light microscope (Nikon SMZ 1500, Nikon, Tokyo, Japan) to identify the adhesive remnant on the bracket base and tooth surfaces. ARI was then scored with 20× magnification. Årtun and Bergland's<sup>14</sup> 4-point scale was used for Adhesive Remnant Index (ARI) scoring: 0, = no adhesive left on the tooth; 1 = less than half the adhesive left on the tooth; 2 = more than half the adhesive left on the tooth; and 3 = all the adhesive left on the tooth with a distinct impression of the bracket mesh.

### Statistical Analysis

The Shapiro-Wilk test was used to assess the assumption of normality of all the obtained data

before nonparametric tests were used. A one-way analysis of variance (ANOVA) test was used to compare the groups, and multiple comparisons were performed using the Tukey honestly significant difference (HSD) method. Differences in non-normally distributed variables among ARI scores were tested using the Kruskal-Wallis test. Then Dunn multiple comparison test was applied to determine any further differences among the groups, and  $p$  values less than 0.05 were considered significantly different. All the computational work was performed with MINITAB (Minitab V. 13.20, 2000 Minitab Ltd., Coventry, United Kingdom).

### RESULTS

The one-way ANOVA test showed statistical differences between the groups. Tukey HSD showed that there was no statistically significant difference between the bond strength of the groups, except for group 5 (Table 1). Specimens contaminated with hemostatic agent and bonded with the self-etching primer showed significantly lower SBS than the other groups ( $p < 0.05$ ). Applying ABS on the bonding surface before applying the self-etching primer decreased the bond strength to half the bonding strength of the self-etch group without ABS contamination. Therefore, cleaning the ABS-contaminated bonding surface with wet surgical gauze increased the bond strength to normal values. However, ABS contamination before standard etch-and-rinse phosphoric acid etching and light-cure primer application had no statistically significant effect on SBS.

The ARI scores showed parallel findings with bond strength (Table 2). The Kruskal-Wallis test showed statistical differences between the groups' ARI scores ( $p < 0.001$ ). The Dunn multiple comparison test showed that groups 5 and 6 had statistically lower ARI scores (Table 2). These results showed that the bond between the tooth surface and the bonding material failed when ABS was used before

**Table 2.** Frequency distribution of Adhesive Remnant Index (ARI) scores

	ARI Score <sup>a</sup>				<i>p</i> <sup>b</sup>
	0	1	2	3	
Group 1	1	3	11	3	a
Group 2	1	2	12	3	a
Group 3	2	7	7	2	ab
Group 4	1	5	10	2	a
Group 5	9	8	1	0	c
Group 6	4	10	4	0	bc

<sup>a</sup> 0 indicates no adhesive left on the tooth; 1, less than half the adhesive left on the tooth; 2, more than half the adhesive left on the tooth; 3, all the adhesive left on the tooth with a distinct impression of the bracket mesh.

<sup>b</sup> Different letters show statistical difference. ( $p < 0.05$ ).

the self-etching primer was applied. Although cleaning ABS before applying the primer increased bond strength, cleaning had a very limited effect on the ARI score.

## DISCUSSION

Several studies have shown that bleeding during orthodontic bonding, in particular with surgical exposure of teeth, is an important problem that must be overcome. Hemostatic agents are a reliable option for stopping the blood flow into the surgery site, but they are likely to decrease the SBS when the bonding surface of the tooth is not thoroughly isolated from or cleaned of these hemostatic agents.

However, cleaning the hemostatic agent can be undesirable because the mechanic effect of cleaning can break the fibrin formation in the area and can therefore lead to increased bleeding.

The generic plant extract ABS, which has been used historically in Turkish traditional medicine, has been approved for use in managing external hemorrhage and dental surgery bleeding. It consists of a standard mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica*.<sup>9</sup> The safety and efficacy of ABS have been reported in several studies that also described its sterility and nontoxicity.<sup>15</sup> The basic mechanism of action of ABS is to form an encapsulated protein network that provides focal points for vital erythrocyte aggregation. This network covers the primary and secondary hemostatic system without disturbing the individual coagulation factor however the exact mechanism of its action remains unknown.<sup>9</sup> Ease of use and relatively fast effect were thought to be advantageous in dental

surgical interventions, when it is especially difficult to obtain an isolated environment. Utility of ABS in dental surgical interventions has been shown in several previous studies.<sup>15,16</sup>

The aim of this study was to demonstrate an efficient way of bonding orthodontic attachments during a surgical intervention when hemostatic agents were used to stop the bleeding. All of the groups except group 5 exceeded the 8 MPa SBS value, which was considered adequate for successful orthodontic attachment bonding.<sup>17</sup> However, different applications of the blood stopper exhibited different amounts of SBS. In particular, SBS of the orthodontic attachments was significantly decreased when ABS was applied to the enamel surface before the self-etching primer was applied. On the other hand cleaning the bonding surface with wet surgical gauze after applying ABS increased the bonding strength of self-etch primers to acceptable levels. Applying ABS before bonding had no significant effect on SBS when the traditional etch-and-rinse technique and light-curing primer system were used.

During orthodontic attachment bonding on surgically exposed teeth, there is a high risk of blood contamination on the bonding site. The residual blood remaining in the bonding site was shown to excessively decrease the SBS of orthodontic attachments independent of the system used for bonding.<sup>18,19</sup> Bonding the attachment at a later visit after surgery has the advantage of easier application in a blood-free environment and does not require a chair-side surgeon; however, in this method, the surgeon must expose more of the tooth and aim for secondary healing of the exposed site. In addition, Becker *et al.*<sup>3</sup> showed that the reliability of bonding in a later visit was much poorer than when performed at the surgery. Therefore, the main aim of the surgeon in this phase of the surgery is to maintain a bloodless field of operation for the bonding procedure. Persistent bleeding from the bone field can be controlled by pressure or bone wax application; soft tissue bleeding may be stopped with electrocautery or ligation of the vessel.<sup>20</sup> All of these techniques require specific knowledge of the procedure and have the disadvantage of prolonging the total surgery time. Hemostatic agents, however, are easier to apply, and their effect can be seen in a relatively shorter time. Liquid etchants should not be used when hemostatic agents are applied in the exposure site because their spread can lead to soft tissue damage and can disturb the fibrin formation.<sup>21</sup> Therefore, self-etching primers could be useful in

such cases as they do not need a separate rinsing protocol.

A one-step self-etch system was used in groups 4, 5, and 6 because of their distinct advantage in the bonding protocol during a surgical intervention. One of the most important disadvantages of the traditional phosphoric acid etching is its damage on enamel tissue, which was reported to occur less frequently with self-etching primers.<sup>10</sup> Self-etching primers have the advantage of being successfully used in bonding orthodontic brackets even after light salivary contamination.<sup>22</sup> In a previous study, two-step self-etch systems were proven to have superior bonding characteristics when there was ABS contamination on the bonding site.<sup>23</sup> However, two-step systems have more application steps, which may be a disadvantage in risky areas. Although there was no significant decrease in bond strength when ABS was cleaned with wet surgical gauze before the self-etch system was used, the ARI scores showed a significant decrease compared with self-etch application without ABS contamination. In addition, there was no statistical difference between the ARI scores for groups 5 and 6, which showed statistical difference in SBS. These results indicate that ABS contamination negatively affected the enamel self-etch primer interface when the bonding surface is covered with ABS.

When the etch-and-rinse technique is used, the tooth surface is usually rinsed with sterile saline.<sup>20</sup> However, salt crystals remaining from dried saline can lead to problems in the bonding interface. To prevent this problem, some practitioners rinse with atomized water.<sup>21</sup> Atomized water was used in our study to prevent any potential problems caused by saline use.

In this study, ABS was directly used on the tooth surface without blood contamination. In several previous studies, no blood contamination occurred before ABS was applied.<sup>8,24,25</sup> Blood was not used because in most cases the blood flow occurs in the margins of soft tissue. Most of the tooth surface is covered only with hemostatic agent.

The ARI scores showed that for groups 5 and 6 the bond failed between the enamel surface and the bonding material, while group 4 did not show any differences in the ARI scores and SBS. The effect of acid and rinsing after the acid was applied may have cleaned the remaining ABS on the enamel surface. Although there was no significant decrease in bond strength, the ARI scores showed that the enamel-primer interface was negatively affected even when

the ABS residue was cleaned with wet surgical gauze before the self-etching primer was applied. Thus, this result indicates that clinicians should be cautious when using self-etch primer on cleaned enamel surface when ABS was used as a hemostatic agent.

The remaining adhesive was scored with  $\times 20$  magnification using a light microscope in our study. Montasser and Drummond<sup>26</sup> asserted that scoring the remaining adhesive under  $\times 20$  magnification was a superior method to scoring with the naked eye or with  $\times 10$  magnification. Similarly, Öz *et al.*<sup>27</sup> reported that ARI assessment with  $\times 20$  magnification was a reliable method compared with computer-aided estimation. ARI assessment with  $\times 20$  magnification was also used in the present study because of its reliability and efficiency.

## CONCLUSIONS

Traditional 37% orthophosphoric acid etch-and-rinse systems and light-curing primers could be safely used for direct bonding of orthodontic attachments on ABS-covered enamel surfaces. Although the bond strength was decreased when self-etching primer was used on ABS-contaminated enamel surfaces, self-etching primers can also be used when ABS contaminant is cleaned with a wet surgical gauze before bonding. However, the use of self-etching primers on ABS-contaminated areas is questionable, and applying self-etch primer directly on the ABS-contaminated bonding surface should be avoided.

Consequently we recommend using traditional etch-and-rinse systems directly on ABS-contaminated enamel surface whenever possible and using self-etch primers only after thoroughly cleaning the enamel surface.

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