ORIGINAL ARTICLE

Effects of hemostatic agents on shear bond strength of orthodontic brackets

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Abstract

Objectives: The aim of this study was to determine the effects of blood contamination and hemostatic agents on shear bond strength (SBS) of brackets and bond failure.

Materials and Methods: The study material consisted of 57 freshly extracted human premolar and randomly divided into four groups: Group I, control group (n = 14); Group II, contamination with blood (n = 13); Group III, contamination with epinephrine (n = 14); and Group IV, contamination with Ankaferd blood stopper (ABS) (n = 16). After the bracket bonding procedure, all bonded teeth thermal cycled in deionized water at 5 ± 2°C to 55 ± 2°C for 500 cycles. SBS was applied using a universal test machine.

Results: According to Kruskal–Wallis test significant differences were found among the groups $P < 0.05$. Furthermore, significant differences were recorded between groups with Mann–Whitney U statistical test with Bonferroni correction (P = 0.0083).

Conclusions: Examples contaminated with blood showed a statistically significant lower *in vitro* SBS than those contaminated with epinephrine, ABS, and control groups.

Clinical Significance: In impacted tooth surgical operations, blood contamination poses a substantial risk of bond failure in bonding attachments applications to the impacted teeth. Epinephrine and ABS may be used on surgical exposed impacted teeth operation for the prevention of blood contamination.

Key words: Brackets, contamination, hemostatic agents, orthodontic bonding, shear bond strength

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Introduction

Several studies have assessed the effect of contamination of the enamel surface of teeth with saliva and blood.[1-4] Bonding of orthodontic attachments with composite resin adhesives requires a dry environment. If the enamel surface becomes wet, resin penetration deteriorates, and this results in inadequate numbers and lengths of resin tags.[5] According to some studies, contamination with blood and saliva at any stage of the bonding procedure has a negative effect on the shear bond strength (SBS) of orthodontic brackets.[4,6] Itoh *et al*. have reported that water contamination did not affect the bond strength but that saliva and blood contamination reduced the bond strength. Saliva and blood showed different characteristics due to the differences in the types and amounts of organic and inorganic substances.[7] Hormati *et al*. studied the effect of moisture on bond strength and found a 1/2 decrease of strength in the presence of moisture.[5]

In orthodontic practice, bonding in contiguity with hyperemic gingival tissue does not permit ideal isolation.[8] There is also a risk of blood contamination on the enamel surfaces, especially in impacted teeth that require surgical

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operations. To expose the enamel of the tooth for the placement of orthodontic attachments, the intraoral tissues that cover the impacted tooth have to be removed or repositioned. When direct bonding procedures are applied in impacted tooth surgical operations, blood contamination poses a substantial risk of bond failure.

Surgical techniques like mechanical obstruction of tissues have been used for bleeding control, as well as hemostatic agents. In recent years, several hemostatic agents have been employed as blood stoppers such as bone wax, cellulose, gelatin, collagen, epinephrine, and Ankaferd blood stopper (ABS).[9-17] Many techniques have been reported to reduce blood loss when combined with the application of adrenalin or epinephrine.[14,18] Epinephrine is a vasoconstrictor. When used as a local anesthetic in oral surgery, it should be applied by infiltration or intraligamentery injection.[19]

Ankaferd blood stopper is a folkloric medicinal plant extract, which has traditionally been used in Turkey as a blood stopper agent. ABS is available in various commercial preparations including solutions, sprays, and tampons. It contains a standardized mixture of the plants *Glycyrrhiza glabra*, *Vitis vinifera*, *Thymus vulgaris*, *Urtica dioica*, and *Alpinia officinarum*.[17] Each of these plants exerts effects on vascular and hematological parameters and cellular proliferation.[20-22]

However, the applications of ABS and epinephrine in bonding of the orthodontic attachments have not been evaluated. The aim of this study was to evaluate the effects of blood contamination and hemostatic agents on SBS of metal brackets and bond failure.

Materials and Methods

Sample preparation

A total of 57 freshly extracted human premolars was collected and randomly divided into four groups: Group I, control group (n = 14); Group II, contamination with blood (n = 13); Group III, contamination with epinephrine (n = 14); and Group IV, contamination with ABS (n = 16). The inclusion criteria for selection of the specimens were: Intact buccal enamel with no damage caused by the extraction forceps and no caries.

The teeth in Group I were bonded with Transbond XT (3M Unitek, Monrovia, CA, USA) as recommended by the manufacturer. In Group II, the surfaces of the specimens were contaminated with fresh human blood from a male donor (researcher). The blood was applied with a brush on the labial surfaces of specimens, and the enamel surfaces were then air-dried. In Group III, the surfaces of the teeth were contaminated with 0.5 mg/ml of epinephrine (Adrenalin Biofarma, Mefar Drug Inc., Istanbul, Turkey). The epinephrine was applied directly on the conditioned enamel surface with a brush and air-dried. In Group IV, the surfaces of the specimens were contaminated with ABS solution (Ankaferd Drug Inc., Istanbul, Turkey), with the solution applied directly on the conditioned enamel surface with a brush and air-dried [Table 1].

The enamel surfaces were etched for 15 s with 37% phosphoric acid (Gel Etch, 3M Unitek, Monrovia, CA, USA), rinsed with water for 15 s, and air-dried for 15 s. The Transbond XT primer (3M Unitek, Monrovia, CA, USA) was then applied to the enamel surfaces. Metal brackets (Master Series, American Orthodontics, Sheboygan, WI, USA) for upper first premolars were bonded to the teeth with Transbond XT adhesive (3M Unitek). The adhesive was cured for 20 s using the same light curing device (Demi, Kerr, Orange, CA, USA) on both the mesial and distal parts [Table 1].

Aging and shear bond strength test

After the bracket bonding procedure, all the bonded teeth were stored in deionized water at 37°C for 30 days and then thermal cycled in deionized water at 5 ± 2°C to 55 ± 2°C for 500 cycles, with 20 s dwelling time in each bath, and 10 s transfer time (Huber GmbH, Offenburg, Germany).

The SBS of each group was measured using a universal testing machine (AGS-X, Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min. Shear force was applied parallel to the long axis of each tooth. The force required to shear off the bracket was directly recorded in Newtons (N) and converted into mega Pascal's (MPa) using the following equation: Shear force (MPa) = debonding force (N)/bracket surface area (mm^2) where 1 MPa = 1 N/mm^2 and the bracket surface area is 10.27 mm^2.

Adhesive remnant index scores

Debonding areas were examined under an optical microscope (Leica Microsystems, Germany) at ×16 magnification to measure the amount of residual adhesive remaining on each tooth. Adhesive remnant index (ARI) scores ranging from 0 to 3 were given as follows: 0 = no adhesive remained on tooth; 1= <50% of the adhesive remained on the tooth; 2= >50% of the adhesive remained on the tooth; 3 = all the adhesive remained on the tooth.[23,24]

Statistical analysis

Statistical analysis was performed with SPSS Statistics for Windows Version 16 (SPSS, Chicago, IL, USA). Descriptive statistics, including the mean, standard deviation, median, and minimum and maximum values were calculated for each of the four groups [Table 2]. The Kruskal–Wallis statistical test was used to determine significant differences in the SBS between the four groups. The Mann–Whitney U test with Bonferroni correction was used to compare the subgroups.

Table 1: Bonding procedures

Groups	Etching	Rinsing/drying	Contamination	Adhesive	Curing
Group I	37% phosphoric acid	Rinsing and drying	Dry	Primer and adhesive (transbond xt)	Light cure
Group II	37% phosphoric acid	Rinsing and drying	Blood	Primer and adhesive (Transbond XT)	Light cure
Group III	37% phosphoric acid	Rinsing and drying	Epinephrine	Primer and adhesive (Transbond XT)	Light cure
Group IV	37% phosphoric acid	Rinsing and drying	ABS	Primer and adhesive (Transbond XT)	Light cure

ABS=Ankaferd blood stopper

Table 2: Descriptive statistics of the four groups tested

Group	*N*	Mean±SD[a]	Minimum	Median	Maximum	Mann-Whitney U grouping[b]
Control	14	143.321±64.559	7.78	12.95	27.27	A
Blood	13	21.577±13.479	0.28	2.15	4.36	C
Epinephrine	14	79.423±29.885	3.71	8.50	14.00	B
ABS	16	6.068.105±19595	2.55	6.04	9.45	B

[a]SD=Standard deviation, [b]Mann-Whitney U grouping. Means with the same letters are not significantly different. ABS=Ankaferd blood stopper

Table 3: Distribution of ARI scores in each test group

Group	Score 0 (%)	Score 1 (%)	Score 2 (%)	Score 3 (%)	Total (%)
Control	7 (50)	5 (36)	2 (14)	0 (0)	14 (100)
Blood	3 (23)	10 (76)	1 (1)	0 (0)	13 (100)
Epinephrine	0 (0)	3 (21)	7 (50)	4 (29)	14 (100)
ABS	0 (0)	6 (37.5)	6 (37.5)	4 (25)	16 (100)

0=No adhesive remained on tooth, 1≤50% of the adhesive remained on tooth, 2≥50% of the adhesive remained on tooth, 3=All adhesive remained on tooth. ABS=Ankaferd blood stopper, ARI=Adhesive remnant index

Results

Table 2 shows the descriptive statistics of the four groups studied. The highest mean SBS value was seen in Group I (14.3321 ± 6.4559 MPa). The lowest mean SBS value was observed in Group II (2.1577 ± 1.34 MPa). The Kruskal–Wallis test indicated significant differences in the SBS between the four groups. According to the Mann–Whitney U test, Group II showed significant differences in the SBS compared with the other groups. Group I also showed significant differences in the SBS compared with the other groups.

Adhesive remnant index scores

Table 3 shows the ARI scores of each test group and also shows the distributions of ARI scores by percentage for all the test groups.

Discussion

Blood and saliva contamination are very important risk factors for bond failure on bleeding areas like an impacted tooth or hyperemic gingival tissue. Studies have shown that blood and oral fluid contamination have a negative effect on bonding procedures.[4,25,26] Bracket and button failures are common problems that can result in treatment failure, especially in orthodontics. To overcome such problems, some attempts can be applied for secure bonding procedure. According to some studies, SBS values of at least 6–10 MPa are needed for most clinical orthodontic bonding requirements.[1,3,6]

In this study, the bond strength of the blood contamination group showed the worst SBS value (2.1577 ± 1.34 MPa). This result was not surprising and has been reported by many clinicians.[3,4,8] Consequently, this value is not enough for secure bonding procedures. This result highlights the need to take care during bonding applications, especially when the oral environment is not ideal. As expected, the best SBS values (14.3321 ± 6.4559 MPa) were obtained in the control group in which the oral environment was ideal. This bonding environment was constructed on the recommendations of the adhesive manufacturers. Various factors in the oral environment such as humidity, blood, and saliva have been shown to affect the performance of the adhesive.[4,27]

Bleeding management has been studied widely, and various hemostatic agents have been developed for the management of hemorrhage in clinical use.[15] ABS is a traditional folk medicinal plant extract agent that is used in the management of external hemorrhage and dental surgery operations in Turkey. It includes a standardized mixture of the plants *G. glabra*, *V. vinifera*, *T. vulgaris*, *U. dioica*, and *A. officinarum*. *G. glabra* has been shown to exhibit antithrombin, antiplatelet, anti-inflammatory, and antiatherosclerotic effects.[21] *V. vinifera* was found to have antitumor, antipathogen, and antiatherosclerotic effects.[20] *T. vulgaris* has been shown to contain varying levels of antioxidants, which may help to avoid *in vivo* oxidative harm.[28] *U. dioica* was found to have a vasodilation effect.[22] *A. officinarum* affected nitric oxide production and inhibited nitric oxide in lipopolysaccharide-activated mouse peritoneal macrophages.[29]

Ankaferd blood stopper exerts a number of effects on blood proteins and red blood cells. ABS shows a

local hemostatic effect by stimulating the rapid (<1 s) formation of a protein network, which acts as an anchor for vital physiological erythrocyte aggregation. Studies demonstrated that it stimulates vital erythroid aggregation in conjunction with spectrin and ankrin receptors on the surface of red blood cells.[17,30] In addition, ABS upregulated the globin transcription factor (GATA)/friend of GATA transcription system, which affects erythroid functions in experimental and anecdotal topical applications.[30] According to studies, no local or systemic adverse effects have been observed in topical and experimental applications of ABS.

In this study, the SBS values for ABS were acceptable following thermal cyclic processing. It is an organic preparation without any systemic effect. Therefore, it can be used safely in dentistry. The application time is very short and it eliminates blood contamination in the area within a few seconds (30 s). In addition to eliminating the blood contamination, it improves the field of vision by clearing the area.

Common local anesthetics used in dental applications include articaine, lidocaine, mepivacaine, and prilocaine, all of which are normally used in combination with a vasoconstrictor. When used in local anesthetic solution, epinephrine enhances hemostasis and slows down the absorption of the solution. It is used in particular by surgeons to stop bleeding during common operations such as impacted third molars and canine or cystic surgery. In the present study, acceptable SBS values were obtained when epinephrine was used as a hemostatic agent under thermal cyclic conditions. Epinephrine can be used in the presence of blood contamination.

Within the limitations in this study, the following conclusions were drawn:

- Blood contamination during orthodontic bonding significantly decreased the SBS of orthodontic brackets to enamel
- Acceptable SBS values were achieved with ABS. ABS has two important benefits: It is an organic product, thereby making it safe for use in dentistry and it rapidly eliminates blood contamination from the operative area
- The epinephrine group had significantly higher bond strength values than the blood contaminated group, suggesting that epinephrine can be used as a hemostatic agent during orthodontic attachment bonding procedures where blood contamination is present.

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