Original Article

Plant Extract Ankaferd Blood Stopper Effect on Bond Strength

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ABSTRACT

Objective: To determine the effect of Ankaferd Blood Stopper (ABS) on the shear bond strength (SBS) of orthodontic attachments.

Materials and Methods: The study material consisted of 60 freshly extracted bovine permanent mandibular incisor teeth. All teeth were cleaned and randomly divided into three groups of 20 specimens and etched with 37% phosphoric acid for 15 seconds, washed, and air-dried. Teeth in groups 1 and 2 were contaminated with ABS and blood, respectively. Teeth in group 3 were only air-dried. Roth Generous maxillary central incisor brackets were bonded with Transbond XT to all teeth. SBS was applied using a universal test machine. The one-way analysis of variance (ANOVA) test was used to determine significant differences in SBS between the three groups, and Tukey honestly significant post hoc test was used to compare subgroups.

Results: The mean bond strengths and standard deviations of groups 1, 2, and 3 were 9.58 $\pm$ 0.95, 4.04 $\pm$ 0.69, and 19.56 $\pm$ 1.84 MPa, respectively.

Conclusions: Specimens contaminated with blood showed a statistically significant lower in vitro SBS than those contaminated with ABS. ABS may be used clinically for obtaining a blood-free tooth surface during application of the brackets on surgically exposed, impacted teeth. (*Angle Orthod.* 2010;80:570–574.)

KEY WORDS: Ankaferd Blood Stopper; Impacted canines; Shear bond strength

INTRODUCTION

An impacted canine tooth is usually easy to diagnose and frequently, dentists refer these patients to orthodontists. The orthodontist then frequently seeks the assistance of an oral and maxillofacial surgeon to gain access to the impacted canine.

The treatment options for the management of unerupted canines include: (1) no treatment if the patient does not desire it (in such a case, the impacted tooth should be evaluated periodically for any pathologic changes); (2) auto transplantation of the canine[1,2]; (3) early removal of the primary canine[3]; (4) extraction of the impacted canine and movement of the first premolar into its position; (5) extraction of the canine and posterior segmental osteotomy to move the buccal segment to the mesial to close the residual space[4]; and (6) surgical exposure of the canine and orthodontic treatment to bring the tooth into occlusion.

Impacted maxillary canines most commonly require surgical exposure and orthodontic guidance during eruption.[5] In this method, auxiliary attachments are placed on the surgically exposed tooth surface and orthodontic forces are subsequently applied to the attachment to move the impacted tooth.

Different methods of attachments to the impacted tooth have been suggested, including crowns, wire ligatures, chain links, bands, and directly bonded brackets.[6] Earlier techniques were limited by the materials available to bond to the tooth. Retentive pins, wire ligatures, and window flaps were originally proposed.[7,8] These techniques were associated with poor periodontal healing, loss of attachment, gingival recession, and gingival inflammation.[7,9] Newer flap designs have resulted in better postoperative periodontal health.[10–13]

As bonding techniques developed in orthodontics, surgical procedures were modified since less of the impacted crown now needed to be exposed. The application of etch and bonding agents has been improved by making these less sensitive to surgical site moisture. Smaller brackets and improved wires led to more predictable results.[14] However, when direct

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bonding procedures are used, blood flow into the area is always a risk for bonding failure.

Complications in the surgical stage include failure of the initial bond at the time of surgery. Caminiti et al[15] observed that bond failure was most often due to the difficulty of obtaining a dry field. In a previous study it was concluded that blood contamination of the enamel surface negatively affected bond strength.[16] In order to minimize initial bond failure during surgical procedures, it is important to obtain a dry surface where the bracket will be placed.

Ankaferd Blood Stopper (ABS) is a plant extract that has been used in Turkish traditional medicine as a hemostatic agent and could be used to obtain a blood-free, dry enamel surface. ABS is a unique folkloric combined medicinal plant extract, which has been approved in the management of postsurgery dental bleedings and external hemorrhage in Turkey.[17] ABS can be used as a spray, solution, and tampon. ABS compromises a standardized mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica*. Each of these plants has some effect on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics, and cell mediators.[18–23]

A recent in vitro study by Goker et al[17] showed that ABS exposure resulted in a very rapid formation (less than 1 second) of a specific hemostatic protein network. This network acts as an anchor for vital physiologic erythrocyte aggregation, covering the classical cascade model of the clotting system without independently acting on coagulation factors and platelets. ABS was found as effective as Surgicel in achieving hemostasis following partial liver excision in an experimental rat model.[24] In light of the above-mentioned data, this study aimed to investigate the effect of ABS on bond strength during bracket bonding procedures.

MATERIALS AND METHODS

Sixty freshly extracted bovine permanent mandibular incisor teeth were collected, cleaned of soft tissue, and stored in a solution of 0.1% thymol. The criteria for tooth selection included intact buccal enamel, no pretreatment with chemical agents (eg, hydrogen peroxide), no cracks caused by the extraction forceps, and no caries.

The teeth were randomly divided into three groups of 20 specimens. Sixty stainless steel, mesh-based mandibular central incisor brackets with a 0.018-inch slot (Roth Generous brackets, GAC International Inc, Bohemia, NY) were bonded to all teeth by a single operator. The enamel surfaces of all teeth were cleaned and polished with pumice and rubber prophylactic cups for 10 seconds and etched with 37% phosphoric acid for 15 seconds; this was followed by thorough washing and air-drying.

In group 1, one drop of ABS solution was applied directly on the conditioned enamel surface and air-dried. The standardized vials of the ABS (ABS patent number 2007-0-1-114485) used in the experiments were supplied by Ankaferd Drug Inc, Istanbul, Turkey. In group 2, all teeth surfaces were contaminated with fresh human blood from a male donor; the blood was applied with a brush on the labial surfaces until they were totally contaminated and air-dried. In group 3, the control group, all tooth surfaces were air-dried only.

A thin coat of Transbond XT primer was applied to the enamel surfaces in all groups. In all groups brackets were bonded with light cure Transbond XT adhesive and cured for 20 seconds. All bonded brackets were light-cured by the same light curing device (Optilux, Kerr, Orange, Calif).

After the bracket bonding procedure, in order to stimulate moisture and temperature changes in the oral environment, all 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 5000 cycles. The total period of exposure to both 5 ± 2°C and 55 ± 2°C was 10 seconds, with a dwell time of 5 seconds in each bath.

Each bonded tooth was oriented with a guiding device, so that its labial surface was parallel to the force applied during the shear bond strength (SBS) test and then embedded in an acrylic mold using a specially prepared cylindrical metal ring. The ring was filled with self-curing, fast-setting acrylic to 3 mm below the bracket. All specimens were mounted in a jig of the universal test machine (Instron 3345, Canton, Mass) and a shear force was applied to the adhesive interface until fracture occurred. The specimens were loaded at a crosshead speed of 3 mm/minute. A computer electronically connected to the testing machine recorded the results of each test. The force required to shear the bracket was recorded, and the bond strengths were calculated in MPa.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows Release 16.0 (SPSS Corporation, Chicago, Ill). Descriptive statistics, including the mean, standard deviation (SD), and minimum and maximum values were calculated for each of the three groups. The parametric one-way analysis of variance (ANOVA) test was used to determine when significant differences were present in bracket bond strength between the three groups. The Tukey honestly significant difference post hoc test was also performed for multiple

Table 1. One-Way Analysis of Variance (ANOVA) Between Groups and Within Groups

Values	Sum of Squares	*df*	Mean Square	F	*P* Value
Between groups	2475.429	2	1237.714	772.265	.000
Within groups	91.354	57	1.603		
Total	2566.783	59			

comparisons to compare subgroups. The results were evaluated with a 95% confidence interval. Significance for all statistical tests was predetermined at $P < .05$.

RESULTS

The results of the ANOVA ($F = 772.7$) indicated significant differences in the SBS between the three bonding procedures ($P < .01$) (Table 1). The Tukey honestly significant difference post hoc test indicated that the mean SBS values of all three groups were significantly different ($P < .01$). Group 3 had the highest strength value, followed by group 1, while group 2 had the lowest strength value (Table 2).

DISCUSSION

Previous studies showed that bovine and human enamel are similar in their physical properties, compositions, and bond strengths.[25,26] Bovine enamel was reported to be a reliable substitute for human enamel in bonding studies.[27] Thus, we used bovine mandibular incisors because they were readily available and inexpensive, and had a close morphologic similarity to human enamel.

Bond failure[7,9,13] is a complication in treating impacted canines. Where a dry field cannot be obtained, the bonding of brackets is unpredictable. When bonding brackets directly to a surgically exposed impacted canine, blood contamination can negatively affect bond strength; and this may lead to bond failure. In previous research, it was indicated that blood contamination during bracket bonding procedures using direct bonding adhesives caused a lower SBS compared to uncontaminated enamel surface.[16] In the present study, specimens in group 2, where all teeth were blood-contaminated, had the lowest SBS values (4.04 $\pm$ 0.69 MPa). Currently, there is no universally accepted minimum clinical bond strength. However, bond strengths of 6 to 10 MPa are suggested as sufficient for most clinical orthodontic needs.[28,29] Although a statistically significant difference was observed between SBS values of group 1 (9.58 $\pm$ 0.95 MPa) and group 3 (19.56 $\pm$ 1.84 MPa), the SBS values of group 1 were between 6–10 MPa. This showed that ABS contamination significantly lowered the SBS in group 1, but that it was clinically acceptable.

Table 2. Comparison of Shear Bond Strengths Of Groups (in MPa)

		n	Mean $\pm$ SD	Minimum	Maximum
Group 1	Ankaferd	20	9.5850 $\pm$ 0.95710	8.30	11.60
Group 2	Blood	20	4.4200 $\pm$ 0.69044	3.02	5.47
Group 3	Control	20	19.5655 $\pm$ 1.84807	15.53	22.60
Total		60	11.0642 $\pm$ 6.59582	3.02	22.60

ABS comprises a standardized mixture of five plants.[18–23] *Glycyrrhiza glabra* has anti-inflammatory, antithrombin, antiplatelet, antioxidant, antiatherosclerotic, and antitumor activities.[21] It inhibits angiogenesis and decreases vascular endothelial growth factor production and cytokine-induced neovascularization.[21] *Thymus vulgaris*, has antioxidative actions, such as prevention of lipid preoxidation.[19] *Vitis vinifera* exerts antitumor and antiatherosclerotic effects.[30,31] *Alpinia officinarum* inhibits nitric oxide production by lipopolysaccharide activated mouse peritoneal macrophages,[18] and *Urtica dioica* causes vasodilation via inducing nitric oxide production by endothelium.[20]

Ankaferd-induced formation of a protein network with vital erythroid aggregation covers the entire physiologic hemostatic process.[32,33] Therefore, ABS could be effectively used both in individuals with normal hemostatic parameters and in patients with deficient primary hemostatic or secondary hemostasis. The topical hemostatic efficacy of ABS has been previously tested in animals with normal[34,35] and defective hemostasis.[36,37] Cipil et al[36] concluded that ABS had in vivo hemostatic actions that may provide a therapeutic potential for the management of patients with deficient primary hemostasis in clinical medicine. Experimental studies have set the preclinical and biochemical safety of the oral systemic administration of ABS to rabbits. The safety and efficacy reports on the product have indicated its sterility and nontoxicity. Acute mucosal toxicity, hematotoxicity, hepatotoxicity, nephrotoxicity, and biochemical toxicity were not observed during the short-term follow-up of the animals.[38]

Those preclinical results reflect a starting point to search any possible confounding systemic effect of ABS when applied to internal topical surfaces. The usage of ABS as a hemostatic agent in external hemorrhages and in dental treatment in humans constitutes the first information on ABS's safety and efficacy in humans.[39] A phase I double-blinded, randomized, cross-over, placebo-controlled clinical study with a 5 days' wash-out period between the cross-over periods in healthy volunteers indicated the safety of ABS. Physiologic cell-based coagulation could be clinically managed via topical ABS application to prevent and treat bleeding in many distinct clinicopathologic states.[40] There are distinct important molecular components of the ABS-induced hemostatic

network. Vital erythroid aggregation takes place with the spectrin ankyrin and actin proteins on the membranes of red blood cells. Essential erythroid proteins (ankyrin recurrent and FYVE bundle containing protein 1, spectrin alpha, actin-depolymerization factor, actin-depolymerizing factor, LIM bundle and actin binding subunit 1 isoform a, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex, mitochondrial NADP [+] dependent malic enzyme 3, ribulose bisphosphate carboxylase large chain [maturase K], and the required ATP bioenergy [ATP synthase, ATP synthase beta subunit, ATP synthase alpha subunit, ATP binding protein C12, TP synthase H+ transporter protein, ADF, alpha-1, 2-glycosyltransferase ALG10-A]) are included in the protein library of ABS. ABS also upregulates the GATA/FOG transcription system affecting erythroid functions and urotensin II.[41,42]

After the approval of ABS for the management of dental bleeding by the Turkish Ministry of Health, ABS has been added to the protocols of prevention and treatment of exaggerated hemorrhage due to dental procedures. Hemorrhagic diathesis during dental surgical procedures can prolong postoperative bleeding, impair wound healing, and increase the risk of infection.[43] In a recent study, Fisgin et al[44] observed that ABS was significantly active against all bacteria investigated in the study. Since ABS is shown to be anti-infective,[45] and to control bleeding, its use is also rational in inhibiting postoperative infections.

There are no published data about comprehensive observations or intraoral applications concerning ABS effect on bond strength. However, the clinical significance of this new blood stopper agent should be further clarified in detail under in vivo conditions. Because lesser amounts of ABS are effective for intraoral use, we may recommend lesser package doses in order to prevent unnecessary, excessive use of the material.

CONCLUSION

- ABS could be used as a blood stopping agent during application of direct bonding brackets to surgically exposed canines to prevent bond failure due to blood contamination.

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