Original Article

Effects of Blood Contamination and Hemostatic Agents on Bond Strength in Primary Teeth Dentin

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ABSTRACT

Background: Contamination is a common problem in pediatric restorative dentistry and there are a few studies that investigate blood contamination, hemostatic agents, and tooth dentin. **Aim:** The purpose of this study was to evaluate the effects of blood contamination and hemostatic agents on the bond strength of two different bonding systems with the dentin of primary teeth. **Materials and Methods:** Buccal and lingual dentin surfaces of 40 primary second molar teeth were used for this study. Specimens were divided into 4 groups according to the contamination and hemostatic agents (Blood-B, Ankaferd Blood Stopper-A, ViscoStat-V, Control-C) and then every group was further divided into two subgroups according to the bonding systems (Clearfil SE Bond-I, All Bond Universal-II, $n = 10$ per group). A bulk-fill composite resin was built-up on the surfaces. The specimens were tested in the micro shear mode at a crosshead speed of 1 mm/min on a universal test machine. Statistical analysis was performed with ANOVA and Tukey's tests at $P < 0.05$. **Results:** Significant differences have been detected in the micro shear bond strengths only between the Ankaferd Blood Stopper (ABS) (AI = 13.72 ± 4.47 and AII = 9.12 ± 4.4) and control groups (CI = 22.78 ± 10.86 and CII = 16.49 ± 6.55) without regards to the bonding systems. The highest scores were obtained in the control groups. Clearfil SE Bond showed better performance than All Bond Universal in all groups. **Conclusion:** It was determined that only the ABS contamination groups showed statistically significant decreases in the bond strengths when compared with control groups.

Keywords: *Blood contamination, bond strength, dentin, hemostatic agents, primary teeth*

Introduction

Blood contamination has a detrimental effect on the bond strength between adhesives and tooth structures.[1] It is a common problem in pediatric restorative dentistry, especially when rubber-dam isolation is not applicable.[2] The gingival margin is a risk area for blood contamination since bleeding can occur as a result of gingival trauma from tooth preparation or gingival inflammation.[3] When resin restorations are performed in such cases, blood macromolecules such as fibrinogen and platelets can form a film on the dentin surface, obstruct dentin tubules, and impair the bond strength,[4] which may further lead to microleakage and secondary caries formation.[5] Thus, to ensure a high bond strength, bleeding control and decontamination are necessary.

Bleeding management has been widely studied and various hemostatic agents have been developed for the clinical management of hemorrhage. "Ankaferd Bloodstopper®" (ABS) and "ViscoStat®" (VS) are

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hemostatic agents used in dentistry with specific contents and mechanism of action.

ABS (Ankaferd Drug Inc®, Istanbul, Turkey) is a standardized mixture of a group of plants (*Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum*, *Urtica dioica)*, each of which has some effect on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics, and/or cell mediators.[6-9] This Turkish folkloric medicinal plant extract combination has been developed for the management of post-surgical dental bleeding and external hemorrhage.[10,11] It can be used as a spray, solution, or a buffer. ABS appears to initiate the rapid formation of an encapsulated protein network that provides focal points for erythrocyte aggregation with aggregated blood cells participating to form a mass with the erythrocytes. ABS exposure apparently provides both tissue oxygenation and physiological hemostasis without affecting any individual clotting factor.[11]

VS (Ultradent Products Inc, South Jordan, UT, USA) is a hemostatic agent that contains ferric sulfate and provides blood coagulation within a few seconds.[12] It has a pH of about 1.0. Hemostasis occurs by means of coagulum plugs pushed into capillaries with this agent.[13]

Adhesive dentistry has progressed greatly in the last decades. With changing technologies, dental adhesives have evolved from total-etch (4th and 5th generation) to self-etch (6th, 7th, and 8th generation) and multi-mode (Universal) systems. Although each breakthrough presents different advantages, two of these systems offer particularly remarkable innovations. The 6th generation bonding systems were introduced to the markets in the late 90s and were a dramatic leap in technology. Currently, these systems are still very popular and have reduced the time and procedure complexity by combining the etching and primer steps or the primer and bonding steps used in the previous generation, although this has not led to a noticeable reduction in the bond strength with enamel and increased the dentin bonding. The other considerable advantage of the sixth generation is that their efficacy appears to be less dependent on the hydration state of dentin than the 4th and 5th generation systems.[14] The most recent and one of the most innovative technologies in adhesive dentistry is the universal bonding systems that have been in clinical use since 2011. This new versatile and modular adhesion philosophy advocates the use of the simplest option of each adhesive strategy.[15,16] Most of these adhesives contain specific carboxylate and/or phosphate monomers like methacryloyloxydecyl dihydrogenphosphate (MDP) that bond ionically to the calcium found in hydroxyapatite,[17] and could increase the bonding effectiveness.[15]

Our study aimed to evaluate the effects of blood contamination and two hemostatic agents before surface treatment on the bond strength with the dentin of primary teeth by using a 6th generation and a universal bonding system.

Materials and Methods

The protocol of this *in vitro* study was approved by the Ethics Committee of the Faculty of Medicine, Pamukkale University. Around 40 intact caries-free human mandibular second primary molars that were extracted in the last month were selected and cleaned of soft tissues for this *in vitro* study, then stored at +4°C in 0.2% thymol solution until use. The teeth were embedded in self-curing acrylic resins (Vertex Castavaria, Vertex Dental, Soesterberg, Netherlands) and the buccal and lingual dentin surfaces were exposed by using a high-speed diamond fissure bur (No: 837XLG FG, Verdent Ltd., Lodz, Poland) with a water spray, and the exposed dentin surfaces were flattened with 600-grit silicon carbide papers until a minimal 3 mm diameter area of flat dentin was exposed.

Following preparation, all specimens were divided into four groups (n = 20) according to the contamination and hemostatic agents used in our study as follows [Figure 1]:

Group B (Blood)

Fresh capillary blood was collected from the fingertip of one of the male researchers of the study. One drop of blood was applied directly as a contaminant to the dentin surface of each specimen with a brush and was left undisturbed for 20 s. Then, the surface was rinsed with water for 10 s and air-dried for 10 s.

Group A (Ankaferd Bloodstopper)

The blood contamination, rinsing, and drying applications were performed as described in Group B. Then, one drop of ABS solution (Ankaferd Drug Inc®, Istanbul, Turkey) [Table 1] was applied with a brush to the dentin surfaces for 20 s, rinsed with water for 10 s and air-dried for 10 s.

Group V (ViscoStat)

The blood contamination, rinsing, and drying applications were performed as described in Group B. The ViscoStat solution (Ultradent Products Inc, South Jordan, UT, USA) [Table 1] was then applied with the original 1.2 mL syringe and working tip on the dentin surface for 2 min, rinsed with water for 60 s and air-dried for 10 s.

Group C (Control)

No blood nor decontamination agent was applied to the dentin surface.

Each group was further divided into 2 subgroups (n = 10, each) according to the bonding systems as follows [Figure 1]:

Subgroup I

A 6th generation two-step self-etch bonding system (Clearfil SE Bond, Kuraray®, Tokyo, Japan) was applied with a self-etching technique according to the manufacturer's instructions [Table 2].

Subgroup II

A universal bonding system (All-Bond Universal, Bisco, Schaumburg, IL, USA) was applied with a self-etching technique according to the manufacturer's instructions [Table 2].

The dentin surfaces were polymerized for 10 s with a multiwavelength LED light-curing unit (VALO Cordless, Ultradent Products Inc, South Jordan, UT, USA) at a light intensity of 1000 mW/cm^2. Following adhesive application, a universal shade bulk-fill composite resin (Filtek™ Bulk Fill Flowable Restorative, 3M ESPE, St. Paul, MN, USA) was built-up on the dentin surfaces using a 3 mm diameter polytetrafluoroethylene mold and polymerized for 10 s according to the manufacturer's instructions. All specimens were stored at 37°C in distilled water for 24 h and thermal cycling was carried out (5°C–55°C, 5.000 cycles, 15 s dwell time) before testing (MTE-101 Thermal Cycler Device, Esetron Smart Robotechnologies, Ankara, Turkey). Figure 1 presents the groups and a summary of the experimental protocol.

After thermocycling, the specimens were tested in micro shear mode at a crosshead speed of 1 mm/min on a universal test machine (Universal Testing Device, Esetron Smart Robotechnologies, Ankara, Turkey) [Figure 2]. The data were statistically analyzed using the SPSS software system, version 20.0 (IBM Corporation, New York, USA). Statistical analysis was performed with t-test, ANOVA, and Tukey's tests. The level of significance for all the analyses was set at $P < 0.05$.

Results

Significant differences have been detected in the micro shear bond strengths only between the ABS (Group AI = 13.72 ± 4.47 MPa and Group AII = 9.12 ± 4.4 MPa) and control groups (Group CI = 22.78 ± 10.86 MPa and Group CII = 16.49 ± 6.55 MPa) without regards to the bonding systems ($P < 0.05$). The highest scores were obtained from the control groups. Clearfil SE bond showed better performance than All Bond Universal in all contamination and hemostatic agent groups but there were significant differences in the blood (Group BI = 20.55 ± 4.80 MPa, Group BII = 13.36 ± 6.70 MPa) and

Table 1: Hemostatic agents used in this study

Ankaferd Bloodstopper® (Ankaferd Drug Inc., Istanbul, Turkey)	Material	ViscoStat® (Ultradent Product Inc., Utah, USA)
Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum, Urtica dioica	Consistent	20% $Fe_2(SO_4)_3$
Forms encapsulated protein network, provides physiological hemostasis and tissue oxygenation	Action	Forms coagulum plugs
A solution in ampoules and syringes	Dental usage form	A solution in bottles and special syringes

Table 2: Bonding systems used in this study

Material	Generation	Composition	Application technique
Clearfil SE Bond (Kuraray, Tokyo, Japan)	6th	Primer: HEMA, MDP, Hydrophilic dimethacrylate, water, ethanol, dl-camphorquinone, N, N-Diethanol-p-toluidine Adhesive: HEMA, MDP, Bis-GMA, Hydrophilic dimethacrylate, dl-camphorquinone, N, N-Diethanol-p-toluidine, silanated colloidal silica	1. Apply primer with a brush for 20 s 2. Gently air dry 3. Apply bonding agent for 10 s 4. Airflow gently 5. Light cure for 10 seconds
All-Bond Universal (Bisco Inc., Schaumburg, IL, USA)	Universal	MDP, Dimethacrylate resins, HEMA, Ethanol, Water, Initiators	1. Apply two separate coats of bonding agent for 10 s 2. Airflow gently 3. Light cure for 10 seconds

Table 3: Microshear Bond Strengths of groups

MPa	Clearfil SE Bond (I)	All-Bond Universal (II)
Control (C)	22.78±10.86[a]	16.49±6.55[a]
Blood (B)	20.55±4.80[a,b]	13.36±6.70[a,b]
Ankaferd (A)	13.72±4.47[b]	9.12±4.40[b]
Viscostat (V)	16.78±7.96[a,b]	13.20±5.44[a,b]

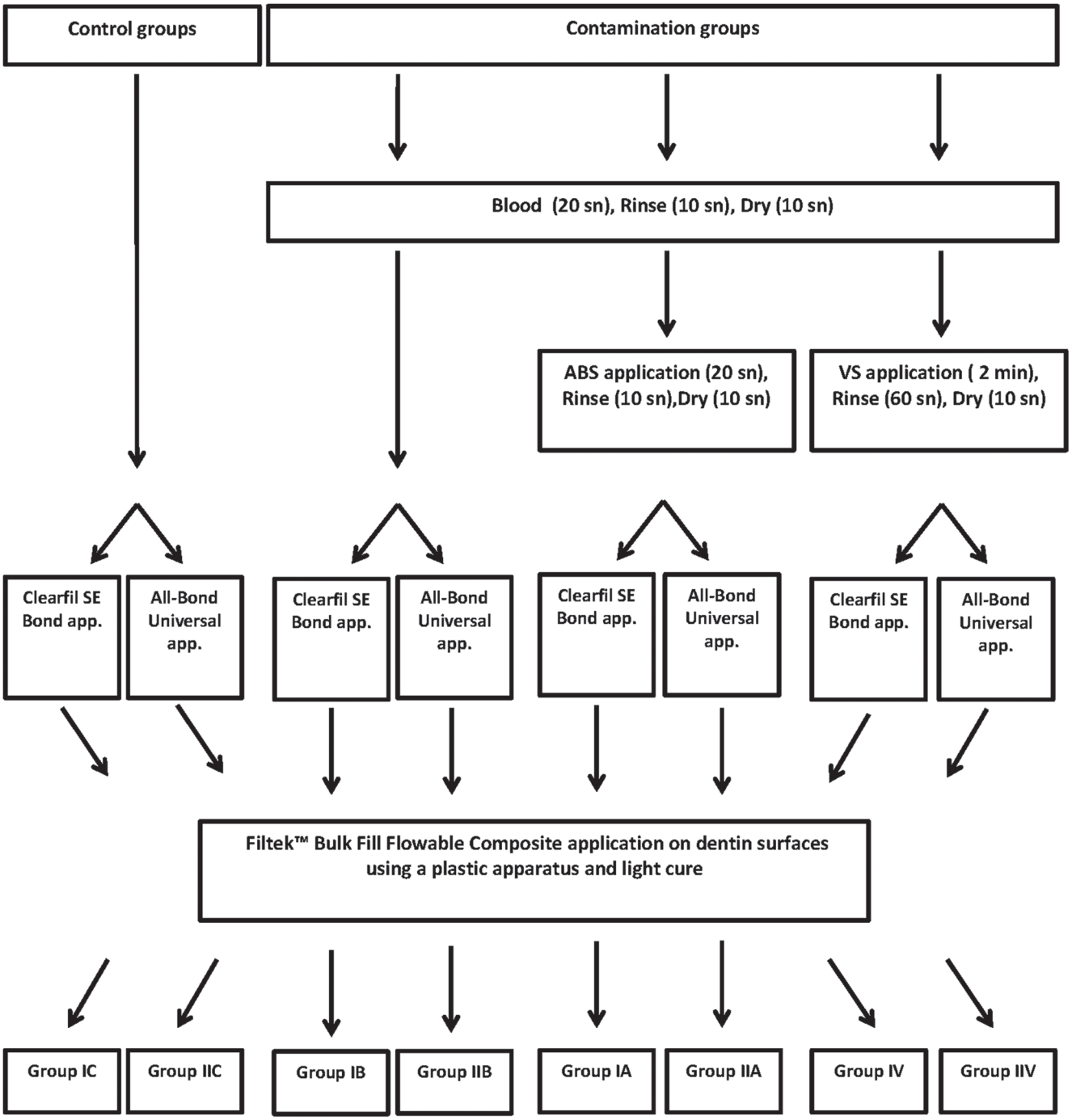

Figure 1: Schematic representation of the experimental design

ABS groups (Group AI = 13.72 ± 4.47 MPa, Group AII = 9.12 ± 4.40 MPa) ($P < 0.05$). The descriptive statistics for the shear bond strengths of the different groups are illustrated in Table 3.

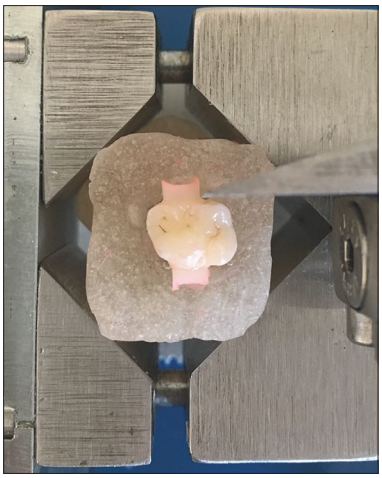

Figure 2: A specimen on a universal test machine

Discussion

Contamination of the dental preparation surface by saliva, blood, or gingival crevicular fluid is one of the major problems in restorative dentistry which negatively affects the success of resin restorations.[18] In the clinical routine, ideal conditions are not always feasible and moisture control could be difficult, especially in the case of caries located at or near the gingival margin. Blood contamination could reduce the resin-dentin bond strength significantly more than salivary contamination.[19] While some previous studies have indicated that blood contamination on the dentin surface causes a significant decrease in the bond strength at the dentin-resin interface,[1,20] other studies have found no significant change.[21,22]

Micro shear bond strength tests have numerous advantages including easy specimen preparation, simple test protocol, and the ability to rank different products according to bond strength values. Thus, it is considered to be useful for evaluating the adhesion strengths of bonding systems.[23]

Since reduced chair-side time has an important role in the behavior management of pediatric patients, self-etch bonding may be considered the best adhesive technique.[24] Self-etch adhesive systems do not require a separate acid conditioning step and moist post-rinse control, thus, they are considered simplified materials.[25] A short application protocol and minimal technical sensitivity are important advantages in pediatric dentistry. Moreover, universal adhesive systems may be used as self-etch adhesives in cases dealing with difficult access, limited time, or poor patient compliance in young patients. Thus, a self-etch bonding system and a universal bonding system without acid etching were considered the appropriate choice in this study performed on the dentin of primary teeth.

There are a few studies that investigate blood contamination, hemostatic agents, and tooth dentin. Kilic *et al*.[26] evaluated the effects of blood contamination and hemostatic agents such as ABS and hydrogen peroxide on the microtensile bond strength between dual-cured resin at the cement-dentin interface on human permanent mandibular molar teeth. They found significant differences between the control and blood-contaminated groups, whereas no significant differences were found between the control and other groups. In contrast to the previous study, where the hemostatic agents and blood were administered separately, blood contamination was done before the application of hemostatic agents (ABS and VS) in the current study, and a cumulative effect may have reduced the bond strengths of these groups despite the rinse step.

Kucukyilmaz *et al*.,[27] evaluated the effects of blood contamination on the dentin of permanent teeth with different processing steps using two universal and a 7th generation adhesive system. Similar results were obtained with the control group in cases where contamination was applied in the following order: bonding application/blood contamination/dry/bonding re-application/light curing for all three adhesive systems, or bonding application/blood contamination/rinse/dry/bonding re-application/light curing for All-Bond Universal. All of the decontamination method sequences used in this study caused a reduction in the adhesive bond strength due to blood contamination occurring after light curing. In the blood groups of the current study, the sequence of blood contamination-rinse-dry-bonding application-light curing was followed and there was no significant difference with the control groups for Clearfil SE Bond and All-Bond Universal. The reason for all the rinse/dry groups not having similar results as the control group may be that the previously applied bonding agents cannot be completely removed with water in the study by Kucukyilmaz *et al*.[27] Similar results in the All-Bond Universal groups may be due to low film thickness and viscosity of this bond that can easily be removed from dentin.

A dentin contamination study by Taneja *et al*.[28] used 180 premolar teeth and evaluated the bond strengths of three different generation adhesive systems when blood application was done separately before and after the bonding steps. The study showed that blood contamination had a strongly negative impact on the shear bond strength with dentin, and the bond strength was affected more if contamination occurred after adhesive application in the self-etch systems.

The most frequently encountered scenario in the clinic is as follows: bleeding occurs, the area is washed with water

and dried, selectively the decontamination agent is used, the bleeding is stopped, the area is washed again, dried, and the restoration process is started. In this study, the most appropriate and predisposed situation was simulated.

Both, the Clearfil SE Bond and All-Bond Universal contain 10-MDP monomers that chemically interact via ionic bonding with the calcium in hydroxyapatite.[29] In Clearfil SE Bond, MDP monomers exist in both bottles and are applied two times on the tooth in clinical practice whereas All-Bond Universal is applied once. The Clearfil SE Bond primer is mildly acidic, with a pH of 2.0, while All-Bond Universal has a pH of 3.2. Weak acidity of adhesive systems can be compromised by the buffering effect of the smear layer resulting in decreased bond strength and durability, and adhesive systems with a low pH are thought to dissolve the smear layer more effectively and increase adhesion.[30] Besides, in the two-bottle systems, the acidic primer is covered with a solvent-free adhesive, rich in dimethacrylates that create strong resin films.[31] These factors may be the reason why Clearfil SE Bond shows higher bond strengths than All-Bond Universal in all groups.

One of the goals of using the self-etching technique is to have an equal depth of demineralization and resin infiltration; however, a number of previous studies have reported that a demineralized zone below the hybrid layer was not protected by the adhesive when one or two-step adhesive systems were used.[32-34] Incomplete resin infiltration into the demineralized dentin is not the only reason for adhesive bond failure. These areas represent an adhesive layer in which the incompletely removed water or fluid from the dentinal tubules inhibits complete polymerization. Tubule density is quite high in primary teeth, nearly three times greater compared with permanent teeth, and they have similar tubule diameter at the superficial dentin.[35] In the current study, significant differences have been observed between ABS and control groups. This result may be due to an inadequate washing protocol and the inability of the plant molecules to be removed from the dentin tubules. In most dental contamination studies that have used ABS, the authors did not prefer to rinse the hard tissue after ABS application.[36-38] This may be because rinsing is not recommended in the ABS manufacturer's instructions. In the current study, as discussed previously, the possibility of ABS penetration into the tooth tissues was thought to be quite high and it would be appropriate to include a washing protocol similar to the study by Kilic *et al*.,[26] to execute a more realistic clinical simulation. However, all of these studies were done on permanent teeth and the high tubule density of primary teeth dentin could require a longer wash time.

Conclusion

Within the limitations of this study, it can be concluded that blood contamination did not have a significant negative impact on the shear bond strength with the dentin of primary teeth. The findings suggest that a standard rinse and dry cleaning protocol would be able to remove the blood and ferric sulphate (VS) more efficiently than removing ABS from the dentinal surface of primary teeth. The 6th generation two-step self-etch bonding system (Clearfil SE Bond) showed better performance than the Universal bonding system (All-Bond Universal) in all contamination and hemostatic agent groups in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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