Do blood contamination and haemostatic agents affect microtensile bond strength of dual cured resin cement to dentin?

Kerem KİLİC[1], Soley ARSLAN[2], Goknil Alkan DEMETOGLU[1], Gokmen ZARARSIZ[3], Bulent KESİM[4]

1- PhD, Department of Prosthodontics, Faculty of Dentistry, Erciyes University, Kayseri, Turkey.
2- PhD, Department of Restorative Dentistry and Endodontics, Faculty of Dentistry, Erciyes University, Kayseri, Turkey.
3- PhD, DDS, Department of Statistics, Faculty of Medicine, Erciyes University, Kayseri, Turkey.
4- PhD, Professor, Department of Prosthodontics, Faculty of Dentistry, Erciyes University, Kayseri, Turkey.

Corresponding address: Dr. Kerem Kilic - Department of Prosthodontics, Faculty of Dentistry, - Erciyes University - Kayseri - Turkey - Phone: +90 352 4374937-29082 GSM: +90 3525335253997 - Fax: +90 352 438 06 57 - e-mail: dtkeremkilic@hotmail.com

ABSTRACT

Objective: The purpose of this study was to evaluate the effects of blood contamination and haemostatic agents such as Ankaferd Blood Stopper (ABS) and hydrogen peroxide (H_2O_2) on the microtensile bond strength between dual cured resin cement-dentin interface. Material and Methods: Twelve pressed lithium disilicate glass ceramics were luted to flat occlusal dentin surfaces with Panavia F under the following conditions: Control Group: no contamination, Group Blood: blood contamination, Group ABS: ABS contamination Group H_2O_2: H_2O_2 contamination. The specimens were sectioned to the beams and microtensile testing was carried out. Failure modes were classified under stereomicroscope. Two specimens were randomly selected from each group, and SEM analyses were performed. Results: There were significant differences in microtensile bond strengths (μTBS) between the control and blood-contaminated groups ($p < 0.05$), whereas there were no significant differences found between the control and the other groups ($p > 0.05$). Conclusions: Contamination by blood of dentin surface prior to bonding reduced the bond strength between resin cement and the dentin. Ankaferd Blood Stoper and H_2O_2 could be used safely as blood stopping agents during cementation of all-ceramics to dentin to prevent bond failure due to blood contamination.

Key words: Blood contamination. Ankaferd Blood Stopper. Hydrogen peroxide. Microtensile bond strength.

INTRODUCTION

Contemporary restorative dentistry places a certain emphasis on adhesion and resin cements because of all-ceramic restorations becoming increasingly popular[34]. Long-term survival of all-ceramic restorations depends on the success of a durable bonding between ceramic, adhesive cement and dentin[8].

Adhesion can be defined as a flexion force between the molecules at the interfaces of different materials[9]. The complex organic structure and the dynamic formation and biological activity of dentin prevent a reliable and durable bonding[27]. Appropriate adhesive agents and luting procedures for bonding all-ceramics to dentin is challenging[15]. All-ceramics can be cemented using conventional dual cure resin-based cements in combination with total etching or self-etching adhesives, or using the recently formulated self-adhesive cements that allow simultaneous bonding between the dentin and the all-ceramic[14,33]. However, properties of adhesive cements can be diminished by various intraoral factors such as saliva, gingival crevicular fluid or blood contamination during the adhesive cementation process[8,12,18,28,33,34]. Blood contamination reduces resin/dentin bond strength significantly more than does salivary contamination[26]. Previous studies indicated that blood contamination on the dentin surface caused

a catastrophic decrease in the bond strength at dentin-resin cement interface[1,7,18,32,34].

Some treatments have been proposed in an effort to reverse the blood contamination effect during the cementation process: resurfacing with rotary instruments, rinsing with water followed by air drying, rinsing with water and primer re-application or re-etching with phosphoric acid[15,17,27]. Additionally, clinical and *in vivo* studies have reported on different hemostatic agents used for the management of hemorrhage in clinical dentistry, such as hydrogen peroxide (H_2O_2), ferric sulfate, aluminum chloride, trichloracetic acid and Ankaferd Blood Stopper (ABS) (Ankaferd Drug INC, Istanbul, Turkey)[14,20-22,31].

ABS is a new, unique folkloric combined medicinal plant extract that has been approved in the management of postsurgery dental bleedings and external hemorrhage, and also could be used to obtain a bloodfree, dry enamel-dentin surfaces in Turkish traditional medicine[16,31]. ABS can be used as a spray, solution and buffer. ABS is comprised of a standardized mixture of the plants *Urtica dioica, Vitis vinifera*, *Glycyrrhiza glabra*, *Alpinia officinarum*, and *Thymus vulgaris*[16]. Each of these plants has some effect on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators[16,23-24]. A recent *in vitro* study by Goker, et al.[16] (2008) showed that ABS exposure resulted in a very rapid formation (less than 1 s) 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[16]. ABS was found as effective as Surgicel in achieving hemostasis following partial liver excision in an experimental rat model[19].

Few studies have investigated the effects of blood decontamination agents such as ABS and H_2O_2 on the bond strengths of resin cements to dentin[30,31,34]. ABS and H_2O_2 may affect the sealing and bonding properties of resin cements. In addition, blood contamination may create a thin film on the dentin surface that hinders adhesive penetration into the dentinal tubules[18,34]. In wake of these concerns, the aim of this study was to evaluate the effects of various dentin surface contaminations (no treatment [control], blood, ABS, H_2O_2) on the µTBS of the resin cement-dentin interface. The null hypothesis was that the µTBS of the resin cement-dentin interface would be similar for all dentin surface conditions.

MATERIAL AND METHODS

The materials used in this study are presented in Figure 1

Preparation of ceramic blocks

Twelve 5×6×8 mm pressed lithium disilicate glass ceramic rectangular blocks (IPS e.max, Ivoclar Vivadent AG, Schaan, Liechtenstein) were fabricated using the lost wax technique, and ingots were injected into an EP 600 furnace (Ivoclar Vivadent AG) according to the manufacturer's instructions. After cooling to room temperature, the specimens were divested and air-abraded with 50-µm Al_2O_3 particles (Korox, Bego, Bremen, Germany) for 14 s from a distance of approximately 10 mm. Block surfaces were flattened with 220, 360 and 600 grit silicon carbide sandpapers to standardize the bonding surfaces. Papers of 220 and 360 grit were used in sequence, each one for 10 s, and a final polishing was done with a 600 grit paper for 60 s. The ceramic blocks were ultrasonically cleaned in water for 10 min to ensure contaminant-free surfaces. The bonding surfaces of the ceramic blocks were etched with 37% phosphoric acid (K-Etchant Gel, Kuraray, Tokyo, Japan) for 5 s, rinsed for 30 s with water spray, air-dried and then silanated with a silane-coupling agent (Clearfil Ceramic Primer, Kuraray, Tokyo, Japan) for 60 s.

Tooth preparation

Twelve caries-free human first mandibular molars were stored in 0.5% chloramin solution at 4°C and used within one month after extraction. All the occlusal enamel and some superficial dentin were removed by cutting off the crown horizontally at the middle, exactly at the top of the pulp chamber, using a low-speed diamond cutting saw (Minitom; Struers, Copenhagen, Denmark). In oder to create a standard smear layer, the exposed dentin surfaces were flattened with wet silicon 220- and 360-grit carbide sandpapers in sequence, each for 10 s, and a final polishing was done with a 600-grit paper for 60 s. The teeth were then rinsed with distilled water to remove any debris.

Experimental design

The 12 prepared teeth were randomly divided into 4 groups according to the following factors (n=3 each):

Control Group: Wet dentin. No contaminant was added.

Group Blood: Fresh capillary human blood (supplied by a single donor) was applied with a microbrush for 20 s, rinsed for 10 s and blotted with absorbent paper.

Group ABS: One drop of ABS solution was applied directly to the dentin surface with a microbrush for 20 s, rinsed for 10 s and blotted with absorbent paper.

Group H_2O_2: One drop of H_2O_2 was applied with a microbrush for 20 s, rinsed for 10 s and blotted with absorbent paper.

Bonding procedures

Panavia F dual-cured resin cement (Kuraray, Tokyo, Japan) was used as adhesive. Equal amounts of ED Primer II Liquids A and B were mixed and applied to the dentin surfaces for 30 s and then the surfaces were thoroughly air dried. Paste A and Paste B of the Panavia F resin cement were mixed and then applied to the ceramic surfaces using a dispenser syringe. A special loading device was used to apply a constant load of 98 N to the ceramic blocks. This load was used to create a uniform resin-luting layer, so as to simulate the film thickness employed for all-ceramic crowns[10]. Initial light curing was performed for 10 s. Excess cement was carefully removed with an explorer. The resin cement was polymerized from each direction (mesial, distal, buccal, lingual, occlusal) with a LED curing device for 40 s (light intensity: 1,000 mW/cm^2; Elipar FreeLight 2 LED Curing Light, 3M ESPE, MN, USA).

Bond strength test

All specimens were stored in a moisture medium at 37°C for 24 h. Using a low-speed diamond cutting saw (Minitom, Struers, Copenhagen, Denmark) at 3200 rpm (0.085 mm/s) under water cooling, the ceramic-resin cement-tooth sets were cut 1-mm thick slabs, starting at the ceramic side through the tooth, perpendicular to the bonded interface. The sectioning continued until 1 mm remained to keep the specimens fixed in position. Then root surfaces of the teeth were cut horizontally and 1-mm thick slabs were obtained. Subsequently, each slab was pasted to acrylic blocks with sircolant wax, and then slabs were cut perpendicular to the bonded interface to obtain 1.0±0.1 mm^2 beam specimens again (Figure 2). The beam specimens (n=25) from each experimental group were obtained and thermocycled for 6,000 cycles between 5±2°C and 55±2°C, with a dwell time of 20 s and a transfer time of 5 s. The thermocycling process was completed in 4.5 days[25]. The ends of each beam were attached to a table top material tester (Micro Tensile Tester; Bisco, Schaumburg, IL, USA) using cyanoacrylate (Zapit; DVA, Corona, CA, USA) and subjected to microtensile testing at a crosshead speed of 1 mm/min until the beams fractured. At this point, the load at failure was recorded in N. The debonded beams were carefully removed from the apparatus and the cross-sectional area at the site of failure was measured with a pair of digital calipers (Sylvac Ultra-Cal III; Fowler Co., Inc., Newton, MA, USA) to calculate the bond strengths at failure in MegaPascals.

Name	Manufacturer	Ingredients	Lot number
IPS e max	Ivoclar Vivadent,	Lithium disilicate, glass ceramic	H36067
Panavia F	Kuraray, Osaka, Japan	Paste A α-quartz powder sil., ba - glass powder sil., titanium dioxide sil., MDP, sodium fluoride, 2,2-Bis[methacryloxy(poly) ethoxyphenyl] propane, 1,2-Bis(3-methacryloxy - 2-hydroxypropoxy) ethane, Neopenthylglycol - dimethylacrylate, 2,4,6-Trimethylvenzoyl -diphenyl -phosphinoxide, triethanol amine Paste B N,N-Diethanol-p-toluidine, inhibitor, pigment	141111
Ankaferd Blood Stopper	Ankaferd Drug, INC, Istanbul, Turkey	"*Urtica dioica* (0.06 mg/ml), *Vitis vinifera* (0.08 mg/ml), *Glycyrrhiza glabra* (0.07 mg/ml), *Alpinia officinarum* (0.07 mg/ml), *Thymus vulgaris* (0.05 mg/ml)	105001
H$_2$O$_2$ Solution	Sifa Kimya Cosmetics Skincare Pharmaceutical Producrts, Konya , Turkey	Hydrogen eroxide, Benzoic acit, Deionized water	27

Figure 1- Materials used in the study

Stereomicroscopy and scanning electron microscopy (SEM) examination

Fractured specimens were examined with a stereomicroscope (Olympus SZ-CTV; Olympus, Tokyo, Japan) at ×40 magnification to determine the mode of failure. Failure modes were classified as adhesive, mixed, or cohesive.

For SEM analysis, two specimens were randomly selected from each group. The debonded beams from each group were sputter-coated (Bal-Tec SCD 050 Sputter Coater; Bal-Tec AG, Balzers,

Liechtenstein) with gold and observed with a scanning electron microscope (LEO 440, Leica-Zeiss, Cambridge, UK).

Statistical analysis

All data sets were subjected to normality tests using the Shapiro-Wilk method, and the Levene's method to check the assumption of homogeneity of variances. The data were presented as mean and standard deviation. One-way ANOVA test was used to compare the groups and multiple comparisons were performed using Dunnett's Method. Failure mode distributions were compared using the chi-square test. For all of the analyses, the level of significance was set at $p < 0.05$. Statistical analysis was conducted using SigmaPlot 12.0 software (Systat Software, San Jose, CA, USA). Three samples in Group Blood and one sample in Group ABS that could not be measured because of spontaneous debonding during thermocycling were evaluated as 0 bond strength.
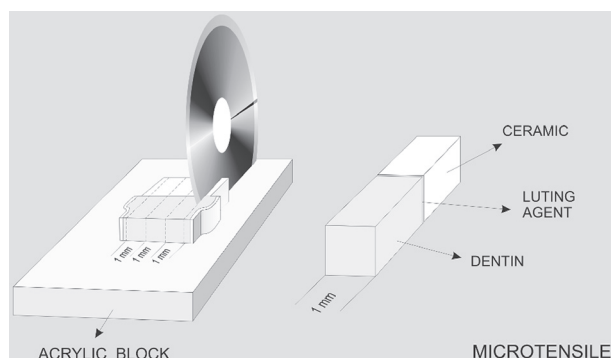
RESULTS

Results of the µTBS test are summarized in Figure 3. One-way ANOVA test revealed significant differences between the groups ($p < 0.05$) and the *post-hoc* Dunnett's Method showed that there were significant differences between control group and Group Blood ($p < 0.05$), but no significant differences were found between the control group and the other groups ($p > 0.05$).

The numbers and percentiles of failure modes in each group are shown in Table 1, There were significant differences for the failure modes among the groups ($p < 0.05$). The results of failure mode analysis demonstrated that in the control group cohesive type failure mode was predominant than in the other groups ($p < 0.05$). In Group Blood, adhesive type failure mode was significantly more than in the ABS and control groups ($p < 0.05$), whereas there were no significant differences between the Blood and H_2O_2 subgroups in terms of

Figure 2- Preparation of the microtensile strength test specimens
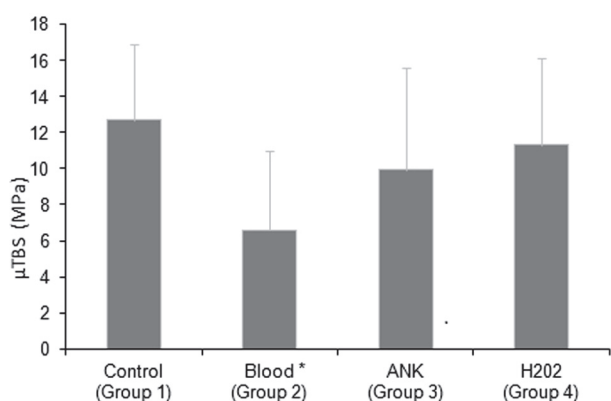

Figure 3- Mean and standard deviations of µTBS (MPa) means of all groups. The asterisk indicates statistically significant difference with control group at $p < 0.05$

Table 1- Failure mode distributions in the groups

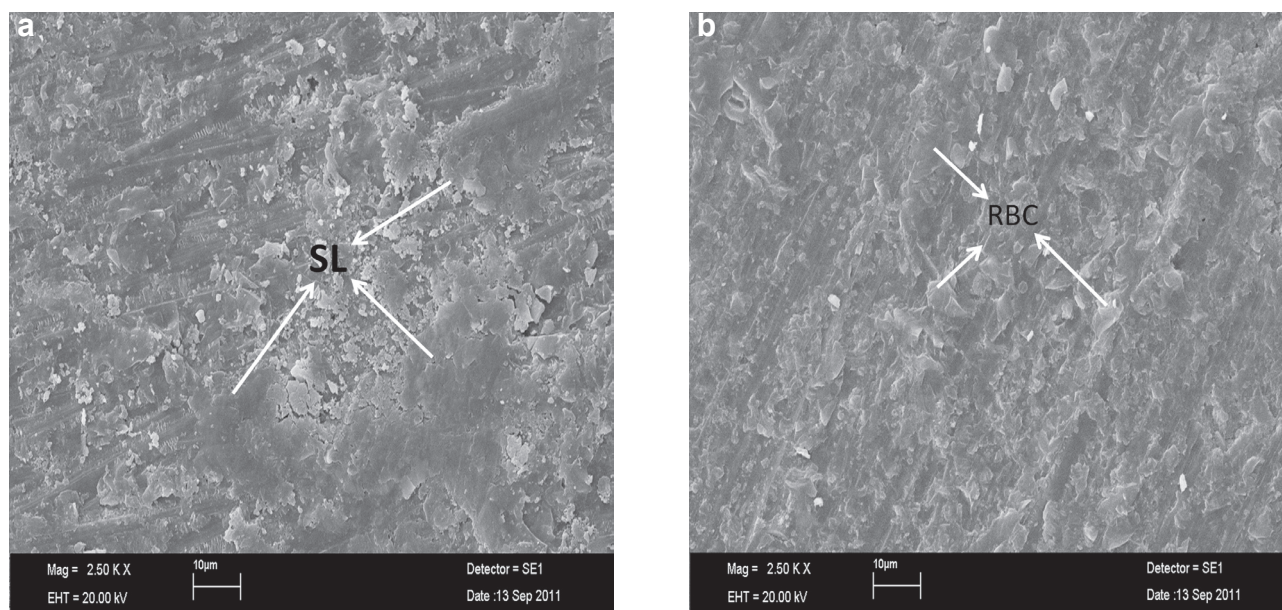| Groups	Failure mode		
	Adhesive	Cohesive	Mixed
Control Group	0	19	6
	0%	76%	24%
Group Blood	16	2	7
	64%	8%	28%
Group ABS	10	3	12
	40%	12%	48%
Group H_2O_2	12	2	11
	48%	8%	44%

Figure 4- Scanning electron microscopy (SEM) images of the fracture modes of debonded beam-shaped specimens (2,500× magnification). (a) Dentinal tubules were covered with smear layer (SL) in the control group. (b) In the Group Blood, dentinal tubules were closed with residues of blood contaminants (RBC). There was a very flat surface that indicated poor adhesion
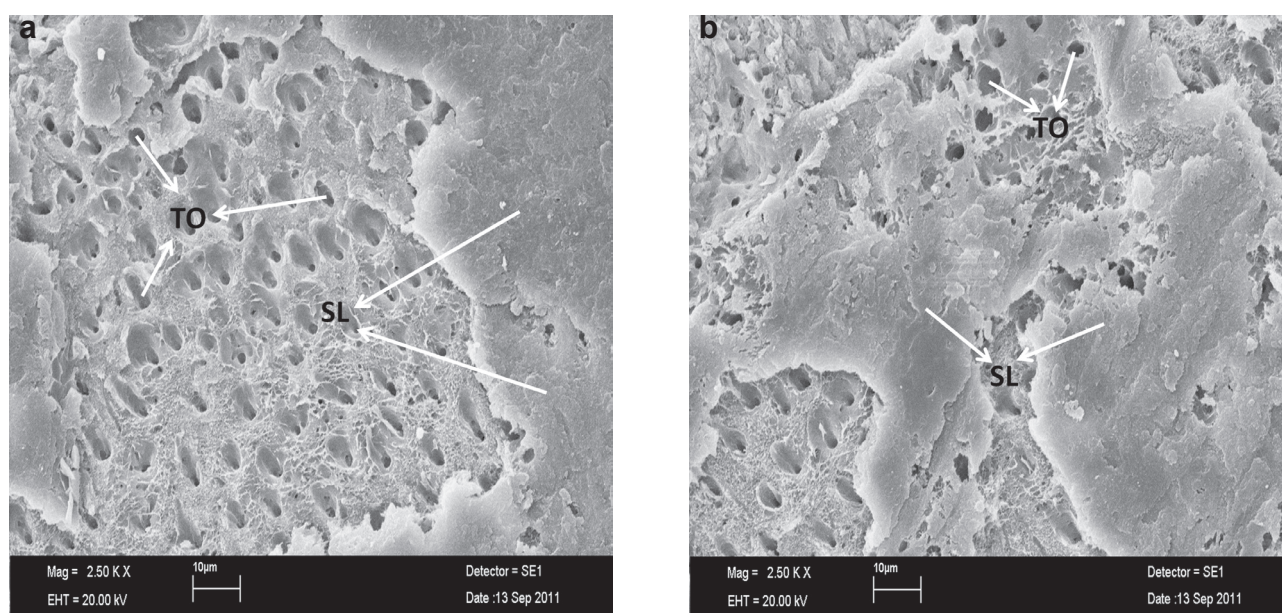

Figure 5- Scanning electron microscopy (SEM) images of the failure modes of debonded beam-shaped specimens (2,500× magnification) (a,b). In Group ABS and H_2O_2, smear layer (SL) was partially removed, and both residual resin and tubular orifices (TO) were observed

adhesive type failure mode (p>0.05). In contrast, mixed type failure mode was significantly more prevailing in ABS Group than in control group (p<0.05).

The SEM images of representative debonded beam-shaped specimens from four groups are shown in Figures 4 (a, b) and 5 (a, b). In the control group (Figure 4a), the tubular orifices were observed to be close over the bonding area and smear layer covered the dentin surface, so, the failure modes of specimens were determined as cohesive failure. Group Blood (Figure 4b), showed in the adhesive failure that dentinal tubules were obliterated with residues of blood contaminants and there was a very flat surface that indicated poor adhesion. In Groups ABS, H_2O_2 (Figures 5a and 5b respectively), both residual resin and tubular orifices were observed, and the failure modes of specimens were determined as mixed failure.

DISCUSSION

In this study, the effects of blood contamination and various haemostatic agent protocols (ABS and H_2O_2) on the µTBS of the resin cement-dentin interface were investigated by simulating a clinical try-in procedure. The results obtained in this study did not support the null hypothesis that the µTBS of the resin cement-dentin interfaces are similar for all dentin surface conditions. In the present study, the blood-contaminated group had the lowest µTBS values in all groups.

During cementation of all-ceramic restorations, there is a relatively high risk of blood contamination on the adherent dentin surface, especially for crown margins in the gingival area[14]. The residual blood remaining on the dentin surface caused an excessive decrease in bond strength at the resin cement-dentin interface, which was shown in previous studies[1,18,32,34]. In the present study, significant differences were found between the blood-contaminated group and the control group, which is similar to the previous studies[18,34]. The influence of blood contamination on bond strength can be attributed to its high protein content that, along with macromolecules such as fibrinogen and platelets, can form a film on the dentin surface, obstructing the penetration of the adhesive system into dentin tubules[7].

SEM images (Figure 4b) also revealed that dentinal tubules were sealed with residues of blood contaminants and a very flat surface was seen on the blood-contaminated specimens compared to the other surface treatments (Figure 5a and 5b), which probably resulted in reduced bond strength due to the lack of interaction of the resin cement with the contaminated surface.

An anionic detergent, H_2O_2 is one of the most common disinfectant and coagulant materials used in dentistry[34]. During bonding procedures, H_2O_2 might break down to oxygen and water, generating bubbles or voids that interfere with resin infiltration into etched dentin. The oxygen severely inhibits the interfacial polymerization of resin-bonding materials[6,34]. Although reduction in bond strength of some adhesive systems applied to enamel and dentin may have been caused by the presence of H_2O_2, as has been shown in some studies[6,29,34], some authors have claimed that H_2O_2 did not affect bond strength[5,29]. İn the current study, 3% H_2O_2 did not reduce adhesion when applied for 20 s. However, it is important to point out that it could have been responsible for the complete removal of remaining contaminants by water rinsing from the surface.

ABS induced formation of a protein network with vital erythroid aggregation that covers the entire physiologic hemostatic process. Therefore, ABS could be effectively used both in individuals with normal hemostatic parameters and in patients with deficient primary hemostatic or secondary hemostasis[16]. The topical hemostatic efficacy of ABS has been previously tested in animals with normal[4] and defective hemostasis[11]. Cipil, et al.[11] (2009) 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 established 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[3].

Except from the study by Trakyali and Oztoprak[31] (2010), there are no published data about comprehensive observations or intraoral applications concerning the ABS effect on bond strength. According to Trakyali and Oztoprak[31] (2010), statistically significant differences were observed between shear bond strength values of the ABS contaminated group (9.58±0.95 MPa) and the control group (19.56±1.84 MPa). However, the shear bond strength values of the ABS contaminated group were between 6 and 10 MPa, which is clinically acceptable. The results of that study contrast with the findings of the present study. Based on the results of the current study, the µTBS values of the ABS application and control group were not found statistically significant. This result may be connected with removing ABS from the dentin surface by water rinsing and it may have inhibited formation of thin hydrophobic film on the dentin surface that provides adhesive penetration into the dentin tubules.

In this study, ABS and H_2O_2 were applied without blood contamination. No human blood contamination was done before the application of ABS in previous articles either[2,31]. In the cementation stage of all-ceramics, bleeding mostly occurs around the gingival margin and the clinician applies the haemostatic agent by swab all around the gingival margin as a blood-stopping agent. Therefore, a large part of tooth surface is contaminated just by the haemostatic agent without blood contamination. Additionally, in this study the authors wanted to investigate the effects of haemostatic agents alone on bond strength of resin cement to dentin. For this reason, hemostatic agent subgroups were created without blood contamination. In future studies bond strength can be evaluated by creating subgroups with and without blood contamination on tooth surface before the application of hemostatic agents.

CONCLUSIONS

Even considering the limitations of an in vitro methodology, the results of the present study showed that blood contamination affected

negatively the µTBS of a dual cured resin cement to dentin. Furthermore, ABS and H_2O_2 were found to give sufficient bond strength and therefore, they could be used safely as blood-stopping agents during cementation of all-ceramics to dentin in order to prevent bond failure due to blood contamination.

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