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Effect of Contamination with Hemostatic Agent on Shear Bond Strength of Composite to Dentin Using G-Premio and Single Bond Universal Adhesives

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ABSTRACT

Objectives: Bond strength of composite restorations plays an important role in their success. This in vitro study evaluated the effect of a hemostatic agent on shear bond strength of universal adhesives.

Materials and Methods: Thirty-six extracted human molars were used in this study. Buccal and lingual surfaces were reduced to obtain flat dentin surfaces and were ground with a silicone paper. The samples were randomly divided into three groups (n=12) based on the application of hemostatic agent: group 1: no contamination (control), group 2: aluminum chloride application, and group 3: ferric sulfate application. Each group was then divided into two subgroups (n=6) for using G-Premio and Single Bond Universal. Resin cylinders (Filtek Z550) were bonded to dentin surfaces according to the manufacturer's instructions. After 1000 thermal cycles, shear load was applied to the specimens using a universal testing machine at a crosshead speed of 1 mm/min until failure. Data were statistically analyzed using two-way ANOVA and Tukey's post-hoc test ($\alpha=0.05$).

Results: There were statistically significant differences in shear bond strength of the three main groups for both G-Premio and Single Bond Universal ($P<0.05$). When the adhesive systems were compared with each other, G-Premio showed higher shear bond strength than Single Bond Universal ($P<0.05$).

Conclusion: Contamination with hemostatic agents had an adverse effect on the shear bond strength of universal adhesives. Moreover, G-Premio yielded a higher bond strength than Single Bond Universal.

Keywords: Hemostatics; Shear Strength; Adhesives

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INTRODUCTION

Optimal bonding to tooth structure is a prerequisite for the success of tooth-colored restorations. Blood and moisture control is imperative for achieving a durable bond [1,2]. Nonetheless, ideal isolation of the area to prevent contamination with the saliva, gingival crevicular fluid, and blood is difficult particularly in areas close to the gingival margin or in proximal boxes [3].

Several strategies have been proposed to prevent or eliminate blood contamination such as re-preparation of the surface with rotary instruments, rinsing the area with water and subsequent air-drying, water irrigation and re-application of primer and re-etching with phosphoric acid [1-3]. Controversy regarding the efficacy of these strategies led to the introduction of hemostatic agents, which can be applied in case of bleeding to prevent tooth surface contamination.

Hemostatic agents can be used in clinical situations where there is a risk of blood contamination. Hemostatic agents can cause protein deposition. They are applied over the bleeding gingival tissue prior to the restoration of the cavity [4,5]. Hemostatic agents can be divided into two groups of astringents (coagulative agents) and vasoconstrictors (adrenergic agents) [6,7]. The hemostatic agents are acidic, and their pH varies from 0.7 to 3. Thus, they can eliminate the smear layer and cause some degrees of demineralization. Their long-term application can even eliminate the peri-tubular dentin [5]. Several types of hemostatic agents are available in the market such as aluminum chloride with 5% to 25% concentration, which has insignificant systemic effects, and can cause vasoconstriction [4]. Iron sulfate with 15% to 20% concentration also serves as a vasoconstrictor and anticoagulant agent [8,9]. The hemostatic effects of iron sulfate are due to the agglutination of blood proteins following the reaction of blood with sulfate and iron ions in acidic conditions [10]. Agglutinated proteins form networks that obstruct capillaries [11]. On the other hand, adhesives used in composite restorations have greatly advanced in the recent years. Universal adhesives are the latest version of dental adhesives, which are becoming increasingly popular due to their fewer clinical application steps and low technical sensitivity [4].

Previous studies have assessed the effect of hemostatic agents on 5th, 6th and 7th generation bonding agents. Some authors showed that hemostatic agents affected the bond strength of bonding agents [12] while some others reported the contrary [13]. However, studies on the effect of hemostatic agents on the bond strength of universal adhesives to dentin are limited [12-14]. Thus, this study aimed to assess the effect of contamination with hemostatic agents on shear bond strength of composite to dentin using G-Premio and Single Bond Universal adhesives. The null hypothesis was that the shear bond strength of composite to dentin contaminated with hemostatic agents would not be significantly different in the use of G-Premio and Single Bond Universal.

MATERIALS AND METHODS

This study was approved by the ethical committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1397.661). In this in vitro experimental study, 36 sound human molar teeth that had been extracted within the past 4 months were cleaned and stored in 0.2% thymol solution for disinfection. Twenty-four hours before the experiment, they were placed in distilled water. Both buccal and lingual surfaces were prepared by high-speed diamond saw (Leitz, Wetzlar, Germany) under water coolant to expose coronal dentin, bringing the actual sample size to 72 [15]. The teeth were then mounted in auto-polymerizing acrylic resin ($1{\times}2{\times}2cm^3$) (Acropars, Tehran, Iran) to 1mm below their cementoenamel junction. The exposed dentin was polished with silicon carbide abrasive paper (3M ESPE, St. Paul, MN, USA) for 30s under water irrigation to obtain optimal smear layer. Next, the teeth were rinsed with water to eliminate debris. The samples were then randomly divided into 6 subgroups based on the type of bonding agent and hemostatic agent used (n=12).

Subgroup 1. G-Premio universal adhesive (GC Corp., Tokyo, Japan) without contamination with a hemostatic agent

Subgroup 2. G-Premio universal adhesive + contamination with 25% aluminum chloride hemostatic agent (ViscoStat Clear; Ultradent, South Jordan, Utah, USA)

Subgroup 3. G-Premio universal adhesive + contamination with 20% iron sulfate hemostatic agent (ViscoStat; Ultradent Product Inc., Utah, USA)

Subgroup 4. Single Bond Universal (3M ESPE, St. Paul, MN, USA) without contamination with a hemostatic agent

Subgroup 5. Single Bond Universal + contamination with 25% aluminum chloride hemostatic agent

Subgroup 6. Single Bond Universal + contamination with 20% iron sulfate hemostatic agent

All applications were based on the manufacturers' instructions (Table 1). Hemostatic agents were applied on the dentin surface (Table 1) and were then rinsed with water spray for 30s [16]. After drying the dentin, the bonding agents were applied and light-cured with a curing unit (Apozoa, Guang Dong, China) for 10s with a light intensity of 450mW/cm^2. For the application of composite, a transparent cylindrical mold with an internal diameter of 3mm and height of 3mm was used. The cylindrical mold was placed on the tooth surface after the application of the bonding agent and fixed.

The mold was then filled with Filtek Z250 (3M ESPE, St. Paul, MN, USA) composite (Table 1) by applying three 1-mm-thick increments of composite according to the manufacturer's instructions. Each increment was light-cured for 20s. The samples were then incubated at 37°C and 100% humidity for 24h. To simulate the oral conditions, the teeth were subjected to 1000 thermal cycles in a thermocycler (Rika-Kogyo, Hachioji, Japan) between 5°-55°C with a dwell time of 30s and a transfer time of 30s. The shear bond strength was then measured by a universal testing machine (Santam, Tehran, Iran) with a crosshead speed of 1mm/min. The shear bond strength was calculated in megapascals (MPa) [16]. Data were analyzed using SPSS version 19 (SPSS Inc., Chicago, IL, USA) via two-way ANOVA and Tukey's HSD test. Level of significance was set at 0.05.

Table 1. Materials and application methods used in this study

Material	pH	Composition	Application technique
G-Premio Bond	1.5	10-MDP, phosphoric acid ester monomer, acetone, MEPS, 4-MET, di-methacrylate, initiator, silicon dioxide	1. Apply adhesive by a microbrush 2. Wait for 10s 3. Dry for 5s with maximum air spray 4. Cure for 10s
Single Bond Universal	2.7	10-MDP phosphate monomer, dimethacrylate resin, HEMA, Vitrebond TM copolymer, filler, ethanol, water, silane, initiator	1. Actively rub adhesive on the surface for 5s. 2. Gently dry with air spray for 5s to allow evaporation of the solvent 3. Cure for 10s.
ViscoStat Clear hemostatic agent	1	25% aluminum chloride gel	1. Apply for 2min 2. Rinse with water spray for 30s
ViscoStat hemostatic agent	1	20% iron sulfate gel	1. Apply for 2min 2. Rinse with water spray for 30s
Filtek Z250 composite		**Organic matrix:** Triethylene glycol dimethacrylate (TEGDMA) < 1–5%; Bisphenol-A-glycidyl methacrylate (Bis-GMA) < 1–5%; Bisphenol-A polyethylene glycol diether dimethacrylate (Bis-EMA) 5–10%; urethane dimethacrylate (UDMA) 5–10% **Fillers:** Zirconia/silica 60vol% inorganic fillers; Particle size 0.01 to 3.5µm	Maximum curing depth: 2mm Curing time: 20s

RESULTS

Table 2 shows the frequency distribution of bond strength values in the study groups. As shown, the G-Premio subgroup without contamination showed the maximum bond strength while the subgroup contaminated with iron sulfate and bonded with Single Bond Universal showed the minimum bond strength.

The Kolmogorov-Smirnov test showed that the data were normally distributed in all groups ($P>0.05$). Thus, two-way ANOVA was applied to compare the groups. The results showed that the effects of type of bonding agent and type of hemostatic agent as the main factors on the bond strength were significant ($P<0.001$), but the interaction effect of them on bond strength was not significant ($P=0.054$). According to the Tukey's post-hoc test, the difference among all subgroups was significant such that the control group had a significantly higher bond strength than aluminum chloride and iron sulfate groups ($P<0.001$). Also, aluminum chloride group showed higher bond strength than the iron sulfate group ($P=0.016$). According to the Tukey's test, the difference in shear bond strength was significant between the G-Premio and Single Bond Universal groups such that the G-Premio groups had a significantly higher bond strength than Single Bond Universal groups ($P<0.001$).

DISCUSSION

In the recent years, the use of tooth-colored restorative materials has greatly increased due to favorable esthetics, no risk of mercury toxicity, more conservative cavity preparation and higher preservation of tooth structure compared with amalgam restorations [17].

However, polymerization shrinkage is one drawback of composite resins. Polymerization stress cannot be directly measured in restored teeth, and only the effects of shrinkage stresses can be assessed by the microleakage, bond strength, and cuspal deflection tests. The shear bond strength was measured in this study, which is a reliable method for assessment of bond strength. It is relatively simple and allows relatively easy screening of adhesive systems and their bond strength at different areas and depths of restorative materials [18].

Cavity preparation is often associated with gingival bleeding, and it is imperative to control blood and moisture contamination to achieve an efficient bond between the composite resin and tooth structure [19]. Hemostatic agents can be used to control bleeding prior to the application of restorative materials. However, several studies have shown that hemostatic agents may stay on the tooth surface, leading to a significant reduction in bond strength to dentin [20]. In this study, aluminum chloride and iron sulfate hemostatic agents were used for this purpose. Aluminum chloride with 5% to 25% concentration is a commonly used hemostatic agent.

It has minimal systemic effects and causes protein deposition, vasoconstriction, and extraction of tissue fluids [21]. Iron sulfate is also effective as a coagulation agent to control bleeding in composite restoration of teeth [22]. These materials are often supplied in gel form to prevent their spread on the tooth surface. Nonetheless, rinsing off the gel is more difficult [23]. In the present study, Single Bond Universal and G-Premio universal adhesives were used.

Table 2. Shear bond strength values (Mpa) in different groups

Bonding agent	Hemostatic agent	Mean±Standard Deviation	Minimum	Maximum
G-Premio	No contamination	10.90±1.40 ^{A}	10.07	11.73
	AlCl3	9.36±1.69 ^{AB}	8.53	10.19
	SO4	7.14±1.18 ^{CD}	6.32	7.97
Single Bond Universal	No contamination	9.17±2.02 ^{B}	8.35	10.00
	AlCl3	6.38±1.03 ^{CE}	5.56	7.22
	SO4	6.20±0.99 ^{DE}	5.38	7.04

Different letters indicate statistically significant differences between the groups

These bonding agents can bond to enamel, dentin, and different types of restorations when applied in total-etch and self-etch modes. In the present study, these bonding agents were applied in self-etch mode. Some previous studies used bonding agents in total-etch mode, which increased the chance of complete rinsing and elimination of hemostatic agents during the etching phase. This complicated the assessment of the effect of hemostatic agents on dentin [14,15]. Thus, in this study, the bonding agents were applied in self-etch mode. These adhesives contain 10-MDP phosphate monomer, which justifies their etching ability and bonding ability to different substrates [24]. When universal adhesives are applied in one-step self-etch mode, they preserve the moisture of demineralized dentin and prevent collagen collapse. These bonding agents have lower technical sensitivity when applied in self-etch mode, and can be easily used in areas where adequate moisture control is difficult, as in posterior teeth [17,25,26].

The current findings indicated significant differences in shear bond strength of the bonding agents used in this study. One important point to remember is the presence of 10-MDP monomer in the composition of universal adhesives used in the present study. In self-etch self-primer application mode of bonding agents, the etched area is not rinsed; thus, calcium and phosphate ions produced by the dissolution of hydroxyapatite crystals form chemical bonds with 10-MDP monomer and yield a higher bond strength than etch and rinse systems [27].

Nonetheless, HEMA and polyalkenoic acid copolymer in Single Bond Universal compete with 10-MDP monomer for bonding to the surface of hydroxyapatite crystals and decrease the formation of calcium-10-MDP salts at the resin-dentin interface [28]. HEMA, present in the composition of Single Bond Universal, deteriorates the mechanical properties of polymerized adhesives due to its mono-methacrylate chemical composition, and has an adverse effect on hydrolytic destruction of the adhesive layer, which results in separation of dentin-adhesive phases [29]. Yoshida et al. [28] demonstrated that it can inhibit the nano-layering of 10-MDP monomer and decrease the adhesive bond strength. Also, previous studies have shown that the presence of alcoholic solvent, compared with acetone solvent, in the composition of bonding agents can decrease the bond strength; however, it is more reliable in terms of technical sensitivity [30,31]. Thus, minimum bond strength was noted in Single Bond Universal. Choi et al. [31] showed that G-Premio bonding agent yielded a higher bond strength than Single Bond Universal, which was in agreement with our results.

It has been shown that the application of hemostatic agents can significantly decrease the bond strength of composite to dentin. Hemostatic agents have acidic pH (0.7-3) and hydrophilic properties; thus, they could interfere with all steps of the bonding process. The use of such hydrophilic hemostatic agents could change the dentin surface morphology, and may influence the bond strength of adhesive resins [32].

According to the current findings, iron sulfate hemostatic agent group had a lower shear bond strength compared with the aluminum chloride and control groups. In contrast, Araújo et al. [20] showed that ViscoStat had significantly higher bond strength than ViscoStat Clear; this difference may be due to the fact that they assessed the bond strength of resin cement. Regarding iron sulfate, changes in dentin surface were probably responsible for this reduction. Previous studies have demonstrated that iron sulfate has a pH of 1 and thus, etches the dentin surface. However, dentinal tubules may remain partially obstructed [33,34]. Also, another study indicated that collagen and plasma proteins may coagulate by the effect of iron sulfate, and this may complicate their separation from dentin surface, especially by self-etch adhesives [23].

The shear bond strength of aluminum chloride group in our study was significantly lower than that of the control group. Residual aluminum ions remaining on the surface and consequent substitution of calcium in the composition of hydroxyapatite crystals with aluminum ions and formation of insoluble $Al(OH)2H2PO4$ is probably responsible for the reduction in shear bond strength [14,20]. This insoluble compound cannot be easily washed off the dentin surface [14].

Saad et al. [35] indicated that contamination with aluminum chloride and iron sulfate hemostatic agents decreased the bond strength of composite to dentin; however, this reduction was not statistically significant for aluminum chloride. It seems that the mechanism of bonding of glass ionomer cements, which is through ionic bonds between the carboxyl groups of polyalkenoic acid and calcium present in hydroxyapatite, and also the micromechanical retention of glass ionomer tags in demineralized dentin, as well as the application of etchant might have neutralized the negative effect of aluminum chloride hemostatic agent on bond strength. Their results were in agreement with our findings. Kuphasuk et al. [14] showed that contamination with aluminum chloride significantly decreased the bond strength of a self-etch bonding agent, which was also in line with our findings [14]. Tuncer et al. [15] demonstrated that in use of Single Bond Universal adhesive in self-etch mode, a significant difference was noted in bond strength of the control group and the group contaminated with aluminum chloride, which was in accordance with our results. Saati et al. [36] indicated that aluminum chloride had no significant effect on bond strength, which was probably due to the difference in rinsing period, which was 5 min, as well as the difference in the type of bonding agents used. Their results were different from ours. Ebrahimi et al. [12] reported that the difference in bond strength of the control group and iron sulfate group in application of AdheSE bonding agent was significant, which was different from the findings of the current study. This controversy is probably due to the use of different bonding agents. The penetration depth of AdheSE One F monomers in dentin is 0.05μm while the penetration depth of AdheSE monomers in dentin is 1.2 to 2.2μm, which explains the difference in bond strength provided by the abovementioned two bonding agents.

Since the current study had an in vitro design and could not perfectly simulate the oral clinical conditions, clinical studies are required to assess the retention of restorations in the clinical setting. Also, hemostatic agents other than those evaluated in the present study should be evaluated. Last but not least, assessment of the effect of blood contamination along with hemostatic agents on bond strength of composite resins can be an interesting topic for further research.

CONCLUSION

Within the limitations of this study, the results showed that the application of hemostatic agents significantly decreased the bond strength of composite to dentin, and this reduction was greater for iron sulfate compared with aluminum chloride. Moreover, G-Premio yielded a higher bond strength than Single Bond Universal.

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CONFLICT OF INTEREST STATEMENT

None declared.

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