Effect of Contamination by Cleaning Agents and Hemostatic Agent on Dentin Bond Strength

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ABSTRACT

BACKGROUND

Contamination by hemostatic agent and rinsing with different disinfectant solutions can influence on micro shear bond strength between dentin and resin composite. The major objective of this study was to investigate the micro shear bond strengths of an etch and rinse technique and self-etch technique to human dentin contaminated with a hemostatic agent containing $AlCl_3$ followed by chlorhexidine (CHX) and boric acid cleaning.

METHODS

A 50 non-carious, permanent, third molar teeth were randomly divided into five groups (N = 10 per group); Group 1: Self etch technique (OptiBond All - In - One, Kerr) was used on dentin contaminated with hemostatic agent (Alustat, CerKamed). Group 2: Etch and rinse technique (Phosphoric acid then OptiBond Universal, Kerr) was used. Group 3: Contaminated dentin was cleaned with CHX for 60 seconds and then self-etch technique was used. Group 4: Contaminated dentin was cleaned with boric acid for 60 seconds and then self-etch technique was used. Group 5: Hemostatic agent was not applied, only self-etch technique was used. Flat occlusal dentin surfaces were prepared and then restored with resin composite rod (Herculite Classic, Kerr) (1 mm diameter × 1 mm height). After thermocycling (1,000 cycles), composite sticks were subjected to microshear until fracture occurred at a crosshead speed of 0.5 mm / min. Statistical analysis was made with one-way analysis of variance (ANOVA) and multiple comparison tests with Tukey.

RESULTS

The mean microshear bond strength values of group 1 to group 5 were 21.89 ± 12.43 , 26.15 ± 22.79 , 20.05 ± 12.03 , 35.08 ± 13.20 and 19.41 ± 6.53 MPa, respectively. When the values of the groups were compared, the difference was not significant. (P = 0.108).

CONCLUSIONS

Boric acid and CHX may be used as alternatives to other materials that are used as cavity disinfectants on contaminated dentin by hemostatic agent.

KEY WORDS

Boric Acid, CHX, Hemostatic Agent, Adhesive Systems, Shear Bond Strength

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BACKGROUND

The success of the bond strength between dentin and composite restoration can be affected by the stability of the hybrid layer.¹ The deterioration in the hybrid layer can develop in 3 ways. First, collagen fibrils which are exposed during etching and rinse may degenerate.² In the second place, demineralized collagen fibrils restrict resin infiltration by collapsing due to hydrogen bonds between peptides, as they are air-dried during bonding.^{3,4} Third, fluid movement within the dentinal tubules due to pulpal pressure causes insufficient leakage of the monomers in the resin into the dentin tubules.⁵ Hence, inadequate binding occurs internally between dentin and adhesive.⁶ In addition to all of these, failures increase even more because of contamination of prepared dentin with internal agents such as saliva, gingival fluids, blood, or external agents such as lubricants from the aerator and hemostatic agents.⁷

Hemostatic agent, which is used to stop bleeding coming from gingival inflammation or gingival trauma from tooth preparation and decreased gingival fluids, have a deleterious effect on bond strength between adhesive and dentine.⁸ These materials have high acidity and include aluminum chloride or aluminum sulfate or ferric sulfate.⁹ According to the studies in the literature, 20 - 25 % aluminum chloride agents are used, and they have been observed to show the cytotoxic effect on gingival fibroblast cells at 48h - 72h time points and lead to demineralization of dentin.^{9,10} Therefore, these hemostatic agent residues need to be removed from the dentin surface to which the bonding will be made. This led to the idea of cleaning the dentin surface with various cleaning agents.

As a cleaning agent chlorhexidine (CHX) is widely used.¹¹ In addition to its antimicrobial effects, researchers have shown that chlorhexidine acts as an MMP inhibitor and limited the collagen breakdown in the hybrid layer.¹¹ In this way, it is thought that the connection between dentin and adhesive will not be disrupted and at the same time, the fibrillar structure of the collagen matrix can contribute to the remineralization of dentin as the fibrillar structure is preserved.¹² Due to the bactericidal, fungicide, and antiseptic properties of boric acid, the idea of it being used as a cavity disinfectant has come up. It is a weak inorganic acid form of boron element.¹³ Furthermore, when used in the treatment of periodontal diseases, a positive response was observed from some clinical measurements such as alveolar bone loss and bleeding on probing.¹⁴

There are studies showing that the connections of self-etch adhesive systems are adversely affected due to changes in the smear layer because of hemostatic agent contaminations.^{15,16}

Most studies on this subject have focused on the effects of cleaning with CHX.^{2,17} There has been little quantitative analysis of how different cavity disinfectants affect dentin bond strength. The major objective of this study was to investigate the micro shear bond strengths of an etch and rinse technique and self-etch technique to human dentin contaminated with a hemostatic agent containing AlCl₃ followed by CHX and boric acid cleaning. Our null hypothesis was that cleaning the dentin with CHX and boric acid after hemostatic agent was applied and performing thermal cycles did not affect the bond strength.

METHODS

50 non-carious, permanent, intact, extracted, third lower human molar teeth were gathered by the Non-Invasive Clinical Research Ethics Committee of Cukurova University Faculty of Medicine, dated 21. 05. 2021, numbered 43. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Our study is an in-vitro study and was conducted between June 2020 and January 2021. To expose flat dentin surfaces, the occlusal enamel of each tooth was trimmed horizontally using carbon separator under water coolant. To obtain a homogeneous dentin surface, the exposed dentin surface was sanded with 600 and 1200 grit water sandpaper SiC for 60 seconds. Then, the teeth were randomly separated into five groups (n = 10).¹⁸ Cotton pellets impregnated with hemostatic agent (Alustat, Cerkamed, Stalowa Wola, Poland) were applied to all dentin surfaces except group 5 for 90 seconds. Then the tooth surface was washed with air - water spray for 15 seconds and dried with the help of oil free air for 5 seconds. Subsequently, the treatment protocol was applied for the subgroups -

- Group 1 - Self-etch technique was used. Applied self-etch adhesive OptiBond All-In-One (Kerr Corporation, Orange, CA, USA). It was scrubbed continuously on the dentin surface for 20 s by brush, air - thin for 5s and light cured for 10 s (LED - Elipar Freelight, 3M - ESPE, USA).
- Group 2 - Etch and rinse technique was used. Dentin was etched with 37.5 % phosphoric acid (Kerr Gel Etchant, Kerr, Orange, CA, USA) for 15 s and rinsed with water for 15 s. The surface was left wet and excess water was removed by blotting with a tissue paper, leaving the surface visibly moist. And then OptiBond Universal was applied. It was scrubbed continuously on the dentin surface for 20 s by brush, air - thin for 5s and light cured for 10 s.
- Group 3 - Cleaning with CHX for 60 seconds and dried with absorbent and then OptiBond All-In-One was applied. It was scrubbed continuously on the dentin surface for 20 s by brush, air - thin for 5s and light cured for 10 s.
- Group 4 - Cleaning with boric acid for 60 seconds and dried with absorbent and then OptiBond All-In-One was applied. It was scrubbed continuously on the dentin surface for 20 s by brush, air - thin for 5s and light cured for 10 s.
- Group 5 - Hemostatic agent was not applied. Only self-etch technique used. Applied self-etch adhesive OptiBond All-In-One. It was scrubbed continuously on the dentin surface for 20 s by brush, air - thin for 5s and light cured for 10 s. The manufacturer and composition of materials used are presented in Table 1.

At the end of all treatment procedures for the build up of the composite micro cylinders, used starch tubes (Nuh'un Ankara Makarnası, Ankara, Turkey) of 1 millimeter (mm) internal diameter and 1 mm height were used for placing the composite (Herculite Classic, Kerr, Orange, CA, USA). LED - Elipar Freelight (3M - ESPE, USA) was used for the polymerization of the samples. It was polymerized for 20 seconds according to the manufacturer's instructions.

They were submerged in 37 °C distilled water for 24h. Prior to testing, the specimens were subjected to thermocycling for 1,000 cycles in water (5 / 55°C) with 30 seconds of dwell time and 30 seconds of transfer time.¹⁷ Finally, the micro shear bond strength (μ - SBS) was measured using an universal testing machine (MOD Dental MIC - 101, Esetron Smart Robototechnologies, Ankara, Turkey) at a crosshead speed of 0.5 mm / minute until failure occurred. The μ - SBS values were calculated in mega Pascal (MPa).

Material	Composition	Manufacturer
OptiBond All - In - One	Acetone, ethyl alcohol, TEGDMA, mineral fillers, ytterbium fluoride, photoinitiators, accelerators, stabilizers, water	Kerr Corporation, Orange, CA, USA
OptiBond Universal	Acetone, 2 - hydroxyethyl methacrylate, glycerol dimethacrylate, ethanol, glycerol phosphate dimethacrylate	Kerr Corporation, Orange, CA, USA
Herculite Classic Composite	Bis - GMA, TEGDMA, Borosilicate - aluminum glass	Kerr Corporation, Orange, CA, USA
Gel etching	37,5 % phosphoric acid	Kerr Corporation, Orange, CA, USA
Chlorhexidine Antibacterial Solution	2 % chlorhexidine gluconate containing disinfectant	Consepsis Ultradent, South Jordan, UT, USA
5 % Boric acid	Boric asit, distilled water	Handmade
Hemostatic agent	25 % Aluminum chloride	Alustat, Cerkamed, Poland

Table 1. Materials Used in the Study

μ - SBS = $P / \pi r^2$, P = load at failure (in newton), π = 3.14, and r = radius of restorative material microcylinder (in mm).

After measuring, each fractured specimen was evaluated using a stereomicroscope (SOIF optical instruments, ST6024 - B2, İstanbul, Turkey) at 40× magnification to make out its mode of failure. The failure mode was defined into the following types:^{18,19}

- Type I - Adhesive failure (failure at the interface between dentin and restorative material)
- Type II - Cohesive failure (failure in the restorative material)

- Type III - Mixed failure (involving both adhesive and cohesive failures)

Statistical Analysis

Statistical analysis was made with one-way analysis of variance (ANOVA) and multiple comparison tests with Tukey. All statistical analyses were conducted at a significance level of $P < 0.05$.

RESULTS

Mean and standard deviation values are summarized in Table 2. When the mean $\pm$ standard deviation of micro shear bond strength values of the groups were compared, the difference between the groups was not significant ($P = 0.108$).

	N	Mean $\pm$ Standard Deviation
Group 1	10	21.89 $\pm$ 12.43 ^a
Group 2	10	26.15 $\pm$ 22.79 ^a
Group 3	10	20.05 $\pm$ 12.03 ^a
Group 4	10	35.08 $\pm$ 13.20 ^a
Group 5	10	19.41 $\pm$ 6.53 ^a
P Value		0.108

Table 2. Mean $\pm$ Standard Deviation of Micro Shear Bond Strength (in MPa) of the Tested Groups

(Group 1: Self-etch technique, Group 2: Etch and rise technique, Group 3: With CHX, Group 4: With boric acid, Group 5: Hemostatic agent was not applied, only self - etch adhesive)

^a Means for groups in homogeneous subsets are displayed with same letters

The percentages of the fracture modes observed with all tested groups are presented in Figure 1. There is no significant difference between the percentages of failure modes according to the groups ($P = 0.610$). In all tested groups, predominant mode of failure was adhesive.

Figure 1. Modes of Failure in Experimental Groups

(Group 1: Self-etch technique, Group 2: Etch and rise technique, Group 3: With CHX, Group 4: With boric acid, Group 5: Hemostatic agent was not applied, only self-etch adhesive)

DISCUSSION

Hemostatic agent, which is containing AlO_3 and having an acidic pH in the range of 0.7 to 3²⁰ might affect the smear layer on the dentin surface and indirectly the bond strength. The dental adhesives are used specifically to bond composites, but their ability to reach dentin tubules and modify smear layer may be hampered by contaminated dentin surface. Therefore, it has been suggested to use various cleansing agents such as CHX and boric acid. This study evaluated the effect of different cleaning protocols on the shear bond strength of a universal adhesive to dentin contaminated with a hemostatic agent. The null hypothesis is not rejected. In the present study, the boric acid group and CHX did not show significant differences among the other methods used. Using etch and rinse technique with universal adhesive had higher μ - SBS than other groups but it was also not showing statistically significant difference among other groups.

There was no statistical difference in the micro shear values between the self-etch groups applied with a hemostatic agent ($P \leq 0.05$). Although there are concerns that hemostat may affect the bonding strength of agents containing ferric sulfate and aluminum chloride, this result may be because washing with water removes the hemostatic agent sufficiently. Harnirattisai et al. (2009) used a hemostatic agent containing 25 % aluminum chloride in their study. They stated that due to the acidity of the hemostatic, it created a demineralization effect on the dentin surface, but this did not affect the bonding strength of adhesives.²¹ Our study corresponded to these results. There are studies that did not support this view. Kuphasuk et al. in their study found that the solution containing 25 % AlCl_3 showed lower bonding value in the used self-etching adhesive groups in the contaminated dentin compared to the normal dentin.¹⁶ Ünlü et al. (2010) and Cebe and Çobanoğlu (2014), showed that hemostatic agent application had a negative effect on the bonding performance of self-etch adhesives.^{19,22} Ünlü et al.¹⁹ applied different hemostatic agents for 2 minutes to dentine contaminated with blood. They stated that the primers of the bonds they used were light (CSE) and medium acidity (ACE, ASEP) and because no extra washing was done, coagulating agents could not be removed sufficiently, and the bonding strength was reduced because of an insufficient hybrid layer. In addition, the self-etch systems used in these two studies contained water as a solvent in their primers.^{19,22} The bonds we used in our study, Optibond All-In-One contained water + ethanol + acetone as a solvent and Optibond Universal contained water + ethanol as a solvent. AlCl_3 can dissolve in organic solvents such as water, acetone, and ethanol.²³ One of the reasons for not having a statistically significant difference between the groups may be that the bond content we used was different from other studies. In this research, we chose the Alustat hemostatic agent containing 25 % AlCl_3 because we frequently used it in clinical practice. Since 90 seconds of application is sufficient to control bleeding in the soft tissues, (it is written recommended contact time up to 5 minutes by manufacturer in the instructions for use) we used this application time to our samples. However, the reason for not having a statistical difference in the group without hemostatic application may be the low demineralization of the dentin surface due to the short application time of the hemostatic agent. Previous studies

have shown that contamination times longer than 4 minutes caused demineralization supported by SEM images.^{20,24}

Dentin - composite bonding occurs with the hybrid layer formed by the access of the bonding agent to the collagen fibrils by modifying the smear layer in self-etch bonding systems.²⁵ The self-etch system we used had a mild acidity ($\text{pH} \geq 2.5$). Mild acidity may be insufficient to modify smear layer and contamination on dentin. Hence the hemostatic agent could not be removed sufficiently in the group 5 wherein no additional washing process was applied, the intended thickness of the hybrid layer may not have been formed and the bonding strength may have decreased compared to the other groups. Chlorhexidine is an antimicrobial cationic detergent. It can be used in more than one step in dental procedures. CHX can be used for cleaning the tooth surface before etching,²⁶ or can be used to reduce the collagenolytic activity in dentin after etching.²⁷ In the present research, we applied before etching. Some researchers showed higher bond strength values,²⁸⁻³⁰ while some showed that it did not affect the bonding statistically.³¹⁻³⁴ In our study, we determined that CHX could be used as a surface disinfectant and did not affect bonding. Boric acid, which has bacteriostatic agent, anti-inflammatory, osteoblastic activity, is versus with CHX, which has been accepted as the gold standard in cavity disinfection in recent years. In our study, 5 % CHX concentration was used as a cavity disinfectant. Although it did not show statistically significant difference from other groups, it showed the highest bonding value between groups.

In addition, it is possible to talk about the limited effect of boric acid $\text{pH}^{5.1}$ on dentin structure.³⁵ Ercan et al. found that the self-etch adhesive systems were not statistically different from the control group in their bond strength after cleaning the cavity with 5 % boric acid.³⁶ Also, Aktürk et al. found that the bond strength of self-etch and universal adhesive systems was not affected after disinfection with 5 % boric acid.³⁷ Our study corresponded with other results. Moreover, boric acid has the ability to dissolve in ethanol and acetone.³⁸ The adhesive we applied also contained acetone and ethanol, so using boric acid as a cavity disinfectant may not affect the bonding strength. Therefore, the antibacterial effect of boron can make boric acid a suitable alternative only as a cavity disinfectant. Boric acid group showed the highest μ - SBS. It was followed by a 37 % phosphoric acid applied universal bond group using the etch and rinse method. Kuphasuk et al. found that when phosphoric acid was applied with the total etch technique in dentin contaminated with 25 % aluminum chloride solution, it did not show statistically significant different μ - SBS values.¹⁶ In another study, the bonding strength values of the control group and the contamination groups were similar.¹⁹ It may be that 37 % phosphoric acid, which has a very strong acidity, completely removes all hemostatic agents affected by contamination owing to its pronounced etching effect.³⁹ Etching enamel with phosphoric acid also increases bonding. Bonding in dentin is more difficult than enamel due to its high organic matrix, water content and its heterogeneous structure that includes dentin canals, collagen fibers, hydroxyapatite crystals, and odontoblast extensions.⁴⁰ This may be another reason why our results do not show significant differences.

Shear or tensile bond strength tests are among the most frequently preferred test methods to evaluate the performance of adhesive systems in the laboratory. It is

believed that shear stresses contribute the most to the breakdown of adhesion of restorative materials in vivo.⁴¹ Therefore, we used the shear bond strength test to predict the clinical performance of the materials. Considering the limitations of this in vitro study is that the dentin surface is not contaminated with saliva and fresh blood. However, in clinical practice, there is not always blood and saliva contamination into the cavity, but the bleeding stopper may touch the cavity due to the application of the bleeding stopper with cotton pellet.

CONCLUSIONS

After the dentin is contaminated by hemostatic agent for a short time, it is easily removed with water. Contamination with hemostatic agent did not reduce the shear bond strength of both etch and rinse and self-etch adhesive systems. Application of chlorhexidine and boric acid after the removal of hemostatic agent did not affect the bond strength. Boric acid and CHX can be used as cavity disinfectants when dentin is contaminated by hemostatic agent.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.

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