Effect of Haemostatic Agent Application on the Shear Bond Strength of Contemporary/Multi-Mode Adhesive Systems

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Abstract

Objectives: The aim was to determine the effect of blood contamination and haemostatic agent application on the shear bond strength (SBS) of different adhesives to dentin.

Materials and Methods: Seventy-two extracted human molars were used in this study. Teeth were mounted acrylic in moulds. Mesial and distal surfaces were removed to obtain flat dentin surfaces (n=144) and grinded with a 600 grit sandpaper. The samples were randomly divided into three main groups (N=48) based on the adhesive system and application procedures.

Group 1: No contamination (control),

Group 2: Blood contamination,

Group 3: Blood Contamination+Haemostatic agent application.

Each group was further divided into four subgroups:

Subgroup I: Single Bond 2 (etch-and-rinse)

Subgroup II: Clearfil SE Bond (two-step self-etch)

Subgroup III: Single Bond Universal (multimode, etch-and-rinse)

Subgroup IV: Single Bond Universal (multimode, all-in-one self-etch) (n=12). Resin cylinders (Filtek Z550) were bonded to the dentin surfaces according to the manufacturers' instructions. A shear load was applied to the specimens using universal testing machine at a cross-head speed of 0.5 mm/min until failure. Data were analyzed statistically ($p<0.05$).

Results: Statistically significant differences were observed between no contamination (control) and blood + haemostatic agent contamination groups for both Single Bond 2 (etch-and-rinse) and Single Bond Universal (multimode, all-in-one self-etch) ($p<0.05$). When the adhesive systems were compared to each other, no statistically significant differences were found in all contamination groups and control group for the mean SBS values ($p>0.05$).

Conclusion: When blood contamination is inevitable two step self- etching adhesive systems may be the choice of adhesive system in terms of shear bond strength.

Key Words: Multi-mode Adhesive, Shear Bond Strength, Blood Contamination, Haemostatic Agent.

Introduction

It is of a dentist's duty to optimize his skill, knowledge, contemporary material selection and application in an ideal way to obtain the best clinical results in every field of dentistry. In particular, the clinical protocols of restorative dentistry require many operative steps that need special technique sensitivity.

Dentin bonding agents, along with other dental materials, are sensitive to moisture and blood contamination. However, many carious lesions are located near the gingival margin where blood and/or crevicular fluid contamination is more likely to occur [1,2]. Preventing or eliminating gingival bleeding or contamination by crevicular fluid is very critical for the longevity of the restoration in such situations; sub-gingival caries, class V cavity preparation, taking impression, cementation of all-ceramic restorations with margins near the gingiva, and chronic gingival inflammation [3].

There is of course a dilemma for a dentist to form a blood and/or moisture free surface before bonding procedures when contamination is inevitable since contamination with blood and/or moisture reduces bond strength of the adhesive to tooth structure [4,5]. In fact, blood contamination produces pronounced reduction in the bond strength compared to salivary contamination alone [6].

Blood has high-protein content (approximately 6.7%) and fibrinogen macromolecules, on the other hand, because of protein attraction property of dentin, blood proteins can form a layer, which prevents resin infiltration into the dentin structure and reduces 30-70% of bond strength [7].

In recent years, haemostatic agents have been used to control gingival bleeding and reduce crevicular fluid. For this purpose, epinephrine, aluminum chloride, and ferrous sulfate containing formulations are being frequently used. Additionally electro surgery and lasers are also used to prevent cavity contamination with blood and crevicular fluids [8].

Today's adhesive systems were described as: etch-and-rinse adhesive systems, self-etch adhesive systems, and glass ionomer adhesives. Etch-and-rinse system's bonding mechanism to dentine is diffusion-based and depends on hybridization of the resin within the exposed collagen mesh as well as into the dentine tubules [9], creating a micromechanical interlocking of resin within the exposed collagen fibril scaffold. This technique utilizes 30–40% phosphoric acid to remove the smear layer [10]. Self-etch adhesives dissolve smear layer only partially and do not demineralize dentin as deep as etch-and-rinse adhesives. The incorporation of smear layer, resin, collagen and mineral into hybrid layer and the superficial portion of resin tags may prevent postoperative sensitivity that occurs with etch-and-rinse adhesives because of incomplete infiltration of resin monomers into the collagen network [11].

Several one step/all-in-one self-etch adhesives have been recently developed to simplify and shorten the application time making the

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clinical procedure easier [11]. A novel approach in advanced dental adhesive technology is to develop a universal or multi-mode one bottle adhesive systems that can be applied in either etch-and-rinse, self-etch or selective-etch protocols after deciding on the most suitable modality for a specific cavity preparation [12].

The aim of this in vitro study was to evaluate the effect of blood contamination and haemostatic agent application on the shear bond strength of different contemporary adhesive systems to dentin.

Materials and Methods

72 caries-free human molars were selected and disinfected in 0.5% chloramine solution and stored in distilled water until specimen preparation. In order to facilitate handling of the samples, the teeth were mounted in self-cure acrylic resin molds. Mesial and distal enamel surfaces were removed with a water-cooled diamond saw in a cutting machine until 1/3 outer dentin surface is reached. A total of 144 exposed dentin surfaces were grinded with a 600-grit sand paper under running water for 30 seconds to obtain a uniform and standardized smear layer. The teeth were then rinsed with distilled water to remove any debris. Then the specimens were randomly assigned to 3 groups (N=48) with respect to the following contamination protocols:

Group 1 (control group): No contamination.

Group 2 (blood contamination): All dentin surfaces were covered with fresh human blood. The blood was applied to the dentin surfaces as droplets by using a syringe, waited for 15 seconds and then rinsed for 10 seconds with a water stream from an air-water syringe and dried with a gentle blast of air.

Group 3 (blood contamination + Hemoban): Dentin surfaces were contaminated with fresh human blood as in group 2 and blood was cleaned with cotton pellet soaked with haemostatic agent (25% Aluminum Chloride containing Hemoban, Sultan, Hackensack, NJ, USA) for 15 seconds and then dried with cotton pellet.

Each group was further divided into four subgroups according to the following adhesive systems and application procedures (n=12) which were all applied in accordance with the manufacturers' instructions (*Table 1*).

Subgroup I: Single Bond 2, 3M ESPE, St. Paul, MN, USA (etch-and-rinse adhesive system)

Subgroup II: Clearfil SE Bond, Kuraray, Osaka, Japan (two-step self-etch adhesive system)

Subgroup III: Single Bond Universal, 3M ESPE, St. Paul, MN, USA (multimode, etch-and-rinse adhesive system)

Subgroup IV: Single Bond Universal, 3M ESPE, St. Paul, MN, USA (multimode, all-in-one self-etch adhesive system)

A resin composite (Filtek Z550, 3M ESPE, St. Paul, MN, USA) was built up on the dentin surface of each specimen by packing the material into a cylindrically shaped plastic mold with an internal diameter of 2.86 mm and a height of 3 mm with the incremental technique. The specimens were polymerized with a LED curing device (light intensity: 1000 mW/cm^2; Smartlite PS, Dentsply De Trey, Konstanz, Germany) for 20 seconds and stored in distilled water for 24 hours at room temperature before testing. A shear bond strength test was performed. Shear load was applied to the specimens using universal testing machine (LRX, Lloyd Instruments, Fareham, England) at a cross-head speed of 0.5 mm/min until failure. Maximum loads at bond failure were recorded in Newtons (N), and bond strengths were calculated in megapascals (MPa) by dividing the maximum loads at failure by the surface area of the resin composite. Data derived from shear bond strength test were analyzed with two-way ANOVA and multiple comparisons were made with Bonferroni test at a significance level of 0.05.

Results

When contamination groups were compared, statistically significant

Table 1. *Materials used in the study.*

Material(Batch number)	Composition	Self-etch strategy	Etch-and-rinse strategy
Adper Single Bond 2 (N388576)	1. Etchant: 35% phosphoric acid (Scotchbond Etchant) 2. Adhesive: bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly(itaconic) acids,10% by weight of 5 nm-diameter spherical silica particles		1. Apply etchant for 15 s 2. Rinse for 10 s 3. Blot excess water 4. Apply 2–3 consecutive coats of adhesive for 15 s with gentle agitation 5. Gently air dry for 5 s 6. Light polymerize for 10 s
Clearfil SE Bond (Primer: 01196A – Bond: 01802A	1. Primer: water, MDP, HEMA, camphorquinone, hydrophilic dimethacrylate 2. Bonding: MDP, bis-GMA, HEMA, camphorquinone, hydrophobic dimethacrylate, N,N-diethanol p-toluidine bond, colloidal silica	1. Apply primer to tooth surface and leave in place for 20 s 2. Dry with air stream to evaporate the volatile ingredients 3. Apply bond to the tooth surface and then create a uniform film using a gentle air stream 4.Light polymerize for 10 s	
Single Bond Universal (471010)	1. Etchant: 35% phosphoric acid (Scotchbond Etchant) 2. Adhesive: MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	1.Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. If necessary, rewet the disposable applicator during treatment 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely 3. Light polymerize for 10 s	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Air dry 2 s 4. Apply adhesive as for the self-etch mode
FiltekZ550 Nanohybrid Composite (N388576)	Bis-GMA, UDMA, Bis-EMA, TEGMA and PEGDMA, Surface-modifiedzirconia/silicafillers 3000 nm (3 µm or less),non- agglomerated/non-aggregatedsurface-modifiedsilicaparticles 20 nm, 82 wt % 68 vol %		

Table 2. *Mean shear bond strength values ± SD (MPa).*

Adhesive systems	Control (No contamination)	Blood Contamination	Blood Contamination + Haemostatic Agent	p
Single Bond 2	12.58 ± 4.46[a]	9.17 ± 2.74	8.37 ± 2.33[a]	0.043
Clearfil SE Bond	12.69 ± 5.07	8.56 ± 2.83	11.35 ± 4.46	0.062
Single Bond Universal (Etch-and-rinse)	13.69 ± 5.79	12.58 ± 4.83	10.53 ± 5.36	0.171
Single Bond Universal (All-in-one self-etch)	15.11 ± 4.68[b]	11.17 ± 1.83	8.14 ± 3.08[b]	<0.001
p	0.422	0.079	0.171	

Same letters in the same row indicates statistical significance

differences were observed between control (no contamination) and blood + hemostatic agent contamination groups for both Single Bond 2 (etch-and-rinse) and Single Bond Universal (multimode, all-in-one self-etch) ($p<0.05$) (*Table 2*).

When the adhesive systems were compared to each other, no statistically significant differences were found in all contamination groups and control group for the shear bond strength values ($p>0.05$) (*Table 2*).

Discussion

Avoiding any kind of contamination of the prepared cavity walls before application of resin composite and adhesive systems is a fundamental prerequisite to obtain a successful adhesion and durable bond. However, in many clinical situations, rubber dam application may be difficult to place or may not be feasible in a very busy practice. In such cases, the contamination of the operation field with blood or saliva is almost inevitable [13].

In this study, the effect of blood contamination and application of aluminum chloride based Hemoban haemostatic agent application on the shear bond strength of contemporary adhesives was investigated. The rationale beneath this in vitro research was that the rich protein content and macromolecules of residual blood will form a film thickness on the dentin surface to be bonded obstructing the penetration of the adhesive system into dentin tubules [14]. Freshly drawn blood was used as the contaminant for this study since blood coagulation might be an important factor in the effect of blood contamination on bonding [13].

In dental literature, it was previously well reported that blood contamination during restorative procedures causes a decrease in the bond strength of the resin-based materials [15]. However, this research seeks an answer to the question that what a dentist can use as an adhesive system if contamination is inevitable without compromising the bond strength of the adhesive systems to dentin by using shear bond test protocol which was used to quantitatively analyze the performance of adhesive systems on both enamel and dentin [16].

To reach this goal, the present study evaluated the shear bond strength values of an etch-and-rinse, a self-etch and a multi-mode adhesive systems after haemostatic agent application. The current study, along with other studies in literature, reported that blood contamination leads to decreased shear bond strength of all tested adhesive systems to dentin at varying degrees with respect to their respective control groups, but only Single Bond 2 (etch-and-rinse adhesive system) and Single Bond Universal (all-in-one, self-etch) demonstrated statistically significant decreased shear bond strength values after blood contamination + haemostatic agent application.

Previous studies have demonstrated that haemostatic agents are highly acidic and their pH varies from 0.7-3.0 [17,18]. Aluminum chloride is an acidic agent with a concentration between 20%-25% and dentin surfaces treated with this agent demonstrate varying degrees and patterns of demineralization [18] at different concentrations and application periods. It has been shown that dentin surfaces treated with 21.3% aluminum chloride exhibit various degrees of demineralization. Complete smear layer removal with some dentin demineralization can be observed after applying this agent for five minutes [18]. In this research, Hemoban was applied for 15 seconds.

Some studies have reported that contact of some astringent agents on tooth structures resulted in decreased bond strength between a composite and tooth structures [4,19]. In a study demonstrated decreased bond strength, lower bond strength values were obtained after haemostatic agent application and SEM evaluations demonstrated remnants of aluminum chloride in tooth structures [4].

Because of the weak acidity of the primer of self-etch adhesives in comparison with phosphoric acid, it was predictable that they could not dissolve also Hemoban material so that their penetration to deeper areas of dentin was impaired [7] demonstrating lower shear bond strength values after haemostatic agent application. O'Keefe et al. [19] showed that rinsing ferric sulfate and aluminum chloride hemostatic agents with water before using self-etch adhesives causes higher bond strength than non-rinsing ones. This fact also may be further investigated in future studies.

Clearfil SE and Single Bond Universal are both self-etching adhesive systems; however, Clearfil SE is a two-step self-etch adhesive system with a pH of 2.0 whereas Single Bond Universal is an all-in-one self-etch adhesive system with a pH of 2.7. The reason why Clearfil SE demonstrated higher performance than other material after Hemoban application may lie on the fact of different pH values and the demineralization effect of the systems.

The lower shear bond strength value of etch-and-rinse adhesive system in our study may be due to the technique sensitivity of these systems which are susceptible to variations in the degree of dentin moisture, arising from the wet bonding technique [4].

Conclusion

Within the limitations of this in vitro study, the results revealed that when blood contamination is inevitable and haemostatic agent had to be used to prevent blood contamination, two step self-etching adhesive systems may be the choice of adhesive system in terms of shear bond strength. The finding suggests that shear bond strength is lower when all-in-one self-etch adhesive and etch-and-rinse adhesive systems, respectively were applied.

Conflict of Interest

All my affiliations, corporate or institutional, and all sources of financial support to this research are properly acknowledged, except when mentioned in a separate letter. I certify that do not have any commercial or associate interest that represents a conflict of interest in connection with the submitted manuscript.

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