

Evaluation of the Effect of Different Contaminants on the Shear Bond Strength of a Two-step Self-etch Adhesive System, One-step, Self-etch Adhesive System and a Total-etch Adhesive System

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Abstract:

Background: Adhesive procedures have become an integral part of today's restorative dental procedures. This study was carried out to evaluate the effect of contaminants such as saliva, blood, and hemostatic agents on the shear bond strength of a self-etch adhesive system Clearfil SE Bond, one-step, self-etch adhesive system Adper™ Easy One and a total-etch adhesive system Adper™ Single Bond 2.

Materials and Methods: A total of 111 extracted sound human molars were collected and divided into 5 groups for each of the 3 bonding agents. For each adhesive, specimens were divided as follows 5 teeth (control group), 8 teeth contaminated by saliva, 8 teeth contaminated by blood, 8 teeth where Viscostat was used, 8 teeth where Viscostatclear was used. For the control group of Groups I, II, and III adhesive application was done directly followed by composite build-up. Specimens in each group were tested in shear mode using a chisel-shaped rod in a universal testing machine at a cross head speed of 0.05 cm/min. The data for each group was subjected to one-way ANOVA followed by Studentized Newman-Keuls test to make comparisons among the groups.

Results: The total-etch adhesive system Adper™ Single Bond 2 showed better bond strength when compared to the one-step self-etch adhesive system, Adper Easy One and self-etch two-step adhesive system, Clearfil SE when contaminants like saliva, blood, hemostatic agents are used.

Conclusion: When bonding to contaminated surfaces the total-etch system, Adper™ Single Bond 2 showed better results when compared to the one-step self-etch adhesive system Adper Easy One and self-etch two-step adhesive system, Clearfil SE.

Key Words: Adhesives, contaminants, hemostatic agents

Introduction

Patient demand for esthetic restorations has generated interest in the advancement of adhesive dentistry. The achievement of high-strength, durable bonds between tooth structure and restorative materials have been a long-term goal of the dental profession. Enamel and dentin bonding has progressed from the first- and second-generation adhesive systems to current sixth and seventh-generation adhesive systems. However, a problem that compounds the entire procedure of bonding is the effect of contaminants on the effectiveness of the bond.¹⁻³

The emergence of new materials and applications in adhesion is profoundly changing the way dentistry is being delivered. As adhesive dentistry is taking over, bonding has become an integral part of restorative dentistry.

Contemporary dentin adhesives use one of two strategies to interact with the dentin smear layer: The total-etch technique or the self-etch technique. Total-etch materials use 30-40% phosphoric acid to etch dentin and enamel before the clinician applies the adhesive to the preparation. Etching dentin removes the smear layer and opens up the dentinal tubules.^{4,5}

Self-etch adhesives, which are being used increasingly, do not require a separate acid-etch step and do not remove the smear layer.⁶

The bonding mechanics of these systems either relies on the entanglement of resin monomers with dental substrates or on hybridization, which is now considered the fundamental mechanism for retention of resin-based composite restorations.⁷

As the technique of bonding has been simplified, complications have increased. The self-etch technique has met incessant challenge from the conventional total-etch technique. Though the basic bonding mechanics of the two techniques is more or less similar, they vary substantially in their formulations, method of delivery and technique sensitivity. Available literature does not seem to be giving a clear picture of the

superiority of one technique over the other. There have always been conflicting reports on the current status of the two bonding techniques.⁸

Bonding to dentin remains a variable and technique-sensitive procedure; compounding upon this existing problem are the profound effects of contaminants on bond strengths.

Even though contemporary dentin adhesive systems are easier to use and less technique sensitive, salivary and blood contamination may still occur during bonding procedures affecting bond strength. While preparing for and placing restorations around the gingival margins, hemostasis is of utmost importance in maintaining the ideal, contaminant-free working field.^{9,10}

In these situations, hemostatic agents are used. There are various hemostatic agents like 2% zinc chloride, 13% ferric sulfate, Viscostat (20% ferric sulfate solution), Viscostat Clear (25% aluminum chloride solution). Viscostat is used to efficiently provide isolation from sulcular fluids, including blood and saliva.¹

This study will attempt to provide valuable inputs to literature on the effect of contaminants such as blood, saliva, and hemostatic agents on the shear bond strength (SBS) of a two-step self-etch adhesive system Clearfil SE Bond, one-step, self-etch adhesive system Adper™ Easy One and a total-etch adhesive system Adper™ Single Bond 2.

Materials and Methods

After receiving clearance from the ethical committee of Bapuji Dental College and Hospital, this study was performed on 111 extracted human molars. They were stored in distilled water at 4°C.

Preparation of the samples

Teeth were cleaned with an ultrasonic scaler to remove soft tissue. They were embedded in self-curing acrylic resin up to their cemento-enamel junction. The occlusal surfaces of the teeth were reduced perpendicular to the long axis of the tooth on a water-cooled, model-trimming wheel to create flat dentin surfaces. The prepared samples are shown in Figure 1. The teeth were divided into 5 groups for each of the 3 bonding agents. For each adhesive, specimens were divided as follows 5 teeth (control group), 8 teeth contaminated by saliva, 8 teeth contaminated by blood, 8 teeth where Viscostat was used, 8 teeth where Viscostat clear was used. The contaminants were applied as described in Table 1. Product description of the contaminants used for the study is given in Table 2.

Adhesive application

Manufacturer’s instructions were strictly followed in the application of all bonding agents.



Figure 1: Prepared samples after reducing occlusal surfaces.

Table 1: Application of contaminants.	
Contaminants	Application and removal
Saliva	Artificial saliva was applied to the surface with a brush for 30 s. It was rinsed with water and dried for 10 s
Blood	Uncoagulated human blood was applied and left undisturbed for 1 min, followed by a firm water spray rinse for 1 min
Viscostat	It was applied for 1 min, rinsed with a firm water spray for 1 min and air-dried
Viscostat clear	It was applied for 1 min, rinsed with a firm water spray for 1 min and air-dried

Group I: Bonding agent, Clearfil SE Bond (Kuraray Medical Inc., Tokyo, Japan) two-bottle, self-etch adhesive system.

The primer was applied to the prepared tooth surface with the saturated brush and left for 20 s. Then, it was dried with a mild air stream to evaporate the volatile ingredients. Then, the bonding agent was applied to the prepared tooth surface. After applying bonding agent, a uniform film was created by using a gentle air stream. Light curing was done using a light-emitting diode (LED)-curing device (Elipar® Free Light, 3M ESPE) for 10 s.

Group II: Bonding agent - Adper™ Easy One (3M ESPE), single-bottle, self-etch adhesive system.

The prepared surface was dried thoroughly, and the adhesive was scrubbed using a cotton tip applicator. The adhesive was applied for 20 s, air thinned for 5 s and light cured using an LED-curing device (Elipar® Free Light, 3M ESPE) for 10 s.

Group III: Bonding agent - Adper™ Single Bond 2 (3M ESPE), two-step, total-etch system with Scotchbond™ etchant (3M ESPE).

The Scotchbond™ Etchant was applied to prepared tooth surfaces and left for 15 s. The etchant was rinsed for 15 s. Excess water was blotted using a cotton pellet. Immediately after blotting, 2-3 consecutive coats of the adhesive were applied for 15 s with gentle agitation using a fully saturated applicator. Air thinning was performed for 5 s to evaporate the solvent. The adhesive was then light cured for 10 s using an LED curing device (Elipar® Free Light, 3M ESPE).

For the control group of Groups I, II, and III, adhesive application was done directly followed by composite buildup using the Teflon mold.

Restorative technique

- To bond a cylinder of resin-based composite to each specimen a circular Teflon mold (inner diameter 3 mm and height 3 mm) was attached to the flat surface of dentin after adhesive application. Filtek™ Z350 resin composite was packed into the mold.
- The material was placed in two increments with adequate compaction and light cured for 30 s using LED curing light (Elipar® Free Light, 3M ESPE).
- After polymerization, the mold was removed and specimens were placed at 37°C in distilled water for 24 h.

Bond strength measurement

The above prepared specimens were then mounted on universal testing machine for SBS measurement. The load was applied with cross head speed of 0.05 cm/min. until fracture. SBS required to cause fracture was calculated by dividing the failure force (Ff) by the cross-sectional area of specimen (n)

$$SBS = Ff/n$$

In Figure 2, the sample is placed under the universal testing machine.

Results

A statistical analysis using one-way ANOVA and Studentized Newman Keul's test revealed a statistically significant difference between Viscostat Clear and the other contaminants. Results revealed statistically significant difference between Viscostat Clear and the other contaminants.

So according to this study, the SBSs of both self-etch systems are reduced when Viscostat Clear is used as a contaminant, whereas it has no effect when total-etch system is used. Comparison of SBSs of 3 main groups done by ANOVA is shown in Table 3.

The results of the study reveal that the contaminants such as blood, saliva, Viscostat have no effect on the bond strengths of the 3 bonding agents, and their values are comparable with that of the controls. Statistical tests using one-way ANOVA and Studentized Newman Keul's test showed highly significant difference between Groups I and III, Groups II and III. Group III showed better bond strengths than Groups I and II in all the situations where different contaminants were used. The total-etch adhesive system showed better results than the self-etch systems.

Discussion

Current adhesive research focuses on the simplification of the application procedure. Reduction of a number of application

Table 2: Product description of the contaminants used for the study.

Product	Composition
Artificial saliva	NaCL - 0.400 g KCL - 0.400 g CaCl ₂ H ₂ O - 0.795 g NaH ₂ PO ₄ - 0.69 g Na ₂ S ₉ H ₂ O - 0.0005 g Urea - 1 g Distilled water - 1000 ml
Viscostat	20% ferric sulfate solution
Viscostat clear	25% aluminum chloride

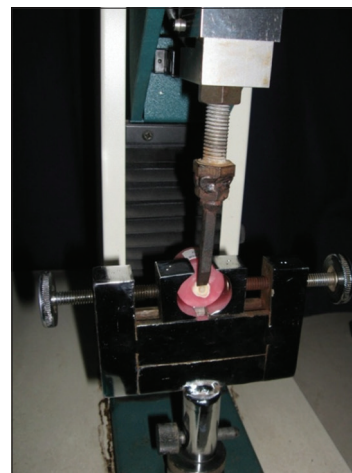


Figure 2: Sample under universal testing machine.

steps should reduce manipulation time, and abate technique sensitivity, thus improving bonding effectiveness. This trend in adhesive dentistry has LED to the introduction of self-etch adhesives, of which the one-step self-etch adhesives or the so-called all-in-one adhesives are the most user-friendly adhesive systems nowadays in the market. Their application procedure involves a single step, combining etching, priming and bonding.⁷

Clinically, there are many factors that affect adhesion and retention of resin-containing restorative materials. Moisture such as gingival fluid, blood, hand piece oil and in particular saliva can affect quality of the bond leading to microleakage at the interface. As a result loss of restoration, recurrent caries, post-operative sensitivity and discoloration may occur.¹⁰

In the clinical situations where blood contamination can occur, dry operative fields can be obtained after the application of hemostatic agents to control bleeding and decrease gingival fluid. Examples of these materials are Viscostat and Viscostat Clear.¹¹

The results of the study reveal that the total-etch adhesive systems showed better bond strengths than the self-etch systems, in all the situations where different contaminants were used.

Table 3: Intergroup comparison of shear bond strengths of 3 main groups.

Contaminants	Clearfil SE 2-Step	Adper easy one, self-etch one step	Adper single bond 2, total etch	P* value, sig	Significant pairs**
Control					
Mean±SD	19.45±0.54	19.33±0.51	24.87±0.91	P<0.001 HS	I and III (5.4), II and III (5.5)
Saliva					
Mean±SD	18.76±0.62	18.80±0.64	24.21±0.92	P<0.001 HS	I and III (5.4), II and III (5.4)
Blood					
Mean±SD	18.42±0.86	18.43±0.72	24.12±0.66	P<0.001 HS	I and III (5.6), II and III (5.7)
Viscostat					
Mean±SD	18.49±0.59	19.43±1.06	24.00±0.37	P<0.001 HS	I and III (5.5), II and III (4.5)
Viscostat clear					
Mean±SD	14.59±0.39	13.63±0.45	24.11±0.80	P<0.001 HS	I and III (9.5), II and III (10.4)

SD: Standard deviation

The SBSs of both self-etch systems are reduced when Viscostat Clear is used as a contaminant, whereas it has no effect when total-etch systems are used.

All dentin bonding systems employ acids of one type or another to facilitate adhesion to the tooth tissues. Acidic treatment of dentin and/or enamel creates a zone of demineralization, which is subsequently (total-etch) or concurrently (self-etch) infiltrated with various bifunctional primers and resins.¹²

Etch and rinse approach

This adhesion strategy involves at least two steps and, in its most conventional form, three steps with successive application of the conditioner or acid etchant, followed by the primer or adhesion promoting agent, and eventually, application of the actual bonding agent or adhesive resin. The simplified two-step version combines the second and third step but still follows a separate “etch and rinse” phase. Adper Single Bond 2, which is commonly used as representative of the two-step total-etch systems, was used in this study.¹²

Self-etch approach

Probably, in regard to user-friendliness and technique sensitivity, clinically, the most promising approach is self-etch. It no longer needs an “etch and rinse” phase, which not only lessens clinical application time but also significantly reduces technique-sensitivity or the risk of making errors during application and manipulation. A self-etch approach involves either a two- or one-step application procedure.¹²

Adper Easy One was used in this study. It is a single-bottle; self-etch, ethanol-water based adhesive system.

The greatest drawback of the single-bottle, self-etch systems is the hydrolytic instability of the ester bonds of the methacrylated phosphoric acid esters which are present to perform the self-etching action. The hydrolytic stability of the phosphoric

acid esters increases in the following order: Dialkyl hydrogen phosphate < trialkyl phosphate < monoalkyl dihydrogen phosphate. This phenomenon occurs due to the incorporation of water as an essential ingredient of the self-etching systems.

However, even with the most hydrolytically stable 2-methacryloyloxyethyl phosphoric acid, the solution does not remain usable when stored at room temperature for several weeks; therefore, refrigeration is required.¹³

In an attempt to eliminate this instability of the self-etch systems, Clearfil SE Bond has been developed as a two-bottle, self-etch system. Liquid A primarily consists of water and Liquid B contains the phosphoric acid esters; thereby, water is separated from the acidic esters, which increases the stability of the latter. This material has been tested over time and is considered to be one of the best in this category of bonding systems.³

Clearfil SE Bond or the two-bottle, self-etch adhesive system was used in this study.

The heavily filled light activated composite resin used in the present study was Filtek Z350 which is a nanocomposite, which according to the manufacturer, delivers both the strength of a hybrid and the beautiful, lasting polish of a microfill in one dependable product. The combination of nanomer-sized particles to the nanocluster formulations reduces the interstitial spacing of the filler particles. This provides for increased filler loading, better physical properties when compared to composites containing only nanoclusters.¹⁴

In general, during the definitive cementation procedure, the prepared tooth should be free of contaminants to achieve a lasting bond between the luting agent and tooth structure.⁹

However, appropriate contamination control might not always occur, especially near or along the gingival margin. Blood and

gingival crevicular fluid due to gingival inflammation may be present on the prepared tooth surface.

The most common procedures used to control bleeding and decrease the flow of gingival fluid involve the use of a topical hemostatic agent. This procedure is important for avoiding any blood contamination along the interface between the prepared tooth surface and the luting agent.

A hemostatic agent-containing an aqueous solution of aluminum chloride (AlCl_3) is frequently used in restorative dentistry. Aluminum compounds with a concentration range of 20-25% have the ability to precipitate protein, constrict blood vessels and extract fluid from tissue.¹⁵

In the present study, Viscostat or 20% ferric sulfate solution and Viscostat Clear or 25% aluminum chloride solution were used as contaminants.

Viscostat is used to efficiently provide isolation from sulcular fluids, including blood and saliva. Viscostat is 20% ferric sulfate solution. Hemostasis using this solution is achieved by the formation of coagulum plugs and their incorporation into the capillary openings.¹

The curing unit used in this study was Eliparfreelight 2 (3M ESPE) in a standard mode. It has a high intensity of 1000 mW/cm² and has got a narrow emission spectrum, which reduces curing time by 50% as compared to conventional curing units.

The universal testing machine was used in this study at the cross head speed of 0.05 cm/min. This cross head speed is in accordance with results of a study done by Hara *et al.* where different cross head speeds were used to evaluate the SBS test on dentin surface. The study concluded that cross head speed of 0.50 mm/min and 0.75 mm/min result in more adhesive failures and is therefore preferred in SBS tests.¹⁶

Shear method of bond strength testing was used in this study. The SBS test method draws more criticism than approval. In a shear bond test, two materials are connected via an adhesive agent and loaded in shear until fracture occurs. The SBS is calculated by dividing the maximum applied force by the bonded cross-sectional area. It is easier to perform and does not require special equipment to carry out the test.¹⁷

In the present study, Group III or the total-etch adhesives exhibit greater bond strength than the self-etch adhesives.

The results of the study reveal that the total-etch adhesive system showed better results than the self-etch systems, in all the situations where different contaminants were used.

The total-etch adhesives require a separate step of etching, which is usually performed by phosphoric acid. The depth of

demineralization promoted by phosphoric acid determines the thickness of the hybrid layer, as the application of phosphoric acid before the adhesive acts by removing the smear layer, demineralizing the dentin structure, and consequently exposing the collagen fibrils to allow formation of the hybrid layer.¹⁸

The exposed collagen may provide reactive groups that can chemically interact with bonding primers. The solvent used in Adper Single Bond 2 is ethanol, which competes with and replaces moisture, promoting infiltration of monomers through the nanospaces of the exposed collagen network. This collagen network serves as a framework for the creation of a resin-demineralized dentin hybrid layer, resulting in strong micromechanical interlocking between the dentin and the superficially demineralized dentin.

Hence, total-etch adhesive systems showed better bond strength than self-etch adhesive systems.¹⁹

In the present study, Groups I and II or the self-etch adhesives exhibit lower bond strength than Group III or the total-etch adhesive.

For the control group, the mean SBS of Adper Single Bond 2, Adper Easy One, and Clearfil SE Bond is 24.87, 19.33, and 19.45 MPa, respectively.

The principle mechanism of bonding of the self-etch systems, as in the case of the total-etch systems, is by the formation of a hybrid layer in dentin; though the hybrid layer that is formed is shallower and the resin tags short (2 μm). This has been attributed to the low pH of the acidic methacrylate monomers when compared to that of 37% phosphoric acid. These monomers contain one or more acidic functional groups and have comparatively low first acid dissociation constants (pK_a1). Water is required to dissociate these monomers to release the hydronium ions (H_3O^+) which bring about demineralization. These agents also generally contain hydroxyethyl-methacrylate, which acts as a transitional polymerisable solvent since most of the acidic monomers are only mildly miscible in water. However, the simultaneous etching and the infiltration of the resin monomers is expected to eliminate any discrepancy in the extent of demineralization and resin infiltration. This mild self-etching action does not completely deprive the collagen fibers of their hydroxyapatite (HAP) content, which is in contrast to the total-etch systems. The residual HAP serves as a receptor for additional intermolecular interactions with specific carboxyl or phosphate groups of the functional monomers. Therefore, what ensues is "chemo-mechanical" bonding mechanism.^{7,20}

Self-etching systems incorporate a significant amount of water as a solvent to promote ionization of the acidic monomers, making these dental adhesives permeable membranes that

are highly susceptible to the degrading effects of water. After solvent evaporation, the adhesive layer can be very thin, and its mechanical properties may be low.¹³

Self-etching adhesive systems rely on acidic monomers to simultaneously demineralize and infiltrate enamel and dentin. This acidity must be neutralized by the mineral content of the tooth structure to allow complete polymerization of the adhesive film.

With total-etch adhesives, the smear layer and the dissolved mineral are removed during the rinsing step.^{21,22}

As there are residual acidity and an inability to remove the smear layer, so the bond strength of self-etch adhesives is lower.

Results of this study showed similar bond strengths of one-step and 2-step self-etch adhesives.

The reason could be the presence of similar composition. They both contain functional monomers, cross-linking monomers, solvents, inhibitors and activators. However, the amount of ingredients applied on the tooth surface differ considerably among one and 2-step adhesives.³

The results of the study showed that the bond strength of self-etch and total-etch adhesive systems are not affected when saliva is used as a contaminant.

This result of the study is in accordance with the previous studies done by El-Kalla and Garcia-Godoy,²³ Vargas *et al.*²⁴ Hitmi *et al.*²⁵

According to these studies, there was no statistically significant decrease in bond strength to tooth surfaces contaminated with saliva when testing modern adhesive systems that incorporated primer in adhesives and saliva contamination did not affect the formation of the hybrid layer.

According to the earlier school of thought, dry tooth surface was essential for achieving good adhesion and Buonocore concluded that etched tooth surfaces readily absorb salivary constituents thus reducing their surface energy and rendering them less favorable to bonding. According to the recent *in vitro* studies, the modern adhesive systems may be more forgiving of contamination on dentin than the earlier adhesive systems.^{26,27}

The results of the study showed that the bond strength of self-etch and total-etch adhesive systems are not affected when Viscostat is used as a contaminant.

According to the manufacturer, Viscostat is completely soluble in water and a firm water spray rinse is recommended prior to any bonding procedures.

Viscostat has no effect on the bond strength of total-etch adhesive systems. The reason for this result is that phosphoric acid reportedly breaks down and removes ferric sulfate.⁹

For this study, the application time was 1 min, a sufficient time to achieve hemostasis. Most situations will require less time for hemostasis, and, therefore, teeth will have less contact time with the hemostatic agents.

The results of the study showed that the bond strength of self-etch adhesive systems is not affected when Viscostat is used as a contaminant. This result is in accordance with the previous studies done by O'Keefe and Salama where Viscostat does not affect self-etch adhesive systems if it is rinsed prior to the adhesive procedures.¹¹

For the self-etching adhesive used in this study, the contamination of dentin with $AlCl_3$ solution showed significant reduction of the bond strength.

$AlCl_3$ treated enamel revealed inhibition of the demineralization process of HAP, which was exposed to a demineralizing solution even though the aluminum concentration was low. This mechanism has been explained by displacement of calcium in the HAP by aluminum, which results in the very soluble $Al(OH)_2H_2PO_4$ compound.

Because HAP is also the major part of dentin-like enamel, the influence of $AlCl_3$ solution on dentin could be similar to enamel.²⁸⁻³⁰

Since the Clearfil SE primer has weak acidity, with the pH being approximately 2 the demineralizing effect on dentin contaminated with $AlCl_3$ solution might be similarly inhibited.³¹

For self-etching adhesives, the dentin bonding mechanism is due to the exposed collagen network and smear layer modification by self-etching primer incorporated into resin adhesives. As a result, less dentin etching effect of the primer could result in a decrease of bond strength as shown in this study.

In this study results showed that Viscostat Clear has no effect on the bond strength of the total-etch system. The bond strengths of the control and contaminated group were comparable. This might be due to the aggressive etching effect of phosphoric acid, with pH 0.5, which simultaneously demineralized and removed all contaminants on the dentin surfaces.³²

Conclusion

When bonding to contaminated surfaces the total-etch system, Adper™ Single Bond 2 showed better results when compared to the one-step self-etch adhesive system Adper Easy One and self-etch two-step adhesive system, Clearfil SE.

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