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Effect of Saliva and Blood Contamination on Bond Strength of Three Different Dentin Bonding Agents - Invitro Evaluation Study.

[1]Dr.Anand Raj, MDS, Assistant Professor, Department of Conservative Dentistry and Endodontics, PSM Dental College, Thrissur

[2]Dr. Shruthi Raj, MDS, Assistant Professor, Department of Oral Pathology and Microbiology, PSM Dental College, Thrissur

Corresponding Author: Dr. Shruthi Raj, MDS, Assistant Professor, Department of Oral Pathology and Microbiology, PSM Dental College, Thrissur

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Introduction

Aim: To evaluate and compare the shear bond strength of 3 different dentin bonding agents on contamination with saliva and blood.

Methods: 90 extracted human premolars were divided into 5 groups based on the type of contamination it was subjected to and when contamination occurred (before or after adhesive application). Each group is subdivided into 3 sub groups based on the 3 different dentin bonding agents based on the solvent potential i.e.; acetone based, ethanol based or water based. Fresh saliva and fresh human blood were applied either before or after application of bonding agent.

Result: Shear bond strength obtained was compared between 3 groups and was found that ethanol based bonding agent have highest shear bond strength compared to water based bonding agent and acetone based bonding agents have the lowest shear bond strength. Bond strength was significantly reduced after contamination with blood as compared to saliva.

Conclusion: Ethanol based bonding agent have more shear bond strength compared to water based and acetone based. Saliva and blood contamination significantly reduced the bond strength of adhesive to dentin .

Keywords: Blood contamination, Saliva contamination, Self-etching adhesive, Shear bond strength.

Introduction

The increasing demand for esthetic restorations has generated intensive research of adhesive materials. Successful adhesion to dental hard tissues is a fundamental requirement prior to the insertion of resin based composites.(1)Any contamination of the prepared surface by saliva, blood or gingival crevicular fluid should be avoided in order to achieve a successful and durable

bond between the resin composite and the tooth[4-5] . Achieving good moisture control is a common problem encountered in restorative dentistry, especially when rubber dam isolation is not feasible. Many carious lesions, which require the use of dentin bonding agents, are found in areas for which it is difficult to obtain appropriate isolation, especially when the site is near or at the gingival margin, where blood contamination is more likely to occur.[2]

Adhesion to dentin has been a subject of considerable interest because it is a more heterogeneous substrate with much higher organic and water content than enamel. The condition of the tooth structure and the chemical composition of the adhesive agent have shown to affect the bond strength [3]

For contemporary adhesive systems, dentin bonding requires removal or modification of the smear layer and superficial demineralization by acid etching. Although chemical reactions between adhesive systems and dentin have been reported, it is generally accepted that dentin bonding relies primarily on micromechanical interaction similar that of enamel bonding, mediated by the permeation of resin monomers into acid-etched dentin. The entanglement of the polymerized adhesive resin with collagen fibrils and residual hydroxyapatite crystals generates an interfacial structure called “resin-dentin inter diffusion zone” or “hybrid layer”.[7]

Some studies reported that saliva contamination of dentin had an adverse effect on adhesive/dentin bond strength. A 50% reduction in mean bond strength was demonstrated when composite resin was bonded directly to a saliva contaminated enamel and dentin.[5]

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 dentine surface ,obstructing the penetration of the adhesive system into dentine tubules[9]

Blood contamination decreases the bond strength of adhesive materials and may occur in different moments of the adhesive procedure. When blood contamination occurs in between acid etching and application of the adhesive, demineralization caused by etching exposes the collagen network, which is more prone to react with the protein compounds of blood, impairing primer and adhesive penetration and affecting the bond to dental substrate.[6]

Different solvents presented in primer component or in simplified bonding agents are responsible for either carrying excess water out or infiltrating resin monomers into interfibrillar dentin. Water, ethanol and acetone are basically the main solvents in commercial formulations.[9]

Dentin adhesive systems contain high-vapour pressure organic solvents (acetone and ethanol). These chemical agents, known as “water-chases”, increase dentin wet ability and help replace the water on the acid etched and rinsed dentin surface with hydrophilic resin monomers.[7]

The authors suggested that alcohol-based agents resulted in better bond strength than when using an acetone-based bonding agents.[8] Shear bond strength test is a simple evaluation procedure used to test the adhesion of dental adhesives Barmier Cooley 1992. In vitro bond strength test are useful and essential for predicting the performance of adhesive systems and possible correlations with clinical issues. So shear bond strength testing is done with a universal testing machine Instron which is conventionally popular for evaluating the adhesive ability of adhesive/restorations. With the simple technique and relevant results it is considered a benefit for Ranking and marketing purpose.[10]

Materials and Method

90 extracted human maxillary premolars are selected and stored in distilled water containing 0.2 % thymol

solution. The teeth are cleaned using an ultrasonic scaler and mounted in self cure acrylic resin. Samples are wet ground using silicon carbide disc to obtain flat dentin surface buccally.

These teeth are divided mainly into 5 groups based on the type of contamination subjected to, and the step in the bonding sequence when contamination occurred, i.e.; before and after adhesive application. Then each group is subdivided into 3sub groups Group A, B, C. Based on three different bonding agents used.

Commercially available bonding agents based on the solvents i.e.; acetone based, ethanol based and water based of different companies are used for the study. (Prime and bond NT, ExciTE® F, Tetric N-Bond self etch). Table 1

Fresh unstimulated saliva from the oral cavity and fresh human blood from the finger tip of one of the investigator are collected at the time when the specimens are prepared for study.

- **Control group: (n = 18)** :

In this group it is again subdivided into 3 groups:

Group A, B, C. (n= 6) in each group, based on the application of 3 different types of bonding agents respectively.

In group A - acetone based bonding agent is used. (**Prime and bond NT)**

In group B – ethanol based bonding agent is used. (**ExciTE® F)**

In group C - water based bonding agent is used (**Tetric N-Bond** self etch**)**

Here the specimens are not subjected to saliva and blood contamination. Bonding agents are applied based on the manufactures instructions. (Figure 1)

- **Contamination group I:(n=18):**

In this group it is also again subdivided in to 3 sub groups:

Group A, B, C (n=6) in each group, based on the application of 3 different types of bonding agents respectively. Here dentin surface are subjected to contamination with saliva for 15 seconds using a micro brush. After contamination, surfaces are rinsed for 10 seconds with water stream from an air water syringe, followed by a gentle blast of air for 10 seconds to dry the surface. Each bonding agents are applied in each group A, B, C respectively same manner as in control group.

- **Contamination groupII(n=18):**

In this group it is also again subdivided into 3 sub groups:

Group A,B,C(n=6). The same procedure as in group I are followed here and the specimens are subjected to contamination with blood.

- **Contamination groupIII(n=18):**

In this group also again subdivided into 3 sub groups:

GroupA,B,C(n=6).Here the different bonding agents are applied to respective subgroups A,B,C as in control, then the surface are subjected to contamination with saliva for 15 seconds and rinsed and dried as in group I.

- **Contamination groupIV(n=18):**

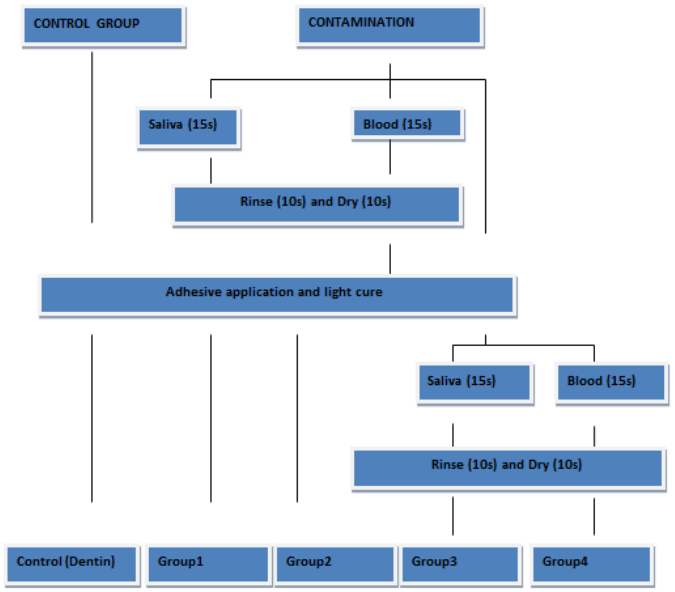
In this group also it's again subdivided into 3 subgroups A, B, C (n=6).Same procedure as in groupIII is followed except that the specimens are subjected to contamination with blood.

A 3 mm diameter poly tetra fluro ethylene mold are used to create an inverted and truncated cone of composite filtek Z-250 Shade A3 –(3M ESPE)and was photocured on the sample surfaces for 20 seconds (bluephase C5 ,Ivoclar-Vivadent).All the specimens were stored at 37^0C in water for 24 hours before testing, to stimulate oral environment.

After storage the specimens were transferred to the universal testing machine individually, and then subjected to shear bond strength analysis at a crosshead speed of 1mm/minute at department of dental materials, Manipal College of dental sciences, Karnataka .The bond strengths were expressed in Mpa.

The data obtained from this study was subjected for multiple comparison by one variable test (ANOVA) Kruskal –Wallis and since the results were highly significant it was followed by Mann-whitney test .

Figure 1: Schematic representation of the experimental protocol for each bonding agents



Results

In control group: The shear bond strength in group A was 9.9+ 0.51, in group B 16.05+0.58, in group C 12.7+0.7. Kruskalwallis test and post hoc analysis by Mannwhitney test shows that each group is significantly differ with each other. P value is <0.01,so in control group B has significantly high bond strength compared to group C and compared to group A. (Graph 1)

In group **I :**the shear bond strength in group A was 7.2+ 0.33, in group B 13.3+0.45,in groupC 9.7+0.33. . Kruskalwallis test and post hoc analysis by Mannwhitney test shows that each group is significantly differ with each other. P value is <0.01,so in control group group B has significantly high bond strength compared to group C and compared to group A (Graph 2)

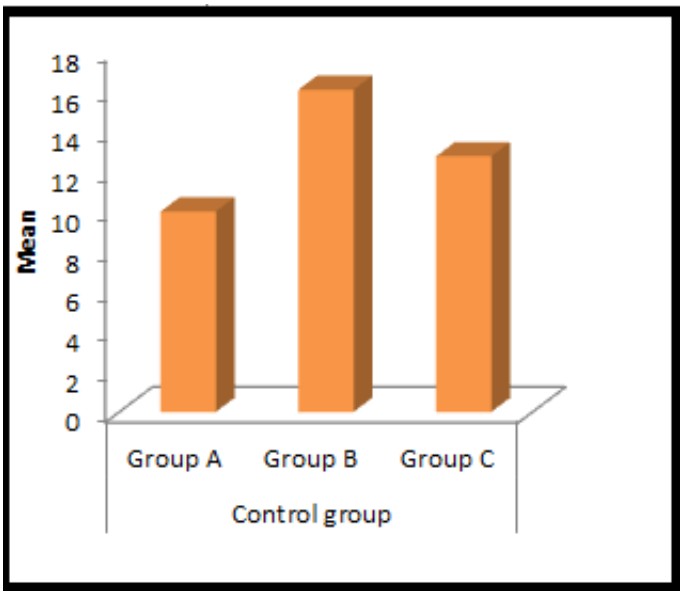
In group **II:**the shear bond strength in group A was 4.4+ 0.29, in group B 8.5+0.24,in groupC 5.4+0.20. Kruskalwallis test and post hoc analysis by Mannwhitney test shows that each group is significantly differ with each other. P value is <0.01,so in control group group B has significantly high bond strength compared to group C and compared to group A (Graph 3)

In group **III:**the shear bond strength in group A was 6.4+ 0.36, in group B 9.3+1.0,in groupC 6.6+1.07. . Kruskalwallis test and post hoc analysis by Mannwhitney test shows group B was significantly high compared to group A and C.where as group A and C have no significant difference (Graph 4)

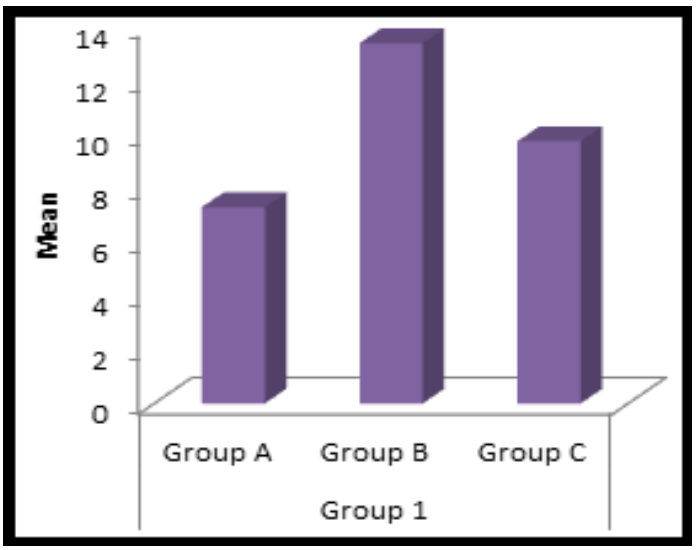
In group **IV:**the shear bond strength in group A was 3.2+ 0.17, in group B 5.3+0.39,in groupC 3.9+0.23 . Kruskalwallis test and post hoc analysis by Mannwhitney test shows that each group is significantly differ with each other. P value is <0.01,so in control group group B has significantly high bond strength compared to group C and compared to group A. (Graph 5)

Post hoc analysis by Mannwhitney test was done to compare the shear bond strength between the Groups(A,B,C).Ethanol based dentin bonding agent(Group B)is found to be better (p value <0.01) even with saliva and blood contamination . (Graph 6, 7, 8).

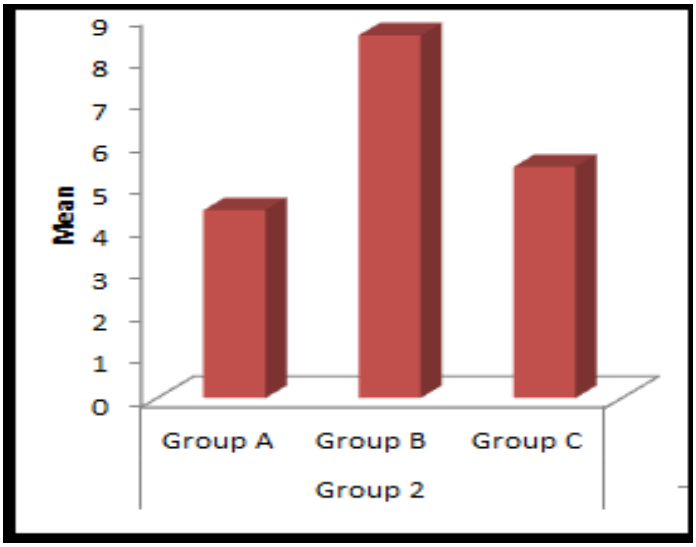
Graph 1



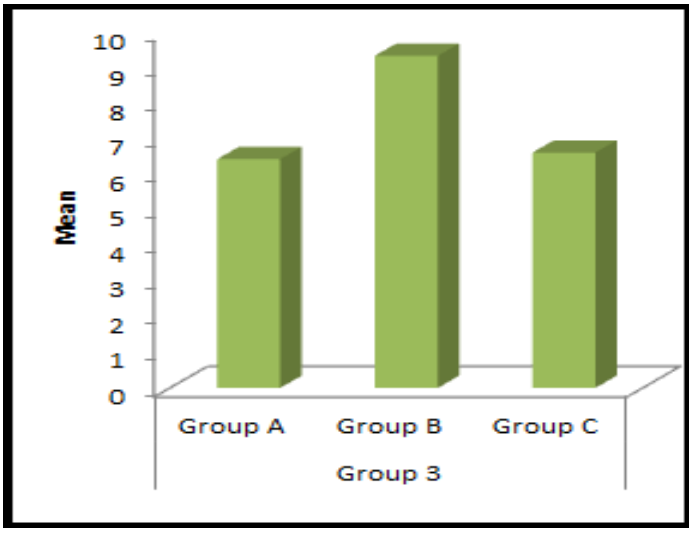
Graph 2



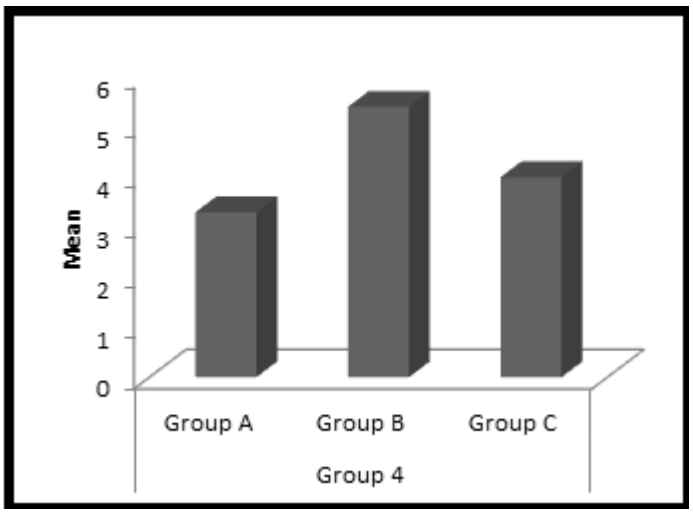
Graph 3



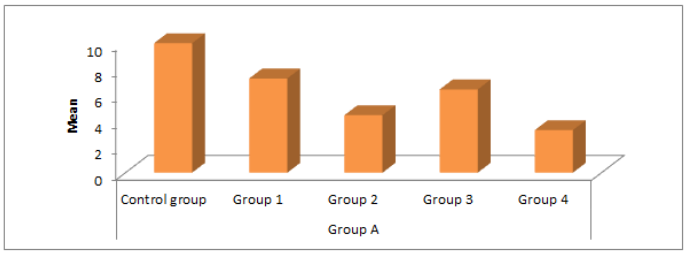
Graph 4



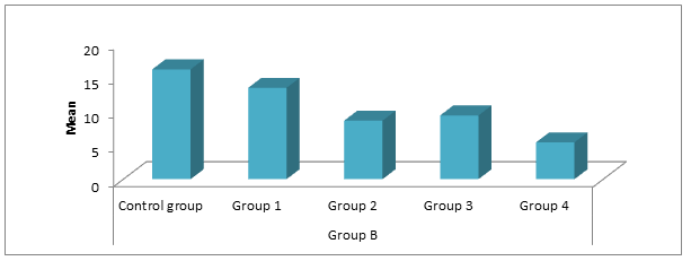
Graph 5



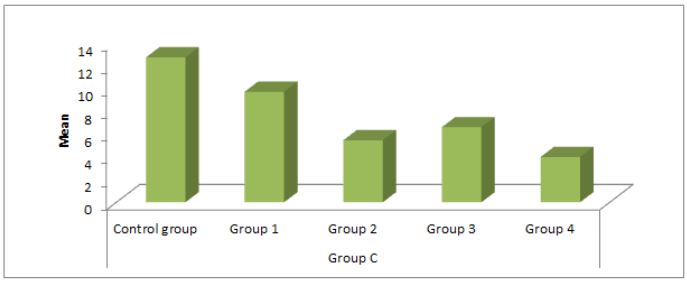
Graph 6



Graph 7



Graph 8



Discussion

Dentin bonding agents, along with other dental materials, are sensitive to moisture and blood contamination. There is of course a dilemma for a dentist to form a blood or moisture free surface before bonding procedures when

contamination is inevitable since contamination with blood or moisture reduces bond strength of the adhesive to tooth structure.[24]

The laboratory parameter most often measured in dentin adhesion is shear bond strength. Flat dentin surfaces are prepared in the extracted tooth, the adhesive system is applied and the composite resin cylinder is bonded over the adhesive. A shear force is then applied at the resin-dentine interface, using a knife-edge probe. The shear bond strength test is only a rough tool for evaluating the relative efficacy of bonding materials. Never the less, they are excellent for screening new materials and for comparing the same parameter among different adhesive systems.[3]

In the present study three different bonding agents based on different solvents present ie,Prime and Bond NT(acetone based),Exite F (ethanol based) Tetric N bond(water based) are used and the shear bond strength were calculated. Exite F shows higher bond strength compared to Tetric N bond(self etch).Prime and Bond NT shows lesser bond strength among the three.

For hydrophilic bonding systems, dentin bonding is optimized in the presence of high-vapour pressure organic solvents. These solvents facilitate deeper and more complete penetration of monomers into dentin to enhance micromechanical retention. It has been reported that to avoid the collapse of the exposed collagen network, adopting a moist bonding protocol is extremely important in acetone-based bonding systems due to the water-chaser effect of acetone .On the other hand, the excess of water can dramatically affect acetone-based shear bond strength to dentin.[7] Acetone based solvents are more sensitive to lack of moisture, because its components evaporate very easily and, in the absence of moisture, without provide adequate diffusion of monomers or even promotes dentin dehydration. When placed in the wet demineralized dentin, acetone is mixed with the waste water causing the diffusion of resin monomers in the space previously occupied by water.[11]

Exite contains hydroxyethylmethaacrylates (HEMA), dimethacrylates, phosphoric acid acrylates ,highly dispersed silicone dioxide, initiators and stabilizers in alcohol solution which reacts quickly and ensures long term stability. Several studies reported that 2-HEMA monomer when applied to conditioned dentinal surfaces enhances the bond strength. Exite being based on alcohol solvent ,its application is more technique tolerent as it is less volatile than acetone and is not as greatly affected by the degree of dentin moisture .Acetone may lead to excessive dehydration of dentine and it evaporates quickly. Acetone is more volatile than ethanol because acetone has vapour pressure 200mmHg at 25^0C where else ethanol has 54.1mmHg. Exite being acetone free solvent represents a good compromise between water and highly volatile solvent , such as acetone. Prime and Bond NT is acetone based adhesive and contains Di and Trimethacrylate resin ,PENTA, Nanofillers, Amorphous silicon dioxide photo initiators,stabilizers, cetylamine hydrofluride. It requires a moist dentin surface to produce adequate bonding it acts as a water chaser and help diffusion of primer into dentin and substrate . It being sensitive to amount of water on the dentin surface ,every a small amount of drying may have a significant role in reducing the bond strength .[15]Tetric N bond contains Bis acrylamide derivatives, bismethacrylamide dihydrogen phosphate,amino acid acrylamide,hydroxyl alkyl methacrylamide nanofiller (sio_2), initiators, stabilizers and water.

Modern dentin bonding agents contain hydrophilic monomers as primers along with a solvent such as acetone or ethanol and an adhesive resin. However, the total etch technique is highly technique sensitive. Ethanol and

acetone act as a carrier and water chaser, delivering the functional monomer into the hybrid layer. Since the vapour pressure (at 27°C) for acetone is 200 mmHg, as compared with 54.1 mmHg for ethanol, acetone is more volatile than ethanol. When acetone-based adhesives are applied to an etched or wet substrate, acetone and water evaporate, leaving resin monomer that covers the exposed collagen network. Ethanol works in a similar manner but it has a lesser capacity for dissolving monomer and a lower vapour pressure compared to acetone. The other reason for the low bond strength on using acetone-based adhesives can be due to the high percentage of acetone (70%), which may not permit the formation of a uniform film on the dentin surface. This is the reason why Prime and Bond NT gives significantly lower bond strength when applied on the dentinal surface than the ethanol-based bonding agents.[36]. When acetone primer contacts with water on dentin. The boiling point of acetone is raised and the boiling point of water is lowered, a process called as 'azeotrophism', causing evaporation of acetone and water and resin is left behind.[19]

- Evidence indicates that the demineralized, denatured collagen fibrils swell after application of the water-containing primers of these products[24]. One study indicates that longer application times for these primers increase the resulting shear bond strength, presumably because the primers more effectively rehydrate the dentin[42].

In the present study dentin substrate are subjected to saliva and blood contamination before and after application of 3 dentin bonding agents and it was found that with all the three dentin bonding agents the contamination with blood and saliva decreases shear bond strength.

The penetration of salivary proteins into the dentin tubules is favourable to bonding success after contamination.

Reduction in the shear bond strength values when contaminated with saliva after light curing of the adhesive. This could be due to the adsorption of glycoprotein onto the poorly polymerized adhesive surface, which results in oxygen inhibition[13].

Dental surface contamination with blood can also occur at two critical times during the bonding procedure: after the tooth surface had been etched and/or after the adhesive system had been applied. As a result, bonding can be compromised at both times. In the present study also the shear bond strength with contamination with blood is also decreased on before and after bonding agent application decrease on shear bond strength[28]

When blood contamination occurred before the adhesive system application. Blood residues or reactants obstructing the dentin tubules can inhibit primer infiltration into dentin thus interfering with hybrid layer formation. Blood contaminants on dentin surface and also caused collagen fibrils to collapse, preventing adhesive monomers to infiltrate leading to reduced bond strength. The blood protein components trapped on the dentin surface interferes with the bonding agent ability to form a uniform surface coating[22]

When blood contamination occurs after adhesive application the interaction between adhesive system and resin can be jeopardized. [21]

The results from the present study clearly indicated that, when the dentin surface was contaminated either before or after application of the adhesive system, reduces the shear bond strength compared to control group without contamination. It is likely that contaminants may have remained on the dentin surface, thus interfering with the formation of a hybrid layer or inhibiting the bonding of adhesive system to resin cement. Therefore, the most important factor for ensuring optimal bonding is to avoid saliva and blood contamination.

Conclusion

Within the limitations of this in-vitro study, it could be concluded that:

Ethanol based dentin bonding agents have higher shear bond strength compared to water and acetone based dentin bonding agents.

Saliva and blood contamination reduces the shear bond strength, and this reduction in bond strength will be more in case of contamination after bonding agent application than before.

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