

Effect of Saliva/Blood Contamination on Enamel Bond Strength

Efeito da Contaminação de Saliva/Sangue na Força de União ao Esmalte

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Abstract

In the routine clinical situation, the contamination by blood and/or saliva in restorative procedures can be happen in non-cooperation of the patient in dental office. The aim of the study was to assess in vitro shear bond strength of a resin sealant associated with two types of adhesives contaminated with saliva and blood. Healthy human molars were used and the specimens and the crowns were sectioned in the bucco-lingual direction, thus obtaining two segments of similar proportions (mesial and distal), totaling 60 surfaces, and the surfaces were randomly divided into 4 groups (n = 15). Group I (control) received no type of contamination and the sealant was applied. In group II, the surfaces were contaminated with 10 µl of saliva/blood and the sealant was applied. In group III, the surfaces were contaminated with 10 µl of saliva/blood and the Single Bond total-etch adhesive system was applied followed by application of sealant. In group IV, the surfaces were contaminated with 10 µl of saliva/blood and the Prime & Bond NT total-etch adhesive system was applied followed by the application of sealant. Samples were tested in the universal testing machine and the analysis of shear bond strength was performed. A difference between Group I (12.61MPa) and the other groups was found; Group II (2.28MPa) was different than Groups III (7.07MPa) and IV (7.79MPa), but Groups III and IV were similar. The application of an adhesive system when there is contamination with saliva/blood is required prior to application of pit and fissure sealants.

Keywords: Pit and Fissure Sealants. Biological Contamination. Shear Strength.

Resumo

Na situação clínica de rotina, a contaminação por sangue e/ou saliva em procedimentos restauradores pode ocorrer em pacientes que não colaboram no consultório odontológico. O objetivo do estudo foi avaliar a resistência ao cisalhamento in vitro de um selante de resina associado a dois tipos de adesivos contaminados com saliva e sangue. Foram utilizados molares humanos saudáveis e os espécimes e as coroas foram seccionados na direção bucal-lingual, obtendo assim dois segmentos de proporções semelhantes (mesial e distal), totalizando 60 superfícies, e as superfícies foram divididas aleatoriamente em 4 grupos (n = 15). O Grupo I (controle) não recebeu nenhum tipo de contaminação e o selante foi aplicado. No grupo II, as superfícies foram contaminadas com 10 µl de saliva / sangue e o selante foi aplicado. No grupo III, as superfícies foram contaminadas com 10 µl de saliva / sangue e o sistema adesivo Single-Bond foi aplicado seguindo a aplicação de selante. No grupo IV, as superfícies foram contaminadas com 10 µl de saliva / sangue e o sistema adesivo de ataque total Prime & Bond NT foi aplicado seguindo da aplicação de vedante. As amostras foram testadas na máquina de ensaio universal e a análise da resistência à ligação ao cisalhamento foi realizada. Uma diferença entre o Grupo I (12,61MPa) e os outros grupos foi encontrada; O Grupo II (2,28 MPa) foi diferente dos Grupos III (7,07MPa) e IV (7,79 MPa), mas os Grupos III e IV foram semelhantes. A aplicação de um sistema adesivo quando existe contaminação com saliva / sangue é necessária antes da aplicação de selantes de fissura e fissura..

Palavras-chave: Selantes de Fossas e Fissuras. Contaminação Biológica. Resistência ao Cisalhamento.

1 Introduction

The development of adhesive systems completely changed the clinical dentistry practice. From this it was possible to add to the restorative procedure (curative or preventive) several advantages, such as preservation of tooth structure and marginal seal of composite resin restorations located in enamel.¹ This last advantage can add benefits to the use of occlusal sealants, which act as a physical barrier to the deterioration of tooth structure, and that this property is closely linked to sealant's ability to adhere to the tooth surface.² In vitro studies have demonstrated that the use of prior adhesive systems to reduce the use of sealant microleakage, improved resistance to shear and increase the vertical penetration of the

sealant thereby reducing the risk of loss of the material and increasing success rates.³

Furthermore, there is another possible advantage of using adhesives with concomitant sealants, since this technique is subject to possible contamination with saliva and / or blood depending on the situation and, in such cases, the use of the adhesive sealant act as an intermediary increasing the binding force between the enamel and the sealant.⁴ Contamination with saliva, moisture, or blood of the etched surface prior to the application of the sealant has been cited as a common clinical problem that contributes to the failure of the technique, thus preventing the proper formation of resin tags and consequently decreasing sealant adhesion.⁵ Contamination with blood

or saliva is almost inevitable in pediatric patients, in whom absolute isolation is difficult to attain, especially in early-aged children, special patients, and young children with newly erupted teeth.⁶ It has been demonstrated that the combination of hydrophilic adhesive systems and sealants decrease the effects of saliva contamination of the etched enamel surface.⁷

There are some considerations regarding adhesives and sealants increased retention against contamination by saliva and/or blood, for example, the type of solvent used in the adhesive (ethanol or acetone) and its chemical composition.⁸ The type of solvent is an important factor affecting the main properties of dentin bonding.⁸ Various properties such as bond strength, penetration capacity, binding efficiency in the presence of residues and humidity are significantly affected by the type of solvent.⁹ The ethanol present in some adhesives is that when an organic solvent replaces the water after etching has shown better results when the immediate and long-term adhesion.¹⁰ Furthermore, these adhesives in the presence of ethanol have been considered as potential candidates to be tested as decontaminants after blood contamination carried out after the acid attack.⁸ Another type of solvent used in adhesive systems is acetone, which promises to increase the compatibility with the moisture through their hydrophilic character and enhance the penetration capacity of the material in the dental structure.¹¹ Furthermore, the presence of acetone promotes a lower pH (pH = 2.3) when compared with those adhesives with ethanolic solvent, which directly affects the bond strength making it the largest.⁸

Therefore, the aim of the present study was to investigate the influence of contamination with saliva/blood on the bond strength of sealants associated with total-etching systems (ethanol or acetone solvents). The null hypothesis was that there would be significant differences in the shear bond strength when the both adhesive system was used with the

sealant conditions of saliva/blood contamination.

2 Material and Methods

The samples were prepared from healthy human molars, caries-free teeth obtained from the Biobank of teeth. The teeth were cleaned with periodontal curettes, polished with Robinson brushes, mounted on a low-speed micromotor, imbibed in water and pumice stone, washed, and then kept at 4 °C in 0.9% saline solution containing an acid solution of 0.4% sodium for 24 hours.

The roots of the teeth were sectioned 3 mm below the cemento enamel junction and the crowns were sectioned in the bucco-lingual direction (Miniton, Struers A/S, Copenhagen, DK-2610, Denmark), thus obtaining two segments of similar proportions (mesial and distal), totaling 60 surfaces. These surfaces were secured to a wax lamina and placed inside PVC rings (Plexiglass®) measuring 1 inch and 1cm in height, which were fixed with epoxy resin (Strues, A/S, Copenhagen, Denmark). Then the test specimens were polished with 600-grit SiC paper (Buehler Ltd. Lake Bluff, IL, USA) under running water. The specimens were then rinsed under tap water and stored in distilled water at 4 °C for 24 hours to facilitate re-moistening of tooth enamel.

The enamel surface area was delimited with the aid of adhesive tape with a 3-mm-diameter central hole punched with a modified rubber dam punch. Then, the specimens were randomly divided into 4 groups (n = 15).

Blood and saliva used as contaminant agents were obtained from one of the researchers. Two experimental groups corresponding to the two etch-and-rinse adhesives used: acetone-based adhesive system Prime & Bond NT (Dentsply Detrey, Konstanz, Germany) and water/ethanol based adhesive system Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA) (Table 1).

Table 1 - Materials used in the study

Materials	Manufacturer	Composition [batch no.]	Technique
Prime & Bond NT	Dentsply Detrey, Konstanz, Germany	Di- and trimethacrylate resins, PENTA (dipentaerythritol penta acrylate monophosphate), nanofillers amorphous silicon dioxide, photoinitiators, stabilizers, cetylamine hydrofluoride; acetone [464890D]	Etch for 15 seconds, rinse, blot dry, apply generous amount of adhesive and allow it to stay 20 seconds. Remove excess solvent by gentle air drying for 5 seconds.
Adper Single Bond 2	3M ESPE, St. Paul, MN, USA	Bis-GMA, HEMA, dimethacrylates, silica nanofillers, polyalkenoic acid copolymer, initiators, water, ethanol [N195722BR]	Etch for 15 seconds, rinse; blot, dry, apply 2 consecutive coats of adhesive for 15 seconds with gentle agitation gentle air dry for 5 seconds
Fluroshied sealant	Dentsply/Caulk, Milford, DE, USA	NCO Monomer, Nupol Bis GMA, TEGDMA, Penta, N-methyl Diethylamine, BHT, 2_n methacrylate, camphorquinone, Cervit T 1000, Silanized Barium, sodium fluoride, Cabosil TS 720 and Titanox 3328. [425788C]	Apply Tooth Conditioner Gel over the entire occlusal surface and leave on for 30 to 60 seconds. Remove the Tooth Conditioner Gel with the help of a high power saliva ejector and a forceful spray of water / air for at least 15 seconds and dry with air. Dispense the sealant directly on dental surface .Light-cure each sealed surface for 20 seconds.

Source: Research data.

In the control group (GI) (Figure 1), the Fluroshield sealant (Dentsply/Caulk, Milford, DE, USA) was applied after acid etching with 37% phosphoric acid for 15 seconds, followed by rinsing and drying for 15 seconds, with no previous contamination of enamel. This group was not contaminated with blood.

In group II (GII), the surfaces were contaminated with 10 µl of saliva/blood (blood/saliva samples used as the contaminant agent were obtained from one of the authors at the same site and time as the specimens were made) for 10 seconds to simulate unnoticed contamination with the aid of a micropipette. After contamination, enamel was carefully dried with the aid of absorbent paper for 5 seconds in a way that the enamel surface remained slightly damp. The Fluroshield sealant was then applied without association of an adhesive system, as described in GI. In Group III (GIII), the surfaces were contaminated with 10 µl of saliva/blood for 10 seconds. After contamination, enamel was carefully dried with the aid of absorbent paper for 5 seconds in a way that the enamel surface remained slightly damp. The total-etch adhesive system Single Bond (Adper Single Bond 2; 3M ESPE, St. Paul, MN, USA) was applied in accordance with the manufacturer's instructions with the aid of a disposable applicator tip (Microbrush-Microbrush Corporation, Orlando, FL 32837, USA) and light cured for 10 seconds with a halogen light source (XL 3000, 3M/ESPE St Paul, MN 55144, USA) at a power level of 540mW/cm², and the sealant application protocol was performed as in GI.

In Group IV (GIV), the surfaces were contaminated with 10 µl of saliva/blood for 10 seconds. After contamination, enamel was carefully dried with the aid of absorbent paper for 5 seconds in a way that the enamel surface remained slightly damp. The total-etch adhesive system Prime&Bond NT (Prime & Bond 2.1; Dentsply/Caulk) was applied in accordance with the manufacturer's instructions with the aid of a disposable applicator tip for 10 seconds and light cured with a halogen light source at a power level of 540mW/cm², and the sealant was applied for 20 seconds as in the other groups.

In both groups, the adhesive system was applied to the delimited enamel surface with the aid a microbush with disposable bristle brush tips to prevent excess and pooling of adhesive along the edges of the adhesive tape, which could compromise stress distribution during testing and therefore the validity of the results.

The specimens were stored in distilled water at 37 °C for 24 hours and shear bond strength testing was performed, in which the samples were placed in a universal testing machine (Mod. MEM 2000; EMIC Ltda, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min and with a 50 kgf load cell.

The debonded specimens were observed under a stereomicroscope (Nikon, Melville, NY, USA) at 40X magnification to assess failure modes, which were classified as adhesive, cohesive or mixed. All examinations were

performed by a single examiner blinded to the groups.

The means (in MPa) and standard deviations were calculated, and data were analyzed statistically by two-way ANOVA. The Duncan test was used for multiple comparisons at a 5% significance level. Statistical analysis was performed with SPSS software for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA).

3 Results and Discussion

In dentistry, particularly in pediatric dentistry, accidental contamination of the operative field with saliva and/or blood is still a problem.³ In many clinical situations, the rubber dam can be difficult to place and contamination may occur, requiring the use an adhesive system for effective bonding even in the presence of contamination.¹²

Retention of resin sealants occurs by means of a micromechanical process established by infiltration and subsequent light curing of the sealant inside the microporosities of the enamel surface created by acid etching, forming the so-called resin tags.¹³ Macro-tags fill the spaces around each apatite crystal, while numerous micro-tags result from the infiltration/polymerization of resin inside the crystals. It is believed that these tags are especially important in providing effective retention of resin to enamel.¹³

Due to the high reactivity of etched enamel, it has been described in the literature that only one second of saliva contamination of the substrate is sufficient to form a film that blocks the micropores,⁶ causing an ultrastructural change in the morphology of the etched enamel⁵ and preventing the formation of resin tags responsible for the mechanical bonding to the tooth substrate.⁵ Therefore, when the formation of resin tags is impossible as a result of inadvertent contact with moisture, saliva, blood, among others during the application procedure of pit and fissure sealants, lower bonding values to enamel are observed.⁴

There are a limited number of studies on the effect of blood contamination, but a direct comparison among studies cannot be performed adequately because of the differences in adhesive systems, times of contamination, type of substrate, type of blood [fresh or anticoagulated), and outcome variables that considerably differ among studies.^{3,14} However, some studies have shown that contamination with blood influences the bonding strength of mineralized tissues,³ but the best way of adequately dealing with contamination remains unanswered. The type of adhesive system used in some studies appears as a decisive element in the adhesive property when it comes to blood contamination. Previous studies have evaluated the effect of using self-adhesive etch against challenge blood contamination on dentin and found that in most cases, the act of washing the place again and reapply the adhesive was sufficient to maintain adhesion.¹⁵ But when it comes to total-etch adhesives, such as those used in this study, with the blood contamination after the collagen fibers are exposed to acid with the application of the adhesive, a new application

of the product would not be able to restore compliance,⁶ it is suggested by some authors the need for an additional step for decontaminating.⁸

Blood contamination is a major clinical problem, which negatively affects the success of adhesive restorations.⁸ Surprisingly, little attention has been paid by researchers to contamination with blood, so there is considerable heterogeneity regarding the type of blood used in contamination studies. While some have used drawn recently blood,¹⁶ others use anticoagulated blood¹⁷ or plasma,¹⁴ while others do not specify the type of blood used in their publications.¹⁸

Based on these clinical difficulties, studies have been developed and they have found that bond strength between the resin sealant and contaminated surface is drastically decreased,¹⁹ which results in complete or partial displacement of sealant in the short term. These results were confirmed by the present study in which we observed a significant reduction in bond strength of the sealant to contaminated enamel with saliva/blood. Thus, the present study the null hypothesis was rejected.

The shear bond strength values for the different groups are shown in Table 2. The results suggest that shear bond strength was significantly influenced by contamination. All the adhesive groups (GIII and GIV) showed lower bond strength than control, but no statistically significant differences were found among the adhesive groups. The groups contaminated (GII) showed significant differences from the other groups (P0.03).

Table 2 - Means and standard deviation (MPa) of shear bond strength

Groups	Mean (Standard Deviation)
Control	12.61 (3.00) ^a
Contaminated	2.28 (3.49) ^b
Contaminated + Single Bond	7.07 (2.13) ^c
Contaminated + Primer Bond	7.79 (3.04)^c

Source: Research data.

The application of an intermediate layer of adhesive prior to the sealant has been suggested to minimize the undesirable contamination effects on the bond strength of adhesives to enamel.³ According to the results obtained in this study, the application of the total-etch adhesive system prior to the sealant application, both Adper Single Bond and Prime & Bond increased shear bond strength values after contamination, with no statistical difference among the adhesive systems (GIII - 7.07 MPa; GIV -7.79 MPa). Even Single Bond Total etch presenting in its composition the polyalkenoic acid would promote a better resistance to the deteriorating effect of moisture, possibly being more efficient in clinical procedures at great risk of saliva contamination,²⁰ in our studies that did not show up higher the Prime & Bond 2.1. This could be because the acetone features that allowed the Prime & Bond 2.1, possibly better dissemination, and greater water displacement capacity.

Percentage of fracture types: Group I: Adhesive – 6.5%; Cohesive – 33.5%; Mixed –60%; Group II: Adhesive – 100%; Group III: Adhesive – 86.5%; Cohesive – 13.5%; Group IV: Adhesive – 60%; Cohesive – 33.5%; Mixed – 6.5%. The analysis of fracture types after shear bond strength testing indicated the predominance of adhesive failures in the specimens that were contaminated and cohesive in the control group.

The increase in bond strength may be related to the ability of the adhesives to penetrate micro-spaces created by acid etching, i.e., the high diffusion coefficient of monomers.¹⁶ Furthermore, the composition of adhesives seems to be particularly suitable for the bonding to contaminated enamel. Due to the hydrophilic nature of adhesives, they can remove saliva/blood (moisture) from the enamel surface, which allows infiltration of the hydrophilic monomer in the enamel pores.²¹ Therefore, the hydrophilic monomers in the adhesives can contribute to increase surface wetting and penetration of resin,²¹ irrespective of the type solvent used, alcohol or acetone, which does not occur when there is contamination with compressor oil, in which case only acetone can remove the oil.²²

Koppolu et al.²³ also found that the application of an adhesive system on the enamel surface contaminated with saliva and blood presented comparatively higher bond strength values than the contaminated surface without an adhesive system, but lower than in the absence of contamination. It is known that adhesives containing solvents such as ethanol and acetone can denature glycoproteins (sugars) and remove salivary contamination²⁴ and thus contribute to better bonding.²³ Another explanation for the result is that the adhesive system cleans or hydrolyzes blood on the enamel surface.²⁵ The present study found a reduction in the shear bond strength values when there was contamination with saliva and blood, but using adhesive system increases shear bond strength.

4 Conclusion

It can be concluded, based on the applied methodology the application of an adhesive system when there is contamination with saliva/blood is required prior to application of pit and fissure sealants.

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