Effect of saliva contamination on the bond strength of an etch-and-rinse adhesive system to dentin

Efeito da contaminação salivar na resistência de união de um adesivo *etch-and-rinse* em dentina

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Abstract

Purpose: The aim of this study was to investigate the effect of saliva contamination on bond strength of an etch-and-rinse system to dentin.

Methods: Fifty bovine incisors were embedded in acrylic resin and divided into 5 groups: G1 (control) – application of the adhesive system (Adper Single Bond 2 – 3M-ESPE); G2 – saliva contamination after acid etching of dentin, rinsing and drying; G3 – saliva contamination after acid etching of dentin and drying; G4 – saliva contamination after adhesive application, rinsing and drying; G5 – saliva contamination after adhesive application and drying. Contamination was performed by using 4 μL of simulated human saliva for 20 s. The adhesive system was applied according to the manufacturer's instructions; a composite resin was built as an inverted cone and was tested after 24 h at a cross-head speed of 0.5 mm/min.

Results: When saliva contamination occurred after the adhesive photo-polymerization, bond strength was significantly reduced. The adhesive strength (MPa) mean values were: G1 = 18.1(±4.7) a; G2 = 20.5(±5.7) a; G3 = 17.3(±3.4) a; G4 = 12.6(±4.0) b; G5 = 9.8(±2.1) b (means followed by distinct letters are statistically different, $P < 0.05$).

Conclusion: Saliva contamination negatively influenced bond strength of an etch-and-rinse adhesive, especially after the final polymerization of the adhesive system; in this condition, treatments were not efficient to recover adhesion.

Key words: Bond strength; adhesion; saliva contamination

Resumo

Objetivo: Investigar o efeito da contaminação salivar na resistência de união de um adesivo condicione-e-lave em dentina.

Metodologia: Cinquenta incisivos bovinos foram divididos em 5 grupos: G1 = (controle) aplicação do sistema adesivo (Adper Single Bond 2 – 3M-ESPE); G2 = contaminação com saliva após condicionamento ácido da dentina + lavagem e secagem; G3 = contaminação após o condicionamento ácido da dentina + secagem; G4 = contaminação com saliva após a aplicação do adesivo + lavagem e secagem; G5 = contaminação com saliva após a aplicação do adesivo + secagem. A contaminação foi realizada com 4 μL de saliva humana estimulada por 20 s. O sistema adesivo foi usado de acordo com as instruções do fabricante. A resina composta foi aplicada na forma de cone invertido, com o teste de tração realizado após 24 h a 0,5 mm/min de velocidade.

Resultados: As médias de resistência de união (em MPa): G1 = 18,1(±4,7) a; G2 = 20,5(±5,7) a; G3 = 17,3(±3,4) a; G4 = 12,6(±4,0) b; G5 = 9,8(±2,1) b, demonstrando que a resistência de união foi reduzida significativamente quando a contaminação salivar ocorreu após a fotopolimerização do adesivo.

Conclusão: A contaminação influenciou negativamente a resistência de união do adesivo somente após a sua polimerização; nesta condição os tratamentos realizados não foram eficientes para recuperar a adesão.

Palavras-chave: Resistência de união; adesão; contaminação

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Introduction

Contamination of the operatory field is one of the major clinical problems that occur during restorative dental treatment (1). The presence of saliva, blood, intrasulcular fluid, and handpiece oil during the bonding procedure may contribute to short-term failure of composite resin restorations (1-3).

The influence of saliva contamination on the retention of brackets (4) and sealants (5) has been extensively investigated. However, studies that investigate the relationship between saliva contamination and the effectiveness of adhesive systems used during the insertion of adhesive-based restorations are currently in need of updating. Each time a new adhesive material becomes commercially available, clinical professionals need to update their knowledge and understanding of factors that influence its performance in order to achieve good restoration results.

In Dentistry, the rubber dam is the most successful method of isolating the operatory field (6); however, only 10 to 17% of dental professionals report using it in daily clinic (7). This is likely due to the fact that use of the rubber dam is a time-consuming procedure; some clinicians also consider it a difficult device to manipulate (8). Furthermore, it is essential to point out that in some clinical situations, such as cavity preparations with subgingival margins, incomplete crown eruption, and in cases where children and special care patients are involved, the use of a rubber dam is not feasible (2). In these cases, the probability of contamination during adhesive application is directly related to the chairside clinical time.

'Etch-and-rinse' adhesive systems are well accepted by clinicians. In this type of system, several steps are required in order to obtain optimum bonding to the dentin substrate. The steps include a separate conditioning phase involving an acid (most frequently 30-40% phosphoric acid), which is applied and rinsed off. This conditioning step is followed by a priming step and the application of adhesive resin, resulting in a three-step application procedure. Single-bottle etch-and-rinse adhesives combine the primer and adhesive resin into one application, but still require acid etching as a separate step. The more steps the adhesive system requires, the greater the opportunity of contamination of the operatory field; thus, the lack of use of the rubber dam is even more detrimental.

In relation to saliva contamination, it was hypothesized that the presence of salivary glycoprotein decreases dentinal permeability up to 65% (9), leading one to suppose that adhesion would be impaired in the presence of saliva (10). However, controversial results have been reported. While some studies using etch-and-rinse adhesives reported that saliva contamination reduces bond strength (2,11,12), other authors observed that the presence of saliva did not influence the adhesion process (6,8,13,14). It therefore seems that investigations that elucidate the effect of saliva contamination on dentin bond strength of commercially available etch-and-rinse adhesive systems would provide practical knowledge of great use to dental professionals.

The aim of this study was to investigate the effect of saliva contamination on bond strength to dentin of an etch-and-rinse system, varying the step of the bonding procedure when contamination occurred (after dentin etching and after adhesive system application) and whether the contamination treatment occurred before or after rinsing was performed.

Methods

A single bottle of commercial etch-and-rinse adhesive system (Adper Single Bond 2, 3M ESPE, St Paul, MN, USA) (SB) and a resin composite (Filtek Z250, 3M ESPE, St Paul, MN, USA) were used in this study. A total of 50 incisors extracted from 2-3-year-old cattle and stored for up to 30 days were used as a substitute for human teeth. After removing the roots using a slow- speed saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA), the buccal surfaces of the bovine teeth were embedded. Specimens were abraded on the labial surface to remove at least 2 mm of tooth structure to expose dentin surfaces on which the bond strength test was performed.

A flat bonding dentin surface was prepared on the labial surface by wet grinding with 400-grit SiC paper discs to expose a 3 mm diameter area of dentin to accommodate the bonding material. The final finishing was accomplished by grinding on a wet 600-grit SiC paper disc. Teeth were randomly divided into five groups (n = 10) as described in Table 1, according to the following variables: step of the bonding procedure when contamination occurred [none (N), after dentin etching (AE), after adhesive system application (AA)] and contamination treatment [none (N), rinse + dry (RD) and dry (D)].

In the specimens of contaminated groups, 4 µL of stimulated human saliva was applied using a disposable micropipette and left undisturbed for 20 seconds (2,15). Following saliva contamination, the surface was either rinsed and dried (RD – the dentin surface was rinsed with air-water spray for 20 seconds followed by drying with oil-free air spray) (14), or dried (D – the dentin surface was dried with oil-free air for 10 seconds).

Table 1. Experimental groups (n = 10).

Step of the bonding procedure when contamination occurred	Contamination Treatment		
	None (N)	Rinse + Dry (RD)	Dry (D)
None (control group) (N)	G1-NN	–	–
After dentin etching (AE)	–	G2-AERD	G3-AED
After adhesive system application (AB)	–	G4-ABRD	G5-ABD

Phosphoric acid (35%) was used on all experimental groups; it was applied to the dentin for 15 seconds, washed for 15 seconds, and dried gently. Two coats of SB, with an air blast applied between, were applied to damp dentin as recommended by the manufacturer. Standard damp dentin was obtained by the application of 1.5 μL of distilled water, as recommended by some authors (16-18).

Composite resin was then placed in a polytetrafluoroethylene mold in the shape of an inverted truncated cone with a diameter of 3 mm at the bond interface as described by Barakat and Powers (19). Incremental insertion of composite resin (Z-250, 3M/ESPE, St Paul, MN, USA) was cured for 20 seconds using a light-curing unit (Astralis 3 – Ivoclar Vivadent) with 600 mW/cm^2 of power as measured by a light meter (Curing Radiometer, Demetron Research Corporation, Danbury, CT, USA).

Specimens were stored in distilled water for 24 hours at 37ºC and debonded in tension on a universal testing machine (Model 4440, Instron Corp., Canton, MA, USA) at a cross-head speed of 0.5 mm/min using a fixture described by Barakat and Powers (19). Bond strength values were expressed in megapascal (MPa). Fracture sites were categorized according to the type of failure (Adhesive/Cohesive Resin/Mixed) under a dissecting microscope at 40X magnification (JSZH-131, Olympus Ltd., Tokyo, Japan).

Means and standard deviations of bond strength were calculated from 10 replications for each condition. Analysis of variance and comparison of means by Tukey's test at the 0.05 significance level were performed using a commercially available statistical software (Minitab Inc. 14, State College, Pennsylvania, USA).

Results

The bond strength values obtained are presented in megapascal (MPa). The descriptive data for each experimental group (n=10) are presented in Table 2.

One-way ANOVA showed statistically significant differences between the experimental groups ($F = 10.45$, $P < 0.001$) when the contamination-free group (control group) was compared to the groups in which saliva contamination occurred after adhesive resin application (G4 and G5). In this bonding step, neither of the post-contamination treatments (RD or D) counteracted the negative effects of saliva contamination.

Discussion

In this study, contamination with saliva after etching dentin for a restorative procedure did not affect bond strength of the etch-and-rinse adhesive used, independent of the manner in which the salivary contaminant was treated. Rinsing and drying or merely drying the contaminant resulted in bond strength similar to that found in the control group, in agreement with other studies (2,8,14). However, Pashley et al. (20) and Fritz et al. (12) observed a reduction in bond strength when etch-and-rinse adhesive was contaminated by water, saliva or blood; they attributed the lessening of adhesion to the presence of salivary proteins occluding the openings of dentin tubules (2,12,14,20). It is important to point out that some studies (18) are performed with an acetone-based solvent adhesive system, which could be responsible for the distinct results.

The etch-and-rinse adhesive system used in this study requires wet dentin (2,21,22). Taking this into consideration, we attribute our findings to the humidity of saliva or to the water used in the rinsing step that may have wet dentin, preventing the collagen fiber network from collapsing and obviating a strong influence of saliva contamination on the bond strength of this adhesive system to dentin (1,6,14, 23-25). In order to clarify our result, the relationship between the amount of saliva and the dimensions of the dentin perhaps should be investigated. Another question also arises from our findings: would we obtain no interference with bonding if we used an adhesive system that requires dry dentin to achieve good adhesion? Contamination studies will always be necessary every time a new adhesive system is developed and available for clinicians.

Our findings revealed a great influence of saliva contamination on the bond strength of specimens that were contaminated after photopolymerization of the adhesive system, similar to the findings of Fritz et al. (12). It is important to point out that even when the polymerized layer of adhesive was subjected to the rinse and dry procedure, bond strength was not recovered. That fact could be attributed to the deposition of salivary glycoprotein over the superficial layer of the adhesive, which may have acted as a physical barrier lessening the effective interaction between the composite resin and the dentin and blocking complete copolymerization (12). Like our results obtained with contamination that occurred after etching, other studies in the literature found no effects of saliva contamination that occurred after the polymerization of the adhesive layer on bond strength (6,8).

Table 2. Descriptive statistics of the experimental groups: Mean (± Standard Deviation)* (in MPa) and [Failure Percent (%) – Adhesive/Cohesive Resin/Mixed].

Step of the bonding procedure when contamination occurred	Contamination Treatment		
	None (N)	Rinse + Dry (RD)	Dry (D)
None (control group) (N)	18.1(±4.7) [a] (40/0/60)	–	–
After dentin etching (AE)	–	20.5(±5.7) [a] (30/10/60)	17.3(±3.4) [a] (90/10)
After adhesive system application (AB)	–	12.6(±4.0) [b] (70/30)	9.8(±2,1) [b] (60/40)

* Means followed by distinct letters are statistically different ($P < 0.05$).

Although reduction in bond strength was not observed in some of the experimental conditions that were tested, the use of a rubber dam when adhesive restorations are performed is still recommended. We consider that, besides avoiding contamination of the operatory field, use of a rubber dam facilitates clinical procedures and also provides other advantages, such as better visualization of the operatory field and reduction of the risk of ingestion of products or materials by the patient.

Based on the results presented here, we believe that contamination of the operatory field with saliva during restorative procedures is deleterious to adhesion. We suggest further investigation of this subject, focusing on the long-term effects of saliva contamination on bond strength. There is also a need for further investigation to elucidate whether the presence of a contaminant in the adhesive interface can explain previous conflicting results obtained in studies of bond strength.

Conclusions

Saliva contamination of the operatory field has a negative influence on the bond strength of the etch-and-rinse system to dentin, especially when it occurs after the photopolymerization of the adhesive resin. Moreover, in this condition, none of the post-contamination procedures performed were able to recover adhesion to dentin.

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