Effect of blood contamination and decontamination procedures on marginal adaptation and bond strength of composite restorations

Efeito da contaminação com sangue e de procedimentos de descontaminação na adaptação marginal e na resistência de união de restaurações de resina composta

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Abstract

Purpose: To evaluate the effect of blood contamination and different decontamination procedures on marginal adaptation and bond strength of a two-step total-etch adhesive system to dentin.

Methods: A total of 135 bovine incisors had the labial surfaces ground to receive cylindrical cavities, and were randomly divided into a control and 8 experimental groups (n=15) according to contamination and decontamination procedures. Freshly collected human blood was applied onto the cavity either before or after light-curing of the adhesive. Four decontamination protocols were tested (drying with paper, water rinsing, phosphoric acid etching, and 10% NaOCl rinsing). The cavities were restored with Adper Single Bond and Filtek Z250 (3M ESPE). The specimens were subjected to thermal cycling before the dye staining test. The cavity floor was removed and the restorations were subjected to a push-out test. Data were analyzed by two-way ANOVA and Tukey's test (α=0.05).

Results: Blood contamination after adhesive light-curing increased marginal gap and yielded lower push-out bond strength values (P<0.01).

Conclusion: Water rinsing seems to be a reliable procedure for cavity decontamination. The decontamination procedures tested do not recover marginal sealing and bond strength when blood contamination occurs after light-curing of the adhesive.

Key words: Blood contamination; dentin bonding agent; marginal adaptation; push-out test; composite restoration

Resumo

Objetivo: Avaliar o efeito da contaminação com sangue e de procedimentos de descontaminação na adaptação marginal e resistência de união de um adesivo convencional de dois passos à dentina.

Metodologia: Um total de 135 incisivos bovinos receberam cavidades cilíndricas na superfície vestibular, previamente desgastada. Os dentes foram divididos em grupo controle e 8 grupos experimentais (n=15), com base no momento da contaminação e nos procedimentos de descontaminação. Sangue recém-coletado foi aplicado nas cavidades, antes ou após a fotoativação do adesivo. Quatro procedimentos de descontaminação foram testados: secagem com papel, lavagem com água, condicionamento com ácido fosfórico e lavagem com hipoclorito de sódio a 10%. As cavidades foram restauradas com Adper Single Bond e Filtek Z250 (3M ESPE). Os espécimes foram submetidos à termociclagem antes da marcação com corante. O assoalho das cavidades foi removido e as restaurações foram submetidas ao teste de push-out. Os dados foram analisados por two-way ANOVA e teste de Tukey (α=0,05).

Resultados: A contaminação após fotoativação do adesivo gerou fendas marginais maiores e resistência de união menor (P<0,001).

Conclusão: A lavagem com água parece ser um método confiável de descontaminação. Os procedimentos testados não recuperam o selamento marginal e a resistência de união quando a contaminação ocorre após fotoativação do adesivo.

Palavras-chave: Contaminação com sangue; agente de união; adaptação marginal; teste de push-out; restauração de resina composta

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Introduction

During adhesive procedures effective isolation of the operative field is necessary to prevent potential damage to the bond of adhesive restorations. Usually, rubber dam isolation is the standard method because it facilitates visualization and keeps the humidity controlled throughout the restorative procedure. When the isolation of the operative field is not effective, contamination may go unnoticed and can affect the bonding procedure in adhesive restorations. Therefore, achieving good moisture control is a common problem in Restorative Dentistry, especially when rubber dam isolation is not feasible (1). Contamination with either water, saliva, or blood during adhesive procedures impairs the bond effectiveness of dentin adhesive systems (2,3). Restorations are very prone to blood contamination in deep proximal boxes, especially when surgical approaches are required for rubber dam placement.

Blood contamination decreases the bond strength of adhesive materials (4-9) 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). Furthermore, in several clinical conditions the contamination could occur after the adhesive application. In order to avoid negative effects on dental bonding, practitioners commonly need to decide between two clinical alternatives: to execute all the adhesive steps again or use decontamination procedures, which is an easier and quicker alternative. There is little information regarding the efficacy of different decontamination methods in cases of blood contamination to avoid its adverse effects on restorations.

Therefore, the aim of this study was to investigate the effect of decontamination protocols for blood contamination, before or after light-curing of the adhesive, by means of the dye staining gap test and the push-out bond strength test. The null hypothesis was that neither the moment of contamination nor the decontamination protocols would affect the gap formation or bond strength.

Methods

The research protocol was approved by the Institutional Committee of Ethics in Research (Federal University of Pelotas, protocol #31/04). A total of 135 freshly extracted bovine incisors were selected, stored in distilled water at 4 °C and immersed in 0.5% chloramine T for one week before tests, according to the ISO TS11405:2003 specifications. Roots were cut off, and teeth were embedded in transparent polyester resin (Cristal 2110/Fiberglass, Porto Alegre, RS, Brazil).

Specimen preparation

The labial surface of the teeth was wet ground with 80- to 600-grit silicon carbide paper in order to expose a flat, superficial dentine with a minimum surface area of 6 mm^2. Standardized cylindrical cavities were prepared in the flat dentine surface using wheel shaped diamond burs no. 3056 (KG Sorensen, Alphaville, SP, Brazil), which were replaced after every five preparations to ensure cut efficacy. The cavities dimensions were: 4.0±0.1 mm of diameter and 1.0±0.1 mm of depth.

Teeth were randomly allocated into 9 groups (n=15), as described in Figure 1. G1 had no blood contamination (control). The other 8 groups were contaminated either before (G2, G3, G4, and G5) or after light-curing of the adhesive (G6, G7, G8, and G9). Decontamination procedures consisted in drying with absorbent paper, rinsing with water, phosphoric acid etching, or application of NaClO (Fig. 1). The materials used in the study are described in Table 1.

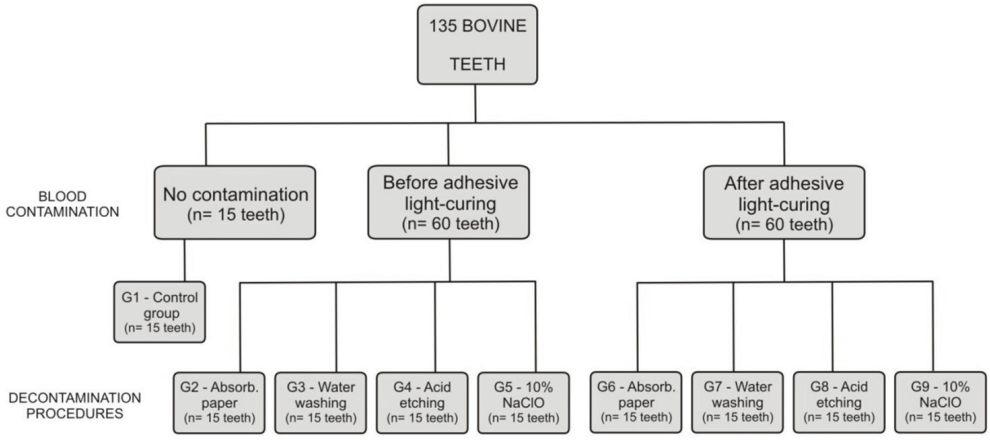

Fig. 1. Schematic diagram of the study design.

Table 1. Description of the materials used in this study.

Material	Composition*	Batch Number	Manufacturer
Filtek Z250	Zirconia/sílica, Bis-GMA, UDMA, Bis-EMA(6), Particle size 0.01-3.5 μm, mean size 0.6 μm (60 vol %)	5BA	3M ESPE, St. Paul, MN
Adper Single Bond	Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiators, water and ethanol	4BC	
Conditioner	Phosphoric acid 37%	4CG	
Blood	Fresh Human blood	–	Laboratory
Sodium hypochlorite	10% Sodium hypochlorite Solution		
Methylene Blue	2% Methylene Blue, buffered pH=7.0	–	

Abbreviations: Bis-GMA = bisphenol A diglycidyl ether dimethacrylate; UDMA = urethane dimethacrylate; Bis-EMA = ethoxylated bisphenol A glycol dimethacrylate; HEMA = 2-hydroxyethyl methacrylate.
* Basic composition based on manufacturers' technical profiles.

Restorative procedure

Cavities were etched with 35% phosphoric acid gel during 15 s, washed for 15 s, and dried using absorbent paper until a clinically moist dentin surface, free of droplets, was achieved. Two coats of the adhesive system Adper Single Bond 2 were applied and air dried for 5 s. Blood was collected from the operator (JLDD) and immediately used to contaminate the cavity preparations. It has been shown that freshly drawn capillary blood is more suitable in laboratory experiments involving blood contamination than heparinized blood, so no anticoagulant agent was used in order to avoid potential interference in adhesion (5). Blood was applied with a syringe onto the entire cavity surface and was kept undisturbed for 15 s.

Four decontamination procedures were tested: in G2 and G6 blood was dried with discs of absorbent paper; specimens of G3 and G7 were washed with 5 mL of distilled water, which was applied with a syringe for 10 s, followed by drying with absorbent paper; specimens of G4 and G8 were rinsed with distilled water, dried, etched with phosphoric acid anew (15 s), washed for the same time, and dried with absorbent paper; in G5 and G9 blood was washed with 5 mL of 10% NaOCl during 10 s, and the surface was dried.

The adhesive was re-applied in groups where contamination occurred before light-curing. In the other groups the restorative procedure followed by inserting the micro-hybrid composite Filtek Z-250 in a single increment. The materials were light-cured according to the time recommended by the manufacturer using a halogen light-curing unit (XL 3000, 3M ESPE, St. Paul, MN, USA), with mean output irradiance of 450 mW/cm^2. The irradiance was constantly measured with a hand held radiometer (Curing Radiometer, Model 100, Demetron/Kerr, Danbury, CT, USA).

The specimens were stored in distilled water at 37 °C for 24 h. The restorations were polished with Soflex polishing system (3M ESPE, St. Paul, MN, USA), and the specimens were submitted to thermal cycling between 5 °C and 55 °C (500 cycles, 30 s of dwell time).

Marginal adaptation

Marginal adaptation at the surface was determined using a staining technique (10,11). A buffered 2% methylene blue (pH=7.0) was applied at the restoration margins for 5 s and followed by copious washing with tap water and drying with absorbent paper. Digital images were obtained using a scanner (Genius ColorPage HR7X, Genius, Taipei, Taiwan) with a 1200 dpi definition. Specimens were positioned beside a metal ruler with scale in mm for software calibration. Images were stored as *.TIFF file with a digitized scale for conversion of pixels into mm. A trained professional measured the dye-stained gaps along the margin of the restorations by image analysis using the measurement tool of the UTHSCSA Image Tool 2.0 software (developed at the University of Texas, Health Science Center in San Antonio, TX, USA). The length of the marginal gap was calculated as the percentage of the entire marginal length:

$$\text{Gap length (\%)} = \frac{\text{Length of stained margin (mm)}}{\text{Total marginal length (mm)}} \times 100$$

Push-out bond strength

The lingual surfaces of the specimens were removed exposing the cavity floor. The specimens were positioned in a xyz coordinate table with a handpiece attached. The handpiece was positioned over the cavity floor using the same wheel shaped diamond bur and moved in the z axis in order to remove it. The diamond burs were replaced after perform five cavities. Then, the diameter of the hole was expanded to 6 mm, exposing the cavity margins (Fig. 2).

Bond strength was assessed by means of a push-out test. Each specimen was positioned with the lingual surface up, towards the loading apparatus. The load was applied using a cylindrical apparatus, with a flat surface of 3.5 mm in diameter, adapted to the universal testing machine (Emic.DL 500, São José dos Pinhais, PR, Brazil), with a cross-head speed of 0.5 mm/min. After the restorations were pulled out by compressive loading on the lingual surface, their diameter (d) and height (h) were measured using a digital caliper (Digimatic caliper, Mitutoyo Co., Kawasaki, Japan). Adhesion surface area was calculated

in mm². Bond strength values computed in MPa by dividing the maximum load by the bonded area of the specimens. The failure mode was analyzed using a stereomicroscope (Tecnival, Biosystems Ltda., Curitiba, PR, Brazil) under 40× magnification and classified as follows: 1) cohesive failure in dentin; 2) cohesive failure in composite; 3) adhesive failure; and 4) mixed failure.

Bond strength data were converted to root square in order to normalize data for the parametric analysis. Both marginal gap formation (dye penetration test) and push-out bond strength data were analyzed by two-way analysis of variance (moment of blood contamination and decontamination procedure) and Tukey's test. Comparisons with the control group were performed using one-way ANOVA. The level of significance was set at 0.05.

Results

Analysis of variance depicted significant influence of the moment of contamination on marginal adaptation and bond strength results ($P<0.001$).

Results of marginal adaptation are shown in Table 2. The control group presented the lowest mean percentage of stained margins. Contamination after light-curing of the adhesive led to higher percentage of stained margins ($P<0.001$) than before light-curing, irrespective of the decontamination protocol used. When blood contamination occurred before light-curing of the adhesive, the application of acid etching as decontamination procedure promoted the highest marginal staining, while washing with distilled water caused the lowest values. On the other hand, when contamination occurred after light-curing, no significant differences were detected between the decontamination procedures used. These groups presented marginal staining statistically higher than the control group (Fig. 2).

Push-out bond strength results were statistically similar, as shown in Table 3. Fracture analysis revealed that either for the groups contaminated prior to light-curing or after light-curing, the predominant modes of failure were adhesive (37% and 19%, respectively) and mixed (63% and 81%, respectively).

Table 2. Comparisons of the mean percentage of stained margins (standard error) among the tested groups.

Moment of blood contamination	Decontamination procedure				
	Absorbent paper	Water rinsing	Acid etching	Hypochlorite rinsing	Control
Before light-curing	B 32.8 (9.6) ^{ab}	A 27.3 (6.4) ^{b}	A 62.6 (7.3) ^{a}	A 42.7 (6.3) ^{ab}	20.0 (4.0)
After light-curing	A 70.1 (6.8) ^{a}	A 50.5 (6.3) ^{a}	A 51.6 (7.6) ^{a}	A 63.3 (5.7) ^{a}	

Notes: Different small letters following means represent statistically significant differences (comparison in line) regarding decontamination protocols ($P<0.05$). Different capital letters before means indicate statistically significant differences (comparison in column) between moment of contamination ($P<0.05$).

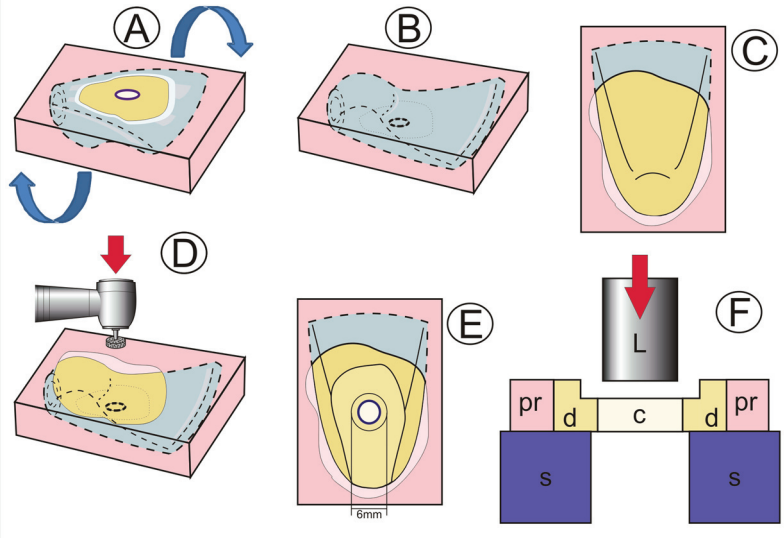

Fig. 2. **(A)** Specimen embedded in polyester resin after the dye staining gap test. **(B)** The specimen was turned upside down, and **(C)** the polyester resin was removed from the lingual side of the tooth. **(D)** Removal of the lingual face of the tooth and of the floor of the composite restoration using a handpiece attached to a xyz-coordinate table. **(E)** Amplification of the hole around the restoration margins up to 6 mm. **(F)** Push-out bond strength test configuration (pr - polyester resin; d - dentin; s - support; c - composite; L - load).

Table 3. Comparison of the mean push-out bond strength (Standard error) in MPa among the tested groups according to the moment of blood contamination.

Moment of blood contamination	Decontamination procedure				
	Absorbent paper	Water rinsing	Acid etching	Hypochlorite rinsing	Control
Before light-curing	4.99 (0.43)	6.43 (1.14)	3.77 (0.54)	6.40 (0.95)	5.36 (0.97)
After light-curing	3.16 (0.49)	2.92 (0.42)	3.45 (0.68)	3.98 (0.49)	

Note: No statistically significant differences were found regarding the decontamination protocols (comparisons in line) and the moment of contamination (comparisons in column).

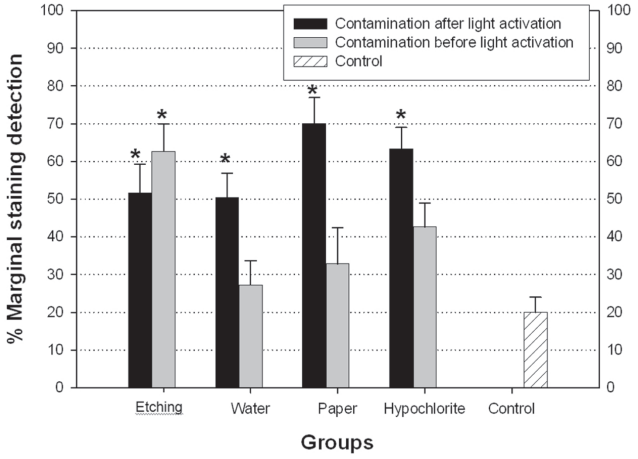

Fig. 3. Mean percentage of dye stained margins and standard error (bar). Asterisks indicate statistically significant difference with the control group.

Discussion

Clinically, contamination with either blood or saliva may occur in different time points during the adhesive procedure, *e.g.*, before acid etching, after acid etching and before application of the adhesive, following the adhesive application, and following light-curing of the adhesive. Previous studies have shown that contamination with blood before application of the adhesive significantly reduces bond strength of total-etch two-step adhesive systems to human dentin (2,6). The present study verified the interference of this contamination in other moments of the adhesive procedure. Blood contamination following light-curing of the adhesive was more deleterious for marginal sealing than before light-curing, supporting that the moment of contamination relative to the hybridization process is determinant for the adhesive sealing effectiveness. Contamination, however, was not a significant factor for push-out bond strength results, neither before nor after light-curing of the adhesive.

Current adhesive systems seem to be incapable of totally sealing the restoration margins (12), and this marginal sealing decreases over time (13). The dye staining gap test was previously described as a reliable alternative method to SEM analysis for assessing marginal gap formation in composite restorations (11). The control group presented up to 20% of stained dentin margins, and the percentage increased with contamination. However, groups 2, 3 and 5 presented statistically similar percentage of stained margins to the control group, revealing that cleansing with absorbent paper, water, and NaOCl may recover part of the adhesion when contamination occurs before light-curing of the adhesive.

When blood interacts with the conditioned dentin surface before the adhesive application, the protein content and macromolecules of fibrinogen and platelets form a thin film on the surface that prevents the infiltration of the adhesive into the treated dentin (2). However, when contamination occurs after the application of the adhesive, expectations are that the adhesive infiltrates properly the dentin tubules and the intertubular dentin.

Adper Single Bond is a water/ethanol-based adhesive. Despite the lower technique sensitivity in comparison with acetone-based adhesives, thick adhesive layers caused by poor solvent elimination have been associated with lower bond strength and poor clinical performance of this adhesive (14). Possibly, blood moisture might have increased the water content of the adhesive, impairing the complete solvent evaporation and affecting the marginal sealing. This effect, though, was not significant for groups 3 and 5, probably because the surface was blot dried after washing with either water or NaOCl. In G2, even though blot drying of the contaminated surface might have trapped some blood constituents in between the first and the second adhesive layers, it seems to be not significant for the marginal sealing of the restorations. In fact, reapplication of the adhesive was important for the recovery of adhesion when contamination occurs prior to light-curing.

Phosphoric acid etching is responsible for the removal of hydroxyapatite and exposure of the collagen network into which hydrophilic monomers infiltrate and micro-mechanically bound in the total etch technique (15). Based on the results of the present study, phosphoric acid etching should be avoided as a cleansing alternative when blood contamination occurs, either before or after light-curing of the adhesive. Speculations are that acid etching in the present study might have affected the previously applied adhesive system, creating porosities. Furthermore, over-etching of dentin may modify its micromorphology and negatively affect adhesion (16), probably due to depletion or degradation of collagen (17).

NaOCl is a strong deproteinizer and has been used for collagen removal in order to create a more stable adhesion (13,18). In the present study, however, it was used to denaturate the protein content of blood (around 6-7%) (2), washing it out. Kaneshima et al. (6) observed recovery of adhesion by removing not only the blood with NaOCl, but also the exposed collagen fibrils. Even though NaOCl may not be strong enough to solubilize the adhesive

and the subjacent collagen network, its action may occur by surface cleaning, justifying the similar results obtained with water rinsing (G3).

Blood contamination after light-curing of the adhesive caused the marginal adaptation of the restorations to decrease significantly. A layer of around 30 µm is normally present in the adhesive surface and is composed by unreacted monomers (19), inhibited by the contact with oxygen (19,20). Contamination of the adhesive reduces the reactivity of the unreacted surface monomers necessary to establish a reliable bond with the composite, and the application of a new coat of adhesive might recover this reactivity. This is probably the reason why groups 6 to 9 presented higher stained margin percentages than the other groups.

Push-out test also was used to evaluate the bond quality after blood contamination. Although push-out tests are indicated when the values expected are low (21), the lack of sensitivity in detecting differences between groups could be related to the test variables, such as the early stress and the pre-ruptures produced during specimen preparation, which could decrease the values obtained (22).

Despite the statistically non significant results of push-out bond strength, a general trend of lower values when contamination happened after light-curing of the adhesive could be detected. This reinforces the fact that the application of a new adhesive layer might be a key for recovery of the adhesion after contamination. According to Yoo and Pereira (23), in the clinical scenario, thorough rinsing and cleansing are important if blood contaminates the preparation, and bonding procedures should be repeated from the beginning. Analyzing contamination between resin increments, Eirikisson et al. (1) also concluded that rinsing and application of a dentin adhesive seem to be necessary whenever blood contamination exists on a resin surface to ensure better interfacial bonding of the next increment.

The null hypothesis of the study was partially rejected since the marginal staining level was affected by blood contamination. On the other hand, no significant difference was observed for push-out bond strength. One could expect that higher bond values could produce better marginal adaptation. However, the correlation between these two parameters has shown to be weak (24,25).

Conclusions

The moment of contamination affects marginal adaptation of restorations. Acid etching should be avoided as a decontamination alternative. Washing with water produced good marginal adaptation and bond strength similar to the control group and, therefore, may be recommended as a reliable method for blood decontamination.

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