Article published online: 2021-11-01

Original Article

Influence of Blood Contamination and Decontamination Procedures on Bond Strength of a Two-Step Etch and Rinse Adhesive System

Rafael Guerra Lund[1], Isadora Augusta Da Silveira[1], Juliana Silva Ribeiro[1], Dayane Rubin[1], Sonia Luque Peralta[2], Carlos Enrique Cuevas-Suárez[1,3], Evandro Piva[1]

[1]Graduate Program in Dentistry, Department of Restorative Dentistry, School of Dentistry, Federal University of Pelotas, Pelotas, Rio Grande do Sul, [2]Department of Restorative Dentistry, Metropolitan Faculty of Grande Fortaleza, Fortaleza, Ceará, Brazil, [3]Dental Materials Laboratory, Academic Area of Dentistry, Autonomous University of Hidalgo State, Pachuca, Hidalgo, México

Abstract

Purpose: This study evaluated the effect of blood contamination and decontamination procedures performed at different stages of bonding, on the microtensile bond strength (μTBS) of an etch-and-rinse adhesive to dentin. **Materials and Methods:** Standardized cylindrical cavities were prepared in bovine incisors and were randomly divided into five groups, each one was treated using these experimental conditions – Control: etching-rinsing/bonding without blood contamination; Group 1: etching-rising/blood contamination/decontamination/bonding; Group 2: etching-rising/bonding/blood contamination/decontamination; Group 3: etching-rising/bonding/blood contamination/decontamination/etching-rising/bonding; and Group 4: etching-rising/blood contamination/decontamination/etching-rising/bonding. Specimens were prepared for μTBS and were evaluated both immediately and after 6 months of storage in distilled water. **Results:** Blood contamination significantly reduced the μTBS ($P < 0.001$). Groups 2 and 3 showed the lowest μTBS values, both for 24 h and 6 month aging. **Conclusions:** Blood contamination tested at any of the different stages of the bonding procedure showed a negative effect on the μTBS. Recovering adhesion of blood-contaminated dentin did not depend only on cleaning with distilled water.

Keywords: Contaminants, decontamination, dentin-bonding agents, microtensile, resin composite

Introduction

Current adhesive systems are applied using either an "etch-and-rinse," "self-etch," or "selective etch" technique, which differs in how the adhesives are applied and how they interact with tooth structures.[1] Etch-and-rinse systems comprise phosphoric acid to pretreat the dental hard tissues before rinsing and subsequent application of an adhesive. In the etch-and-rinse approach, adhesives are applied after phosphoric acid etching, whereas when using the self-etching technique, the acid-etching step is eliminated, simplifying the procedure.[2] The evidence available today suggests that the choice between etch-and-rinse or self-etch systems is often a matter of personal preference.[3] In general, the etch-and-rinse technique is frequently preferred for indirect restorations and when large areas of enamel are still present.[4]

The effectiveness of dentin bonding systems after suitable clinical application protocols is required to ensure the longevity of restorations. Clinically, many factors are known to impair adhesion and retention of resin-containing restorative materials such as the contamination of the operative field with oral fluids and microorganisms.[5,6] To prevent this, the rubber dam is still the most important tool to use to guarantee moisture control.[7,8] However, moisture control is difficult in some clinical situations, such as caries located at or near the gingival margin,[9] and contamination of the operative field with blood or saliva is likely to occur.[6]

Previous studies that have evaluated the effect of blood contamination during bonding procedures have shown that this could lead to premature failure of the bond of light-cured resin

Access this article online

Quick Response Code:

Website: www.ejgd.org

DOI: 10.4103/ejgd.ejgd_57_19

Address for correspondence: Prof. Rafael Guerra Lund, Department of Restorative Dentistry, Pelotas Dental School, Federal University of Pelotas, Gonçalves Chaves Street N. 457, Rm. 503, Centro District, 96015-560 Pelotas, Rio Grande do Sul, Brazil.
E-mail: rafael.lund@gmail.com

How to cite this article: Lund RG, Da Silveira IA, Ribeiro JS, Rubin D, Peralta SL, Cuevas-Suárez CE, *et al*. Influence of blood contamination and decontamination procedures on bond strength of a two-step etch and rinse adhesive system. Eur J Gen Dent 2019;8:71-5.

composites, even after several decontamination methods.[10-12] Contamination of operative field can occur at different critical times of the bonding procedure: before or after acid etching, after application of adhesive without light activation, after application of adhesive and light application, during insertion of a resin composite in increments, and after restoration placement.[10,11,13-15] Furthermore, few studies[16-18] have evaluated the effect of blood contamination in conventional adhesive systems.

Thus, this study aimed to evaluate the bond strength of a two-step etch-and-rinse adhesive system to dentin in the presence of blood contamination and to determine which decontamination protocol is capable of recovering adhesion. The null hypotheses to be tested were that (1) blood contamination will not impair the bond strength to dentin and that (2) the decontamination protocols tested will be able to recover the bond strength to dentin of a two-step etch-and-rinse adhesive.

Materials and Methods

Twenty freshly extracted bovine incisors were collected and stored in 0.5% Chloramine-T solution for 7 days. Teeth were then kept in distilled water at 4°C until use.[19] The criteria for tooth selection included intact buccal enamel free of caries, cracks, and damage due to extraction. Each tooth was examined under a stereomicroscope to eliminate teeth with cracks or hypoplastic defects. All teeth were cleaned using hand scalers and scalpels, and then, their roots were sectioned using a low-speed diamond saw under water cooling, and their crowns were embedded in polyester resin (Resina cristal, Comfibras, Porto Alegre – Brasil), allowing the buccal enamel surface to be exposed. Then, the enamel was removed with an orthodontic grinder, and then, the exposed dentin surface was wet-ground with 400- and 500-grit SiC abrasive papers coupled to a universal polishing machine at a speed of 50 rpm, under constant water irrigation.

Standardized cylindrical cavities were prepared in the flat dentin using a round-wheel diamond bur (No. 3056, KG Sorensen, Alphaville, SP, Brazil) under water irrigation. Diamond bur was replaced after every five preparations to ensure efficient cutting. The cavity dimensions were 4.0 ± 0.1 mm in diameter and 1.0 ± 0.1 mm deep [Figure 1]. The teeth with the prepared cavities were divided into five experimental groups of six teeth each, and these were randomly assigned to one of the five blood contamination and decontamination protocols used [Figure 2]. Fresh human blood was collected from the fingertip of a volunteer (ethics committee approval protocol No. 41/11 Dentistry/UFPel) at the same time that the restoration processes were performed. In the blood contamination groups, the specimens were rinsed with distilled water and were dried with sterile paper towels.[20,21]

The restorative procedures were performed using a two-step etch-and-rinse adhesive system (Single Bond; 3M ESPE, St Paul, MN, USA) applied in accordance with the manufacturer's recommendations. Phosphoric acid gel (Scotchbond etchant; 3M ESPE, St Paul, MN, USA) was applied during 15 s and then rinsed for 10 s. Excess water was removed using sterile paper towels, leaving dentin moist. Then, two consecutive coats of adhesive were applied to the etched dentin using a microbrush. A stream of air was applied for 5 s between each coat. The adhesive was light cured using a light-emitting diode (LED) photopolymerization unit (Radii Cal, SDI Limited, Victoria, Australia) with a light irradiance of 900 mW/cm^2. After light curing the adhesive, a microhybrid resin composite (Filtek Z-250; 3M ESPE, St. Paul, MN, USA) was applied in four increments of approximately 1.0 mm thick. Each increment was light cured for 20 s with a light polymerizing unit equipped with a LED visible light source (Radii Cal; SDI, Bayswater, Victoria, Australia).

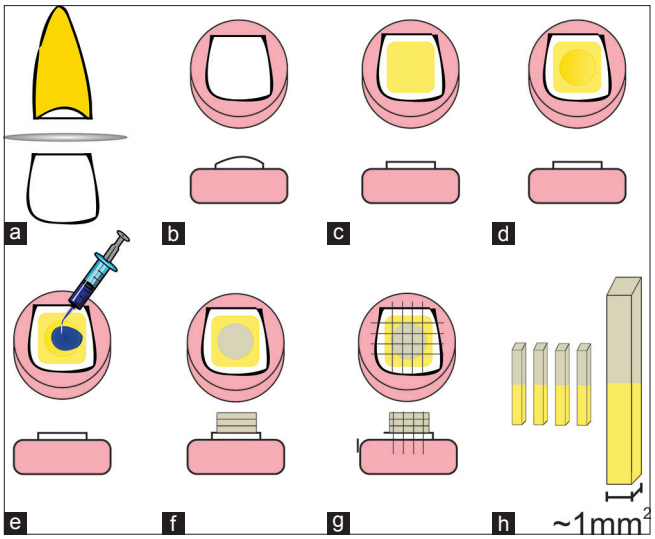

Figure 1: Schematic representation of the microtensile bond strength process used. (a) Root sectioning. (b). Crown embedded in polyester resin. (c) Dentin surface exposed. (d) Cylindrical cavity preparation. (e) Bonding procedures. (f) Increments of micro-hybrid resin composite. (g) Specimens sectioning. (h) Microtensile bond strength beams

After storage in distilled water at 37°C for 24 h, the specimens were sectioned perpendicular to the bond interfaces in the mesiodistal and buccolingual directions, using a slow-speed diamond saw (Isomet Saw 1000 Precision, Buehler Ltd., Lake Bluff, IL, USA) to obtain resin-dentin beams with a cross-sectional area of approximately 0.5 mm^2. Six beams from each tooth were obtained, providing 30 sticks per group for the microtensile bond strength (μTBS) test. Half of the beams were tested after 24 h and the other half, after 6 months of storage in distilled water at 37°C (n = 15). The beams were attached to a microtensile testing device with cyanoacrylate glue (Super Bonder Gel, Loctite® Corp., Henkel Technologies, Diadema, SP, Brazil), and the μTBS was tested in a universal mechanical testing machine (DL 500, EMIC®, Pinhais, PR, Brazil), at a crosshead speed of 0.5 mm/min and a load cell of 100 N.

The μTBS values were expressed in MPa by dividing the load (N) applied at the time of the fracture by the

cross-sectional area of the bonded interface (μTBS = F/A). The fracture modes were evaluated by a single observer, using a light microscope (Mobiloskop; Renfert, Hilzingen, Germany) at ×100 and ×500. Failure modes were classified as adhesive, cohesive within dentin, and cohesive within resin composite or mixed failure.

The data were analyzed to check normality (Shapiro–Wilk test) and homoscedasticity (Levene's test). Two-way analysis of variance (ANOVA) was used to evaluate the effect of contamination step and storage time on the μTBS. Multiple comparison procedures were performed using Tukey's test ($\alpha = 5\%$). Data were analyzed and plotted with SigmaPlot 12 software (Systat Software Inc., San Jose, CA, USA).

Results

The results of the μTBS test are summarized in Figure 3a. The two-way ANOVA test revealed that μTBS was influenced by both the decontamination protocol and storage time ($P < 0.05$); however, the interaction between these two variables was not significant ($P = 0.529$).

In the μTBS test at 24 h, the highest bond strength values were observed for the Control Group, followed by Group 1; the lowest μTBS values were observed for Group 3. After 6 months of storage in distilled water, the μTBS values decreased in all groups; the Control Group showed the highest values, while the Group 2 showed the lowest μTBS values; however, these differences were not statistically significant. The intergroup analysis revealed that μTBS values for Groups 3 and 4 remained stable.

The numbers and percentages of failure modes in each group are shown in Figure 3b. The results of failure mode analysis after 24 h demonstrated that adhesive type failure mode was predominant in all groups, followed by mixed-type failure. After 6 months of aging, the results of failure mode analysis

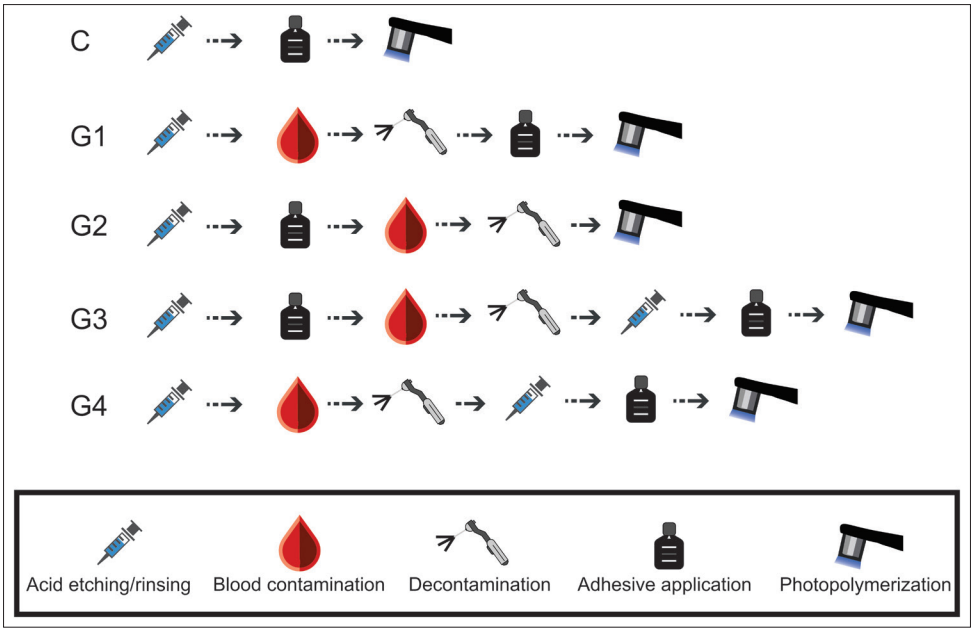

Figure 2: Experimental groups and decontamination protocols

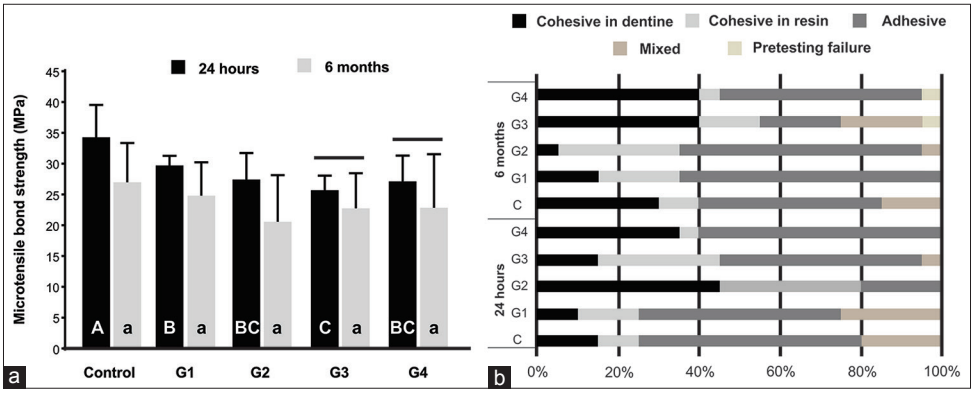

Figure 3: Microtensile bond strength (a) and distribution of failure modes (b). Columns under the same horizontal line indicate no differences between aging times for each group. Different capital or lowercase letters indicate differences between groups within 24 h and 6 months, respectively

demonstrated that the adhesive type failure mode remained predominant in all groups, followed by the cohesive in dentin failure type.

Discussion

In this study, the influence of blood contamination on the bond strength of a two-step etch-and-rinse adhesive to dentin was investigated. Besides, the decontamination protocol that would be capable of recovering the bond strength of this adhesive system was determined. Since the statistical analysis revealed that blood contamination impaired the bond strength of a resin composite to dentin and that none of the decontamination protocols tested were able to recover the bond strength, the null hypotheses tested in this study were rejected.

In this study, freshly drawn blood, collected at the same time that the experiment was being performed, was used to contaminate the dentin surfaces. No anticoagulants were used since studies in the literature have shown that the addition of an anticoagulant may reduce the bond strength.[10,22] Considering this variable, and other factors such as the adhesive system used, the step when contamination occurs, and substrate type, it was difficult to make comparisons with previous studies that investigated the blood contamination of adhesive restorations.

The results obtained in this study proved that when compared with the control group, any blood contamination at any of the stages of adhesive system application decreased the μTBS, both in 24 h and after 6 months. The literature has shown that the blood is capable of interacting with the dentin surface, and the content of proteins, macromolecules of fibrinogen, and platelets may form a thin film on the dentin surface, which may make it difficult for the adhesive to infiltrate into the treated dentin, thereby weakening the bond strength.[23] Furthermore, residual blood proteins could remain on the polymerized bond surface and eliminate an oxygen-inhibited layer, which has the potential of preventing copolymerization between the successive increments of resin composite material.[24]

Among the decontamination protocols, when the contamination occurred after the application of acid and before the application of the adhesive system (Group 1), the bond strength values were higher than those in the other contaminated groups, both in 24 h and after 6 months. These results could be explained by the cleaning processes performed after blood contamination, which were able to eliminate a large part of the blood proteins deposited on the dentin surfaces. In addition, it could be hypothesized the application of primer cleaned or hydrolyzed blood on the dental surface.[25] However, as no values equal to those of the control group were obtained, rinsing with water was shown to be insufficient to achieve complete decontamination of the dentin surface.

When the contamination occurred after the application of the adhesive system (Groups 2 and 3), the decrease in bond strength could be attributed to the degradation of the adhesive components of the contaminated adhesive layer, rather than to its removal. Furthermore, the presence of excessive humidity trapped in the degraded components in the dentinal tubules may have impaired bonding between the subsequent resin composite layers (increments).[26]

Moreover, in this study, it could be demonstrated that after the dentin surface had been contaminated, it was not recommendable to re-etch the contaminated surface. In Groups 3 and 4, in which a re-etching procedure was performed after blood contamination, lower μTBS values were also observed. A possible explanation for this could be that re-etching the dentin surface could produce an excessive layer of demineralized dentin, which could be not totally penetrated by the adhesive system, allowing the formation of a fragile adhesive area.[27]

This study showed the negative effect that blood contamination has on the bond to dentin when using a two-step etch-and-rinse adhesive system. Besides, it could be established that recovering the bond to blood contaminated dentin surfaces did not depend only on careful cleaning with distilled water, and other cleaning agents should be tested in further studies.

Conclusions

The findings of this study proved that blood contamination significantly impaired the bond strength of two-step etch-and-rinse adhesives to dentin. In addition, none of the decontamination protocols tested were capable of recovering the bond strength. Therefore, when the dentin surface has been contaminated with blood during the restoration bonding procedures with the use of two-step etch-and-rinse adhesives, the dentin surface should be re-prepared with a rotary cutter to prevent impairment of the bond efficiency of the adhesive system.

Acknowledgments

This work was supported by the Research Support Foundation of Rio Grande do Sul – Brazil (FAPERGS APE 12/05783 and PRONEX 16.0471-4). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, Brazil. The author, Carlos Enrique Cuevas Suárez, would like to thank PRODEP, México (DSA/103.5/15/6615).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Moszner N, Hirt T. New polymer-chemical developments in clinical dental polymer materials: Enamel-dentin adhesives and restorative composites. J Polym Sci Part A Polym Chem 2012;50:4369-402.
2. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, *et al.* Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials 2007;28:3757-85.
3. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P,

et al. Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges. Oper Dent 2003;28:215-35.
4. Ozer F, Blatz MB. Self-etch and etch-and-rinse adhesive systems in clinical dentistry. Compend Contin Educ Dent 2013;34:12-4, 16, 18.
5. Namgung C, Rho YJ, Jin BH, Lim BS, Cho BH. A retrospective clinical study of cervical restorations: Longevity and failure-prognostic variables. Oper Dent 2013;38:376-85.
6. Elkassas D, Arafa A. Assessment of post-contamination treatments affecting different bonding stages to dentin. Eur J Dent 2016;10:327-32.
7. Feierabend SA, Matt J, Klaiber B. A comparison of conventional and new rubber dam systems in dental practice. Oper Dent 2011;36:243-50.
8. Gilbert GH, Litaker MS, Pihlstrom DJ, Amundson CW, Gordan VV; DPBRN Collaborative Group. Rubber dam use during routine operative dentistry procedures: Findings from the dental PBRN. Oper Dent 2010;35:491-9.
9. Prasad M, Mohamed S, Nayak K, Shetty SK, Talapaneni AK. Effect of moisture, saliva, and blood contamination on the shear bond strength of brackets bonded with a conventional bonding system and self-etched bonding system. J Nat Sci Biol Med 2014;5:123-9.
10. Yoo HM, Pereira PN. Effect of blood contamination with 1-step self-etching adhesives on microtensile bond strength to dentin. Oper Dent 2006;31:660-5.
11. Chang SW, Cho BH, Lim RY, Kyung SH, Park DS, Oh TS, *et al.* Effects of blood contamination on microtensile bond strength to dentin of three self-etch adhesives. Oper Dent 2010;35:330-6.
12. Koppolu M, Gogala D, Mathew VB, Thangala V, Deepthi M, Sasidhar N. Effect of saliva and blood contamination on the bond strength of self-etching adhesive system- an *in vitro* study. J Conserv Dent 2012;15:270-3.
13. Eiriksson SO, Pereira PN, Swift EJ, Heymann HO, Sigurdsson A. Effects of blood contamination on resin-resin bond strength. Dent Mater 2004;20:184-90.
14. Yoo HM, Oh TS, Pereira PN. Effect of saliva contamination on the microshear bond strength of one-step self-etching adhesive systems to dentin. Oper Dent 2006;31:127-34.
15. Damé JL, Torriani DD, Demarco FF, Goettems ML, Rodrigues-Junior SA, Piva E. Effect of blood contamination and decontamination procedures on marginal adaptation and bond strength of composite restorations. Rev Odontol Ciênc 2009;24:283-9.
16. Abdalla AI, Davidson CL. Bonding efficiency and interfacial morphology of one-bottle adhesives to contaminated dentin surfaces. Am J Dent 1998;11:281-5.
17. van Schalkwyk JH, Botha FS, van der Vyver PJ, de Wet FA, Botha SJ. Effect of biological contamination on dentine bond strength of adhesive resins. J South Afr Dent Assoc 2003;58:143-7.
18. Juneja R, Duhan J, Tewari S, Sangwan P, Bhatnagar N. Effect of blood contamination and decontamination protocols on acetone-based and ethanol-based total etch adhesive systems. J Esthet Restor Dent 2014;26:403-16.
19. International Organization for Standardization. ISO/TS 11405:2015 Dentistry Testing of Adhesion to Tooth Structure. International Organization for Standardization; 2015.
20. Gwinnett AJ. Dentin bond strength after air drying and rewetting. Am J Dent 1994;7:144-8.
21. Gwinnett AJ. Moist versus dry dentin: Its effect on shear bond strength. Am J Dent 1992;5:127-9.
22. Dietrich T, Kraemer ML, Roulet JF. Blood contamination and dentin bonding – Effect of anticoagulant in laboratory studies. Dent Mater 2002;18:159-62.
23. Tachibanaa A, Castanhob GM, Vieirac SN, Bona A. Influence of blood contamination on bond strength of a self-etching system. Eur J Dent 2010;4:280-6.
24. de Carvalho Mendonça EC, Vieira SN, Kawaguchi FA, Powers J, Matos AB. Influence of blood contamination on bond strength of a self-etching system. Eur J Dent 2010;4:280-6.
25. Oonsombat C, Bishara SE, Ajlouni R. The effect of blood contamination on the shear bond strength of orthodontic brackets with the use of a new self-etch primer. Am J Orthod Dentofacial Orthop 2003;123:547-50.
26. Tay FR, Pashley DH. Have dentin adhesives become too hydrophilic? J Can Dent Assoc 2003;69:726-31.
27. Peschke A, Blunck U, Roulet JF. Influence of incorrect application of a water-based adhesive system on the marginal adaptation of class V restorations. Am J Dent 2000;13:239-44.