Effect of salivary contamination at different steps of the bonding process on the microleakage around Class V restorations

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Abstract
This study aimed to investigate the influence of the moment of salivary contamination during the bonding procedure (before or after acid conditioning) on the microleakage around composite resin restorations. Sixty bovine incisors received two Class V preparations (one with dentin margins and other with enamel margins). Teeth were randomly assigned into three groups (n=20): 1) control (not contaminated); 2) salivary contamination before etching with 34% phosphoric acid; and, 3) salivary contamination after acid etching. Cavities were restored using Prime & Bond NT (Dentsply) adhesive system and TPH Spectrum (Dentsply) composite according to manufacturer instructions. Teeth were thermocycled (500x, 5-55°C, 60s/bath), immersed in 2% methylene blue buffered solution (pH 7.0), and sectioned into two halves. Three examiners measured the extent of dye penetration on dentin and enamel margins in a stereoscope microscope, using four representative scores. Statistical analysis were performed with Kruskal-Wallis/Wilcoxon tests (α=5%). The results showed that enamel and dentin margins did not present significant differences ($p>0.05$). However, significantly higher dye penetration was observed on substrates etched and further contaminated with saliva. It was concluded that salivary contamination after acid etching increases the microleakage around composite resin restorations, especially at dentin margins. However, acid etching subsequent to the contamination can avoid negative effects on restorations margins.

Key words:
Dentin bonding agents, saliva, dental leakage

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Introduction

The use of adhesive restorative materials preserves dental structure and increases dental resistance through more conservative restorations. However, the dental substrates for adhesion – dentin and enamel – are responsible for differences in bonding quality and durability[1]. Variations on dentin morphology and physiology determines a lack of bonding uniformity compared to enamel bonding[1-2]. Furthermore, the clinical performance of bonding systems depends on the circumstances in which they are applied, and also on the environmental conditions, such as intra-oral temperature/humidity parameters, and salivary contamination[3-4].

An important factor in the placement of resin-based composites is an adequate field control[5]. Although rubber dam usage is strongly recommended during clinical procedures, proper isolation is often neglected by dentists[6-7], who believe rubber dam is both problematic and time-consuming. However, it is important to consider that in some clinical situations, such as restoring young and newly erupted teeth, rubber dam placing is usually not practical. In addition, several caries lesions which require the use of dentin bonding agents for optimum restorations are difficult areas to isolate, and saliva contamination might be more likely[8].

Assessments on the influence of saliva-contaminated dentin and enamel on resin restorative materials indicated a decrease on adhesive material's bond strength[9-13]. Saliva can contaminate dental substrates in different moments during the restorative procedure, i.e. before or after acid etching. For that reason, understanding the influence of the moment of salivary contamination on the marginal integrity is a factor with clinical relevance. The purpose of this *in vitro* study was to evaluate the influence of saliva contamination on the microleakage around Class V composite restorations with enamel or dentin margins. The working hypotheses of this study were that dentin and enamel margins on Class V restorations present different microleakage, and that salivary contamination increases the leakage only when it occurs after the acid etching step.

Materials and Methods

The present study was conducted after the approval of the Committee for Ethics of the School of Dentistry of the Federal University of Bahia. Materials were used according to manufactures' instructions, and their classification and compositions are listed in Table 1.

Sixty bovine incisors were stored in 0.09% NaCl solution at 37ºC. Two Class V cavities with 2.0 mm in diameter and 1.5 mm in depth were prepared with diamond burs (#2294, KG Sorensen, Barueri, SP, Brazil) using a water-cooled high-speed handpiece. Preparations were finished with a cylindrical bur (#57L, Maillefer/Dentsply, Petrópolis, RJ, Brazil) at low speed. Each bur was replaced after six cavity preparations. Cavities were located at the cervical third of the crown (enamel margins) and at the cervical third of the root (dentin margins) of the labial surface. Teeth were randomly assigned into three experimental groups (n=20):

Group 1 (Control)– Preparations were etched with 34% phosphoric acid for 15 seconds and rinsed with distilled water for 15 seconds. The acetone-based adhesive system Prime & Bond NT (Dentsply, Konstanz, Germany) was applied, the composite (TPH Spectrum, Dentsply, Konstanz, Germany) was inserted in one increment, and light-cured for 40 seconds (Optilight 600, Gnatus, Ribeirão Preto, SP, Brazil).

Group 2 – Human saliva was collect from the same donor, who was stimulated by chewing paraffin wax (1.5 g) for five minutes. The saliva (0.2 ml) contaminated the cavity for 15 seconds and the excess was spread by a gentle stream of compressed air before acid etching. Restorative procedures were performed as described in Group 1.

Group 3 – After etching, rinsing and drying the cavity, human saliva contaminated the surfaces for 15 seconds, and then it was spread for 2 seconds with compressed air. The adhesive system was applied, and restorative procedures were finished as described before.

Table 1 – Description of the materials applied: classification and composition

Material	Classification	Composition
34% Tooth Conditioner Gel	Phosphoric acid	Phosphoric acid, highly dispensed silicon dioxide, colorant, water
Prime & Bond NT	Bonding agent	Di- and trimethacrylate resins, PENTA, nanofillers-amorphous silicon dioxide, photoinitiators, stabilizers, cetylamine hydrofluoride, acetone
TPH Spectrum	Composite resin	Bis-GMA, Bis-EMA, TEGDMA, barium aluminum borosilicate, silica (0.0405 µm / 57% vol).

PENTA: dipentaerythritol penta acrylate monophosphate; Bis-GMA: bisphenol-glycidyl methacrylate; Bis-EMA: Ethoxylated bisphenol-A-dimethacrylate; TEGDMA: triethylene glycol dimethacrylate

After restorative procedures, teeth were stored for 24 hours at 37ºC in humidity, and them finished and polished with Al_2O_3 fine and ultra-fine abrasive discs (Sof-Lex, 3M-ESPE, St Paul, MN, USA). After, the root canal and the pulp chamber of each tooth were sealed with epoxy glue (Araldite, Brascola Ltda, São Bernardo do Campo, SP, Brazil). Two layers of fingernail varnish coated the entire tooth surface except the restoration and 1.0 mm of tooth from its margins. Specimens were thermocycled for 500 cycles between 5ºC and 55ºC (± 2ºC) with a 1 minute dwell time at each temperature. They were immersed in 2% methylene blue buffered dye solution (pH 7.0) for 4 hours, washed, and dried.

Specimens were longitudinally sectioned through the center of the restoration using double-faced diamond disks (#7020, KG Sorensen). The dye penetration through restoration margins was qualitative evaluated using an optical stereoscope microscope (x40 magnification; Stemi 2000-c, ZEISS, Germany). Three independent examiners established the extent of microleakage according to the following scores:

0 - No leakage;

1 - Dye penetration up to one-third of the distance between the cavity margin and the axial wall;

2 - Dye penetration up to half the distance between the cavity margin and the axial wall;

3 - Dye penetration until the axial wall;

4 - Dye penetration beyond the axial wall.

Microleakage data were analyzed with nonparametric tests: first the substrate factor was compared in each level of the treatment factor using Wilcoxon test, and then the treatment factor was analyzed in each level of the substrate factor using Kruskal-Wallis test followed by the Multiple Comparisons test. Calculations were assisted by BioEstat 3.0 statistical package with a 5% significance level.

Results

Statistical analysis using Wilcoxon test showed no significant difference on microleakage between enamel and dentin ($p>0.05$). Table 2 presents data from enamel and dentin margins in each treatment group.

The comparison of treatments in enamel margin groups identified a significant difference ($p=0.031$) between G2 and G3, with no difference between these two groups and G1 (Table 3). In groups with dentin margins, there were a significant statistical difference between G3 and the two other groups ($p=0.016$), although there was no difference between G1 and G2 (Table 3).

Discussion

Avoiding saliva or other contaminants is usually hard to achieve during restorative procedures. For that reason, the effects of contamination on restorations quality must be well known by the clinicians. The present study was conducted to access the effect of the moment of salivary contamination, before or after acid etching, on the microleakage at Class V restorations with enamel and dentin margins.

Although enamel and dentin present a different structure and composition, the results obtained in the present did not show any interference of the dental substrate on the microleakage of experimental groups. Therefore, the first working hypothesis had to be rejected. This observation can suggest that current etch&rinse adhesive systems may achieve high quality adhesion to both enamel and dentin[1]. Even though this study used bovine instead of human substrate, previous study indicated that bovine can properly substitute human teeth on dentin or enamel bond evaluations[14].

Nevertheless, the second working hypothesis was accepted, since a greater microleakage was observed in dentin specimens when saliva contamination occurred after etching. Saliva is mostly water 99.4% with 0.6% solids. The solid is composed of macromolecules like proteins, glycoproteins sugars and amylase, inorganic particles like calcium, sodium and chloride and organic particles like urea, aminoacids, fatty acids and free glucose[9]. Etched and contaminated surfaces might absorb salivary constituents, reducing surface energy and rendering the surface unfavorable for bonding. Also, air-blasting saliva-contaminated etched surfaces might result in a dry film of salivary proteins that prevented the adhesive monomer from diffusing and wetting the surfaces, reducing bond strength[9].

In contrast to the findings of the present study, a previous study reported that salivary contamination of dentin before

Table 2 – Microleakage analysis between substrates

Groups	Control		Contamination before etching		Contamination after etching	
	Median	Average Rank	Median	Average Rank	Median	Average Rank
Enamel	1	20.95 a	0.25	19.25 a	1	18.57 a
Dentin	0.75	20.05 a	1	21.75 a	1.5	22.42 a

Same letters indicate no significant statistical difference between substrates in each treatment (Wilcoxon test, α =5%).

Table 3 – Microleakage analysis among treatments

Groups	Control		Contamination before etching		Contamination after etching	
	Median	Average Rank	Median	Average Rank	Median	Average Rank
Enamel	1	29.80 AB	0.25	23.85 A	1	37.85 B
Dentin	0.75	26.52 A	1	26.65 A	1.5	39.32 B

Same letters indicate no significant statistical difference among treatments within each substrate (Kruskal-Wallis test, α =5%).

etching and after adhesive application has no adverse effect on the bonding efficiency of simplified etch and rinse adhesive systems[8]. Those authors justified the lower sensitivity of dentin to saliva contamination by the presence of water in the salivary film, which facilitates penetration of monomers dissolved in acetone carriers[8]. However, the results of other studies do agree with the findings of this study, and reports of negative outcomes of salivary contamination on the bond strength of self-etch and etch and rinse systems[10,12], and on the resin-resin bond[15] can be found in the literature. In addition, it was stated that salivary contamination does not have the same influence at different stages of the bonding process with modern adhesives, and bond strengths decrease significantly when saliva contamination occurs after adhesive application[13].

The bonding agent used in the restorative procedure, Prime & Bond NT, presents the acetone solvent, a high concentration of PENTA monomer, and nanometric fillers[16]. The acetone-based bonding agent seemed able to penetrate between the organic contaminated-layer in enamel surfaces and to create a marginal seal similar to that of control group. Nevertheless, the same performance could not be observed in dentin margins. It is possible that the complexity of dentin structure, its higher organic content or some kind of bond between dentin and saliva constituents could contribute for this different performance. However, this issue should be further investigated.

Significant differences were not observed between groups contaminated before etching and control groups. It is possible that the salivary proteins on enamel and dentin surface were removed by the phosphoric acid, and when the adhesive was applied there was no organic coating preventing it from reaching the conditioned surfaces. Thus, etching procedure was able to bring the dental substrate back to adhesive control conditions.

Microleakage of composite resin restorations is closely related to their durability and longevity. From the results gathered in the present study, it could be concluded that salivary contamination might have a negative effect on restorations marginal quality. However, further research should evaluate if other adhesive materials, such as self-etch systems, water or ethanol-based adhesive systems behave similarly as acetone-based products. By now, clinicians must be strongly advised to re-etch dental substrates at any signal of salivary contamination during bonding procedure. In addition, since salivary contamination jeopardizes the bonding process, the use of rubber dam should be strongly recommended in adhesive dentistry.

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