

Microtensile bond strength of etch-and-rinse and self-etch adhesive systems to demineralized dentin after the use of a papain-based chemomechanical method

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ABSTRACT: Purpose: To evaluate the *in vitro* microtensile bond strength (μ TBS) of etch-and-rinse and self-etch adhesive systems to demineralized dentin after the use of a papain-based chemomechanical method. **Methods:** 36 demineralized human dentin slabs were randomly distributed into two groups according to the method of caries removal: (1). Mechanical removal with manual excavators; (2) Chemomechanical removal with a papain-based gel (Papacárie). Subsequently, three adhesive systems were applied (n=6): (a) an etch-and-rinse adhesive system (Single Bond); (b) a two-step self-etch adhesive system (AdheSE); (c) a one-step self-etch adhesive system (Adper Prompt). The slabs were restored with a microhybrid resin composite and each resin-dentin block was sectioned into 1.0 mm² thick slabs, which were kept in receptacles containing distilled water at relative humidity, for 24 hours, at 37°C. After that, they were subjected to tensile stress in a universal testing machine at a speed of 0.5 mm/minute. Data were submitted to two-way ANOVA and Tukey's test at a 0.05 level of significance. The fractured specimens were observed under a stereomicroscope to assess the failure mode. **Results:** The application of both chemomechanical and mechanical methods on demineralized dentin yielded μ TBS values that were statistically similar among them, regardless of the adhesive system used. Caries removal with a chemomechanical papain-based method did not interfere in the adhesion of the tested adhesive systems to demineralized dentin. (*Am J Dent* 2010;23:23-28).

CLINICAL SIGNIFICANCE: The use of a papain-based chemomechanical method for caries removal did not affect the adhesion of etch-and-rinse and self-etch adhesives to demineralized dentin.

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Introduction

The understanding of caries progress can be considered the first and main principle in minimal intervention dentistry.¹ This knowledge has enabled the traditional "surgical" approach to be replaced by biological and therapeutic treatment,¹ delaying an operative intervention for as long as possible.²

Non-invasive treatments can be applied in caries lesions with little or no evidence of cavitation, with the aim of altering the oral environment, reducing enamel demineralization and promoting remineralization of early enamel and dentin lesions (by the application of topical fluoride). But, if the caries lesion has reached a stage of cavitation that makes it difficult to control the biofilm, a surgical approach is generally required.¹ In the view of minimal invasive dentistry, restorative treatment has only become possible with the advent of restorative adhesive materials, and with them, contemporary operative treatment is able to incorporate the minimal intervention concept in cavity design.³

In this context, the philosophy of caries removal must take into account the subdivision of carious dentin into two levels: one with a high level of infection or infected dentin⁴ that is degraded to a point where it cannot be remineralized;⁵ and the other, with a slow level of infection or affected dentin^{6,7} that is capable of being remineralized and must be conserved.^{5,8,9} However, it is known that the use of conventional rotary instruments and burs leads to excessive wear of dental tissue.¹⁰ In addition, this method of dental intervention has some undesirable aspects, such as thermal damage,¹¹ pressure on pulp

tissue,¹² vibration, noise, pain and need for anesthesia in most cases.¹³ Due to these shortcomings, attention has been paid to alternative methods for caries removal that are suited to the concept of minimal intervention dentistry¹⁴ and are more comfortable to the patient.^{9,13,15,16} Among them, chemomechanical methods for caries removal can be pointed out, such as the Carisolv^a solution, a gel containing amino acids and sodium hypochlorite, which removes carious dentin efficiently and effectively,¹⁷ with a significant reduction in bacterial counts¹⁸ and no attack on healthy collagen fibrils.¹⁹ Most recently, a new gel for chemomechanical removal of caries, based on papain, chloramine and toluidine blue, was developed in Brazil and named Papacárie^b (a word that means "eating caries"). Bussadori *et al*²⁰ demonstrated that Papacárie was found to be easy to manipulate, simple and inexpensive, as well as effective in removing infected tissues.

The influence of these chemical agents for removing carious lesions on the adhesion of adhesive systems to dentin has been reported. Studies²¹⁻²³ showed that the Carisolv system did not affect the bonding of adhesive systems to dentin. Furthermore, the smear layer-dissolving and modifying adhesive systems could potentially benefit from chemomechanical dentin treatment.²¹ Considering the use of Papacárie, Lopes *et al*²⁴ found that it did not interfere in the adhesion of an etch-and-rinse adhesive system to dentin. Correa *et al*²⁵ also demonstrated that the application of the papain-based gel on primary dentin formed an amorphous layer, similar to the smear layer, with few exposed dentin tubules and the occurrence of abundant tag formation after the use of an etch-and-rinse adhe-

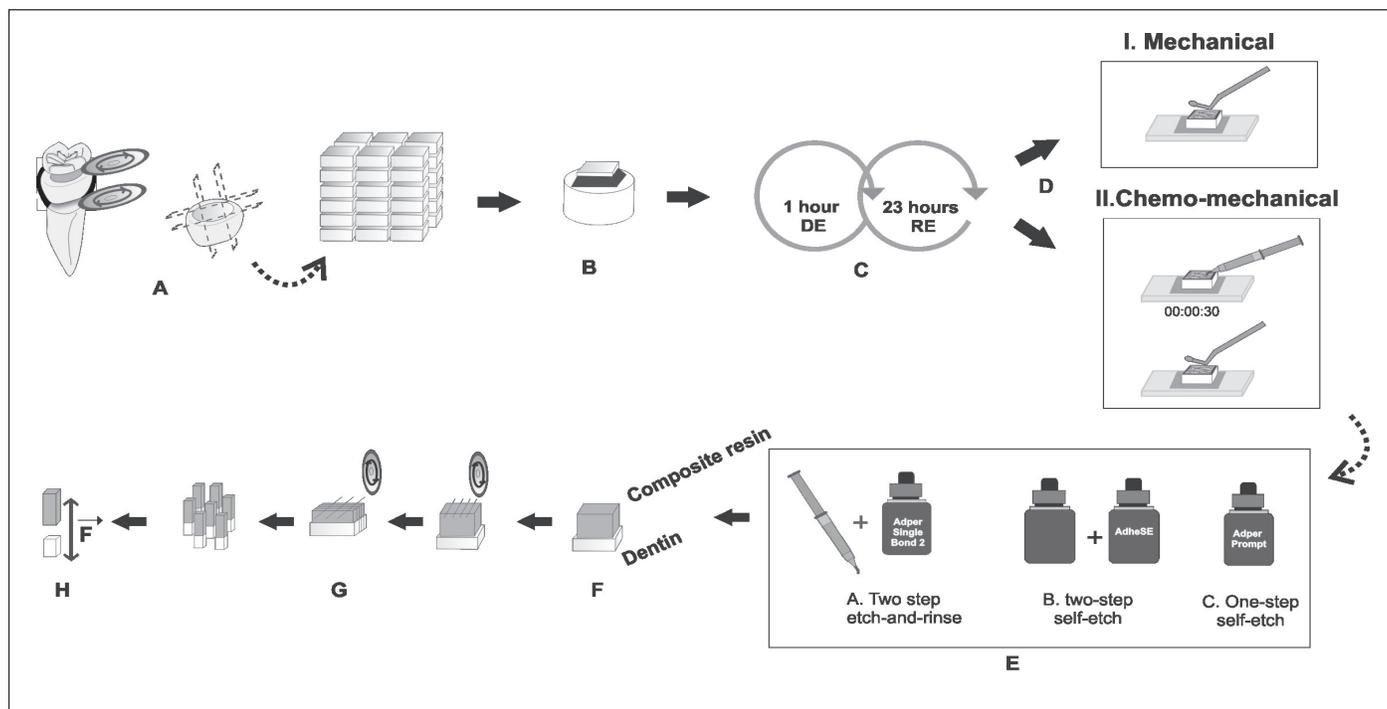


Fig. 1. Study design. (A) Obtaining dentin slabs: roots and occlusal third of crowns were removed to expose superficial dentin. Crowns were sectioned into 3 mm-high specimens and (B) flattened with decreasing granulations of water abrasive paper; (C) Dynamic model of dentin demineralization: 1 hour in demineralizing solution followed by 23 hours in remineralizing solution; (D) Dentin caries removal method: I. Mechanical excavation; II. Chemical treatment - Papain-based gel (Papacarie), followed by mechanical excavation; (E). Adhesive systems tested; (F) Resin composite restoration; (G) Sectioning samples into 1x1 mm sticks (H) and loaded in tension.

sive system. However, it is still not known whether Papacarie has an effect on the adhesion of self-etch adhesive systems.

Therefore, the present study evaluated the microtensile bond strength of etch-and-rinse and self-etch adhesive systems to demineralized dentin after the use of a papain-based chemo-mechanical method.

Materials and Methods

Experimental design - The factors under study were:

Dentin caries removal method at two levels:

- (1) Mechanical removal (removal with mechanical excavators)
- (2) Chemomechanical removal (use of a papain-based gel for caries removal – Papacarie – associated with mechanical removal using the non-cutting part of manual excavators).

Type of adhesive system at three levels:

- (1) Two step etch-and-rinse adhesive system (Single Bond^c);
- (2) Two-step self-etch adhesive system (AdheSE^d);
- (3) One-step self-etch adhesive system (Adper Prompt^e).

The association between dentin caries removal method and adhesive system resulted in six experimental groups. Composition and description of each material used in this study is shown in Table 1. The experimental sample consisted of 60 demineralized dentin slabs, randomly distributed into the six groups (n=6). The response variable was microtensile bond strength means, expressed in MPa. The experimental design was completely randomized.²⁶

Figure 1 shows the experimental design of the microtensile bond strength testing.

Specimen preparation - After approval of the Research Ethics Committee (Protocol No. 2006/0232), non-erupted human third molars, extracted for reasons not related to those of the present research, and stored in thymol (0.1%, pH 7.0) after extraction,

Table 1. Description of materials used in this study.

Brand name	Type	Composition*
Papacarie (PP)	Papain-based gel for chemomechanical removal of caries.	Papain, chloramine, toluidine blue, salts, conservative and thickener (pH=6.97 ± 0.02)**
Adper Single Bond 2 (SB)	Two-step etch-and-rinse adhesive	BisGMA, HEMA, copolymer of acrylic and itaconic acids, water, ethyl alcohol, glycerol 1, 3-dimethacrylate, diurethane dimethacrylate, silane treated silica, water
AdheSE (SE)	Two-step self-etch adhesive	Primer: Mixture of dimethacrylate phosphonic acid acrylate, water, initiators and stabilizers Bond: Mixture of dimethacrylates, HEMA, SiO ₂ , initiators and stabilizers.
Adper Prompt (Pt)	One step self-etch adhesive	Liquid A: DI-HEMA phosphate; BisGMA, ethyl 4-dimethyl amino-benzoate; DL-camphorquinone Liquid B: water, HEMA

* Based on information provided by manufacturers: BisGMA: Bisphenol A diglycidyl ether dimethacrylate; HEMA: 2-hydroxyethyl methacrylate.

** pH measured in our laboratory.

were used in this experiment. Teeth were submitted to debridging with scalpel blades and periodontal curettes.

Teeth were cross sectioned with a diamond blade^e in a low-speed handpiece, separating the occlusal third of the crown and obtaining a large dentin surface in the middle third that was perpendicular to the long axis of the tooth. Specimens presenting cracks or stains were excluded, resulting in 60 dentin slabs.

The dentin slabs were flattened in a water-cooled polishing machine (Politriz Aropol 2V^f) with decreasing granulations (400 and 600) of water abrasive paper. The final fragments were 3 mm

Table 2. Microtensile bond strength means (n=10) and standard deviations in each experimental group.

Method of caries removal	Adhesive system		
	Single Bond 2(SB)	AdheSE (SE)	Prompt (Pt)
(M) Mechanical	18.5 (2.4) Aa	13.9 (3.6) Aa	16.5 (3.0) Aa
(PP) Chemomechanical	15.6 (7.5) Aa	14.4 (5.0) Aa	17.2 (4.6) Aa

Means with the same letters were not statistically significant ($P < 0.05$).

were 3-mm high, measured with a digital caliper.⁸

The pulp chambers were prepared to be filled with a composite resin with the aim of increasing the stick lengths and facilitating their fixation to acrylic devices for microtensile bond strength tests: internal dentin walls were cleaned and etched with a phosphoric acid (Condac 37^h) for 15 seconds, washed for the same time and gently dried with absorbent paper. The adhesive system Adper Single Bond 2 was applied in two consecutive layers; the remaining solvent was evaporated with a brief, gentle dry air jet for 10 seconds and light polymerized for 20 seconds. After that, the pulp chamber of each tooth was filled with a resin composite (Filtek Z250,^c UD color) using the incremental technique and light polymerized with a halogen light curing unit (Ultralux EL¹).

Obtaining demineralized slabs - To obtain artificial caries lesions (demineralized slabs), a dynamic model similar to that used by Hara *et al*²⁷ was used. Each slab was separately immersed in a demineralizing solution for 1 hour. Subsequently, it was washed in distilled and deionized water and placed in a remineralizing solution to complete a 23-hour cycle. When this time had elapsed, slabs were washed again, dried and immersed in the demineralizing solution to start a new cycle. Three cycles were performed, maintaining a temperature of 37°C during the procedures.

Chemomechanical agent application and dentin caries removal - For Group 1, demineralized dentin slabs were submitted to manual excavation only, in which five to-and-from movements were made with the non-cutting edge of the curette (#11½, S.S. White Duflex¹). Manual excavation was repeated with another five to-and-fro movements. The cleaned dentin was rinsed with water.

For Group 2, the dentin slabs received an application of the chemical agent. The manufacturer's recommendations were followed and the product was applied on the dentin surface and left to act for 30 seconds (acute lesions). The dentin was scraped with the non-cutting edge of the curette and five to-and-fro movements were made. The gel was applied again, and manual excavation was repeated with another five to-and-fro movements. Residual gel was removed with absorbent paper.

After cleaning, the fragments were kept in their individual receptacles containing distilled water, at relative humidity for 24 hours.

Application of adhesive systems - All bonding procedures were performed in accordance with the manufacturers' instructions. For Group A (1A and 2A), the etch-and-rinse adhesive system Adper Single Bond 2 was used: Surfaces were treated with the 37% phosphoric acid gel for 15 seconds, rinsed for the same time and gently dried. Then the adhesive system was applied in two consecutive layers; the remaining solvent was evaporated

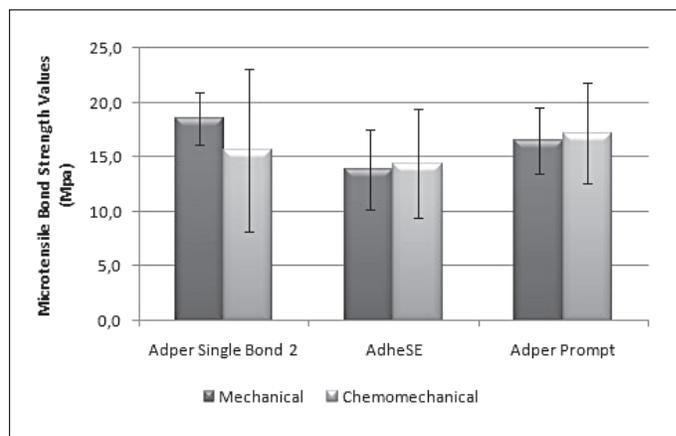


Fig. 2. Microtensile bond strength of the different groups.

with a brief, gentle dry air jet for 10 seconds and light polymerized for 20 seconds.

For Group B (1B and 2B), the two-step self-etch adhesive system AdheSE was used: the primer was applied and dispersed with air for 30 seconds, and dried for 3 seconds. Then the adhesive was applied for 5 seconds, dispersed for 3 seconds and polymerized for 10 seconds.

For Group C (1C and 2C), the one-step self-etch adhesive system Adper Prompt was applied, dispersed for 30 seconds, gently dried for 10 seconds and light polymerized for 10 seconds.

After that, a composite resin block (Filtek Z 250, A1 color), measuring 5 x 5 mm (height x width) was built on the bonding surface, by the incremental technique. Each layer of composite (approximately 2 mm-thick) was individually light polymerized for 40 seconds, with a visible light-curing unit. Finally, the restoration was light polymerized for 20 seconds on each of its two sides. The output of the light-curing unit was periodically measured with a radiometer with a mean range of 620 mW/cm². Throughout the specimen processing, care was taken to avoid dehydration of the samples.

Microtensile bond strength (μ TBS) testing - Tooth-resin blocks were sectioned perpendicular to the bonding surface, into 1.0 mm thick slabs, using a water-cooled diamond disk. By rotating samples 90° and again sectioning them lengthwise, multiple beam-shaped sticks were obtained,^{28,29} each with a cross-sectional surface area of 1.0 mm².

Sticks were kept in distilled water at 37°C, for 24 hours, and subsequently attached to a specific testing device for μ TBS testing, with a cyanoacrylate adhesive (Super Bonder Gel^k). They were subjected to tensile stress in a universal testing machine, at a crosshead speed of 0.5 mm/minute and a 50N load cell until fracture. The bond strength values were expressed in kgf/cm², and converted to MPa after measuring the cross-sectional area at the fracture site with a digital caliper. The comparison was made using the mean value of each tooth (n=10).

Fractured specimens were observed under a stereomicroscope (EK3ST¹) at x30 magnification to assess the failure modes, which were classified as adhesive (lack of adhesion), cohesive in dentin (failure of the dental substrate), cohesive in resin composite (failure of the resin composite) or mixed (adhesive and cohesive failures).

Data were analyzed by two-way ANOVA followed by the Tukey's test. The level of significance adopted was 5%.

Table 3. Percentage of fracture types of μ TBS samples as analyzed by stereo microscopy.

	M-SB	M-SE	M-Pt	PP-SB	PP-SB	PP-Pt
Adhesive	57.1	75.6	59.5	66.7	71.4	73.8
Mixed	0	0	0	0	0	0
Cohesive enamel	16.7	9.8	9.5	14.3	9.5	0
Cohesive resin	26.2	14.6	31.0	19.0	19.0	26.2

Results

The means and standard deviations of microtensile bond strength are presented in Table 2. Data analysis revealed that the application of both chemomechanical and mechanical methods on demineralized dentin yielded μ TBS values that were statistically similar among them, showing that the papain-based gel did not influence the adhesion of different adhesive systems to demineralized dentin (Fig. 2, Table 2). Fracture mode assessment showed a predominance of adhesive failures in all groups, irrespective of the method for caries removal and adhesive system (Table 3).

Discussion

As one of its most important principles, the current minimal intervention dentistry concept proposes minimal surgical intervention of dentin caries lesions,¹ with the aim of preserving dental tissue that can be further remineralized. In this context, there is a need for adopting conservative treatments that preserve the structures affected by caries lesions. It has been clearly noted that conventional dentin caries removal with rotary instruments and drills leads to excessive wear of dental tissue.¹⁰ Therefore, the chemomechanical removal of caries seems to be a suitable method of dental treatment within the philosophy of preservative dentistry,^{10,30} since its application allows the removal of only the outer layer of carious dentin (infected), thus preserving the bottom uninfected and demineralized dentin.⁴

Carisolv, a chemomechanical method for caries removal, is a product that contains sodium hypochlorite and three amino acids. The reaction of these amino acids with sodium hypochlorite neutralizes the aggressive effect of Carisolv on healthy dental tissues and reduces the effect of whole collagen denaturing, thus rupturing only the link between the affected collagen fibrils. As a result, Carisolv has been shown to be an effective method that removes dentin caries selectively, with no adverse effect on healthy collagen fibrils and consequently on the adhesion of restorative materials to dentin.²¹⁻²³ Moreover, it appears to be more comfortable for most patients.¹⁶

In view of the advantages of Carisolv and with the aim of reducing the cost of the final product, a new gel formulation, based on papain, namely Papacárie was developed in Brazil, in 2003. Papain is a low-cost Brazilian raw material²⁰ which is similar to the human pepsin enzyme and acts on necrotic tissues and secretions, without reacting with sound tissues close to the lesion. This is possible because the infected and necrotic tissue does not have 1-anti-trypsin, an enzyme that inhibits protein digestion.³¹ Therefore, the papain has a free way to break molecules of collagen partially degraded by the caries process.³⁰ According to Bussadori *et al*³² the papain interacts with exposed collagen by the dissolution of dentin minerals through

bacteria, making the infected dentin softer, and allowing its removal with non-cutting instruments without local anesthesia and burs. This new biomaterial did not demonstrate cytotoxicity *in vitro* in cultures of fibroblasts.³³ Also, it has a neutral pH, near to 7 (6.97 ± 0.02). The other components of Papacárie are toluidine and chloramine. Chloramine affects the structure of collagen, softening the carious tissue and facilitating its removal.³⁴

Demineralized dentin for laboratory studies can be obtained by the use of extracted teeth with natural carious lesions, but it must be considered that these teeth differ from one to another regarding size, shape, surface irregularity and depth of caries-affected dentin, which may influence bonding studies. In the present study, chemical treatments were used to simulate caries lesions because they are the simplest and most useful laboratory caries models.³⁵ Furthermore, these dynamic models of pH cycling (remineralizing and demineralizing) can be done under highly controlled conditions³⁶ and properly simulate the caries-affected dentin surfaces for bond testing.³⁷

In the present study, the application of Papacárie gel had no effect on the adhesion of all the tested adhesive systems to dentin that was demineralized according to the dynamic model of pH cycling similar to that used by Hara *et al*.²⁷ These findings corroborate the studies conducted by Lopes *et al*²⁴ in which the Papacárie gel did not affect the bonding of an etch-and-rinse adhesive system. These findings may be correlated with the fracture types, in which a prevalence of adhesive failures was observed in all groups, irrespective of the method of caries removal. Others²¹⁻²³ reported that chemomechanical removal with Carisolv did not interfere in adhesion to caries-affected dentin.

It has been suggested that morphological alterations in demineralized dentin may occur due to chemomechanical caries removal with Carisolv, but the findings are varied. Banerjee *et al*³⁰ indicated that Carisolv removed the smear layer and exposed dentin tubules, although others^{38,39} reported that Carisolv treatment did not totally remove the smear layer. Moreover, a layer of cutting debris and fine marks^{38,39} are left by hand instruments.³⁹ The blunt hand instrument was used to remove the gel with degraded dentin, because it was aimed to follow strictly the manufacturer's instructions, but if there is a hypothesis that mechanical excavation may have an important influence on the formation of these smear layers, and if this procedure were performed in both mechanical and chemomechanical caries removal methods, the resultant superficial layer of demineralized dentin may be morphologically similar. This may explain the results of the present study, in which the chemomechanical removal of caries did not influence the adhesion of different adhesive systems. Furthermore, the literature only demonstrates morphological findings after chemomechanical caries removal with Carisolv. Correa *et al*²⁵ demonstrated that Papacárie formed an amorphous layer, similar to the smear layer and few exposed dentin tubules in primary dentin; and the application of an etch-and-rinse adhesive system led to abundant tag formation. To date there has been little information about the effects of Papacárie gel on the topography of demineralized and permanent dentin as well as on the morphology of adhesive interfaces of both etch-and-rinse and self-etch adhesives on this resultant dentin surface. Further studies are required to confirm this.

Despite the possible morphological features in demineralized dentin after chemomechanical caries removal with Papacarie, bonding was not affected with any of the adhesive systems used in this study. Three types of adhesive systems, according to the classification of Van Meerbeek *et al*⁴⁰ were used: a two step etch-and-rinse adhesive system (Single Bond 2), a “strong” one step self etch adhesive system (Prompt) and an “intermediately strong” two-step self-etch adhesive system (AdheSE). In theory, the underlying bonding mechanism of a “strong” one-step self-etch adhesive is primarily diffusion-based, similar to the etch-and-rinse mechanism. But in healthy dentin, literature shows the μ TBS of both two-step etch-and-rinse and two-step self-etch adhesive systems to pooled dentin are similar, with the significantly least favorable results recorded for the one-step self-etch adhesives.⁴¹ In caries-affected dentin, it has been observed that an etch-and-rinse adhesive system exhibits higher bond strengths to caries-affected dentin than the self-etch systems⁴² because the caries-affected dentin contains dentin tubules that are filled with acid-resistant minerals that can be solubilized with the application of phosphoric acid on the dentin,⁴³ thereby contributing to better resin retention. Hara *et al*³⁶ using a single model of dentin demineralization with an acidic-buffered solution that allowed the formation of highly demineralized dentin, observed that a two-step etch-and-rinse adhesive system was able to penetrate into dentin forming an extensive resin-dentin interdiffusion zone, which was similarly reported in natural caries. Erhardt *et al*³⁷ also found differences in morphological pattern and μ TBS of an etch-and-rinse adhesive system to sound and artificially created caries-affected dentin with a proposed pH-cycling model (eight cycles, demineralization for 3 hours followed by mineralization for 45 hours). The present study used a different dynamic pH cycling model with a few hours in the demineralizing solution (three cycles, demineralization for 1 hour, followed by remineralizing for 23 hours). Despite that Hara *et al*²⁷ had already demonstrated the efficiency of this demineralizing protocol to evaluate caries inhibition around adhesive restorations in dentin, it is not clear how the morphological aspect of the resultant demineralized dentin obtained under the conditions of the present study is similar to natural caries. When considering natural caries-affected dentin, the demineralized portion of caries-affected dentin below the hybrid layer serves as a defect in the adhesive interface in terms of mechanical strength.⁴⁴ Çehreli *et al*⁴⁴ found a prevalence of cohesive failures with dentin, even within lower bond strength values than those obtained in the present study. This weakening of dentin substrate may have not occurred within the pH cycling model used here to obtain demineralized specimens and the predominance of adhesive failures might have occurred because microtensile bond strength must improve to force failure of the interface between the adhesive and dentin, rather than if dentin fails cohesively.⁴⁵ Moreover, three types of adhesive system were used, and there are few reports comparing the adhesive interface morphology among these types of adhesives and artificially-created caries affected dentin. Additional morphological studies would be of relevance to describe the topography of demineralized dentin obtained with a dynamic Ph cycling model and its effect on bond strength values and the adhesive interface morphology. In this context, the results of the present study

would be better explained.

Finally, it was noted that bond strengths in the present study appeared to be somewhat low compared to other studies of bonding to actual caries-affected dentin surfaces created by other excavation techniques, such as the Carisolv chemomechanical method,²¹ round steel bur in a slow-speed handpiece and Er:YAG laser.⁴⁶ On the other hand, Cehreli *et al*⁴⁴ found lower bond strength values for conventional bur; Carisolv, a sonic preparation system and air abrasion. In all these studies, bond strength testing was performed in extracted human third molars with natural caries lesions, and not in third molars that were submitted to a dynamic model of pH cycling, as done in the present study. In addition, the adhesive systems differ from one experiment to another. These points show that there is a difficulty in comparing results among other techniques for caries removal. So, in an attempt to obtain a consensus regarding bond strength in caries-affected dentin, further studies should be focused on comparisons of caries removal techniques in a single experiment, using a controlled model of pH cycling, as used in this study.

Based on the results of the present study, the microtensile bond strength of etch-and-rinse and self-etch adhesive systems to demineralized dentin was not affected by the use of a papain-based chemomechanical method for caries removal.

- a. MediTeam, Gothenburg, Sweden.
- b. Formula e Ação, São Paulo, SP, Brazil.
- c. 3M ESPE, St. Paul, MN, USA.
- d. Ivoclar, Schaan, Liechtenstein.
- e. KG Sorensen, Barueri, SP, Brazil.
- f. Arotec, São Paulo, SP, Brazil.
- g. Mitutoyo, Tokyo, Japan.
- h. FGM Produtos Odontológicos, Joinville, SC, Brazil.
- i. Dabi Atlante, Ribeirão Preto, SP, Brazil.
- j. S.S. White, Rio de Janeiro, RJ, Brazil.
- k. Henkel Ltda., Itapevi, SP, Brazil.
- l. CQA, São Paulo, Brazil.

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