Original Research
Cariology

Bonding of universal adhesive system to enamel surrounding real-life carious cavities

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Abstract: This study aimed to evaluate the bond strength of a universal adhesive system to enamel surrounding real-life carious cavities. Twenty-eight permanent molars (n = 7) with carious lesions in dentin were subjected to selective carious tissue removal to firm dentin and had their crowns sectioned longitudinally. A universal adhesive system (Single Bond Universal [SBU] used in either etch-and-rinse and self-etch strategies) was compared with an etch-and-rinse Adper Single Bond 2 (ASB) and a self-etch Clearfil SE Bond (CSE) adhesive systems (control systems). Adhesives were applied on the enamel, assumed demineralized, surrounding the cavity margins and on sound enamel (control substrate). Composite cylinders were built (0.72 mm^2) and microshear bond strength (μSBS) test was performed after 24 h of water storage. The μSBS values (MPa) were analyzed using two-way ANOVA and Tukey's post hoc tests ($\alpha = 0.05$). Bond strength values obtained in demineralized enamel surrounding carious cavity margins were significantly lower than that obtained in sound enamel (distant from carious cavity margins) ($p = 0.035$). The bonding strategy of the SBU did not influenced the bond strength values, which were higher than that obtained with ASB. CSE showed similar μSBS values to ASB and SBU in the self-etch mode. In conclusion, the bond strength to enamel assumed demineralized is lower than to sound enamel. The enamel surrounding carious cavities jeopardize the bonding of universal adhesive system. The bond strength of universal adhesive is similar, regardless to bonding strategy.

Keywords: Dental Enamel; Dental Caries; Adhesives; Dental Materials; Dental Bonding..

Introduction

The technique of selective removal of carious tissue[1], formerly known as partial caries removal, is widely adopted as a minimally invasive approach in the restorative treatment of primary and permanent teeth to reduce the risk of pulp exposure,[2,3,4] treatment costs,[5] post-operative complications, and the overextension of the cavities that weakens tooth structure increasing the risk of restoration failure.[6] In selective removal of carious tissue, peripheral enamel and dentin should be prepared to hard/sound tissue while soft or firm carious tissue should be left over the pulp.[7,8] This strategy for carious tissue removal is the treatment of choice for both dentitions and, furthermore, it is a less time-consuming and user-friendly technique, along with the use of universal adhesive systems.

These versatile adhesives, also called multi-mode systems,[9,10] can be used in strategies with total or selective acid conditioning in enamel or in self-etch mode. Recently, some studies evaluated the performance of these adhesives to dental enamel and most of them indicated that etch-and-rinse strategy provided higher bond strength to enamel.[11,12,13,14,15]

More attention has been given to the dentin-pulp complex when referring to the selective removal of carious tissue, including the arrest of the caries process[3,16] and preservation of pulp vitality.[2,3,4] However, bonding to enamel should be a concern, since the stability of the resin-bonded dentin[17] and effectiveness of marginal sealing[18] depends on the bonding to surrounding enamel. In both the non-selective and selective removal to firm dentin approaches, the excavation of carious dentin is performed only with manual or low-speed rotary instruments[16,19] and the access to the lesion is performed with high-speed rotary instruments only when needed.[4] Thus, it is possible that this conservative enamel preparation may result in some degree of demineralized enamel left at the cavity margins. Furthermore, the visual-tactile inspection is a subjective yet widely used criteria to determine the end-point of caries removal.[20,21]

Although few studies have considered the demineralized enamel as a substrate in bond strength evaluations, bonding to demineralized enamel seems to be lower than to sound enamel,[12,22] probably due lower mineral content, higher porosity of the surface,[23] widened intercrystalline spaces, and consequently a larger pore volume than sound enamel,[24,25] producing an unsatisfactory etching pattern and infiltration of resin monomers.[22]

Therefore, considering that enamel surrounding carious cavities after carious tissue removal is probably demineralized despite the absence of clinical signs of demineralization, this study aimed to evaluate the bond strength of a universal adhesive system to enamel surrounding real-life carious cavities. The null hypothesis was that there is no difference in the bonding values regardless the enamel proximity of carious cavities.

Methodology

The influence of the enamel proximity to cavities on the bond strength values was assessed by microshear bond strength (μSBS) test. Enamel surrounding caries was assumed demineralized and enamel distant from caries was considered sound enamel. The experimental design of the study is presented in Figure 1. The research protocol received previous approval from the Local Ethics Committee.

Sample preparation

Twenty-eight extracted human permanent molars were selected from a pool of extracted teeth according the following inclusion criteria: presence of carious cavities involving dentin on occlusal or occlusal-proximal surfaces (International Caries Detection and Assessment System - ICDAS 5 or 6);[26] no restorations, cracks, enamel defects or signs of previous restorative intervention. Teeth were stored in 0.5% chloramine T solution at 4ºC for up to 30 days before being used.

Teeth were randomly allocated into four groups (n = 7) by a program to generate random number list (Random.org - Randomness and Integrity Services Ltd., Dublin, Ireland) according to the adhesive system: Single Bond Universal (SBU) (3M ESPE; St Paul, USA) in both self-etch (SE) and etch-and-rinse (ER) application modes; Adper Single Bond 2 (ASB) (3M ESPE; St Paul, USA) an etch-and-rinse system; and Clearfil SE Bond (CSE) (Kuraray Noritake; Tokyo, Japan), a self-etch system.

A single experienced operator performed the selective removal of the carious tissue. The decayed tissue of the sidewalls was completely removed by using low-speed metal burs and/or hand excavator, according to the size of the cavity[4] using the visual-tactile criteria in assessing the end-point of carious tissue removal. On the cavity floor, carious tissue removal was continued until firm dentin ('leathery' consistency) was encountered and was left over the pulp.[27]

The presence/absence of white spot lesion in the enamel was verified after the selective carious tissue removal. If the enamel surrounding cavity margins presented some visible white spot lesion (opaque enamel with a dull-whitish aspect) the tooth was excluded from the study and replaced. Afterwards, the crowns of all teeth were longitudinally sectioned at the center of the

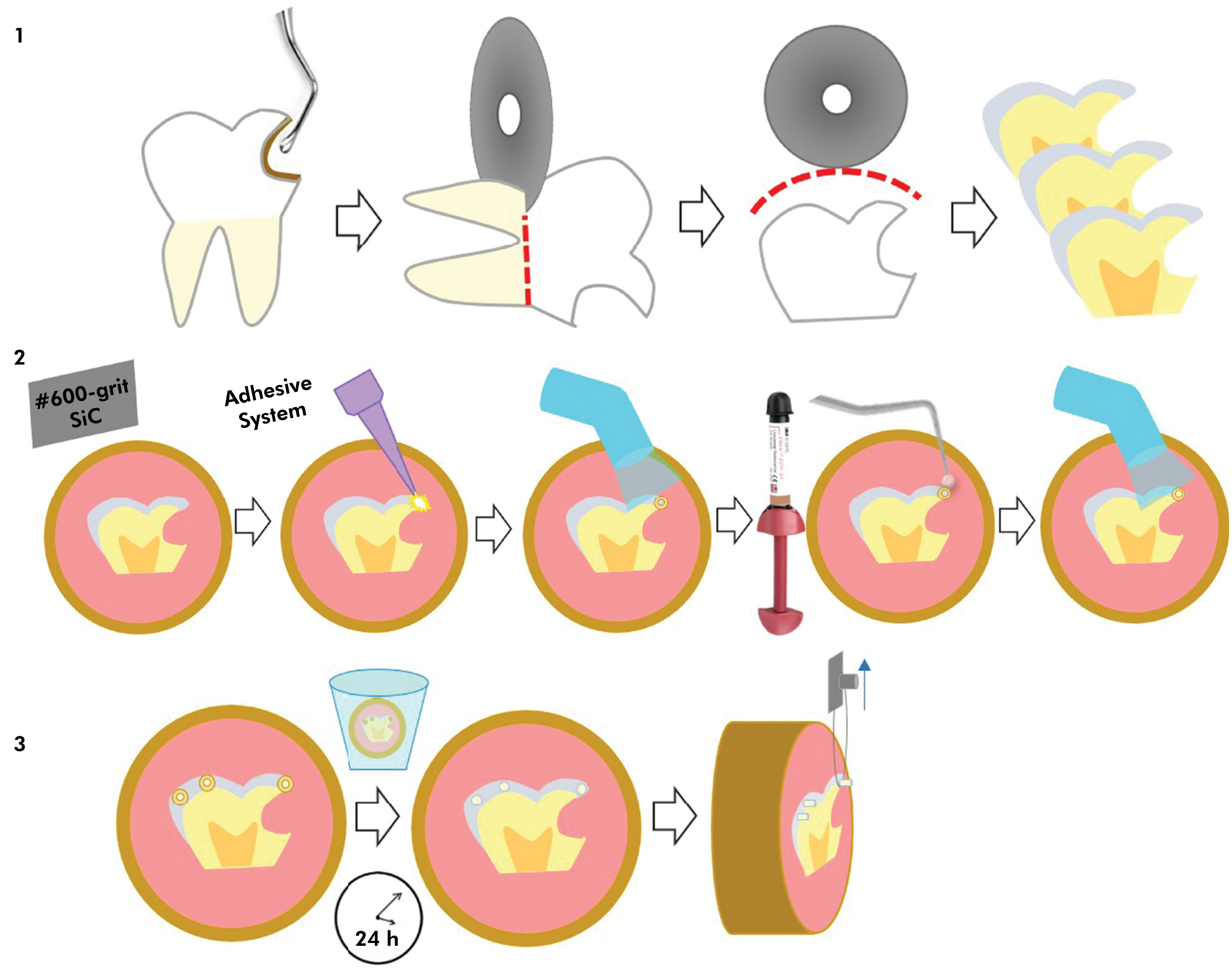

Figure 1. Experimental design of the study: 1 - Sample preparation. 2 - Restorative procedures. 3 - Microshear bond strength test.

cavities using a low-speed water-cooled diamond saw in a cutting machine (Labcut 1010, Extec Co., Enfield, USA) to obtain at least two sections (specimens) of each tooth. Thereby, flat transversal enamel surfaces were obtained, as required for the microshear bond test, preserving the presumed-demineralized enamel. Each section was glued with cyanoacrylate in PVC rings embedded with self-curing acrylic resin (JET Clássico®, São Paulo, Brazil), constituting a specimen. Specimens were manually ground with 600-grit SIC paper under running water for 60 s to create standardized flat enamel surfaces.[28]

Bonding and restorative procedures

The adhesive systems were applied to enamel according to the manufacturers' instructions (Table 1). Starch tubes (Isabela, Ind. e Com. Food, São Paulo, Brazil) with 0.96 mm of internal diameter and 1.0 mm in height,[29] were positioned over the enamel surface prior the light curing the adhesives with a light emitting diode curing unit (Emitter B, Schuster, Santa Maria, Brazil) with a light output of at least 1,250 mW/cm^2. The devices own radiometer quantified the output power. The tubes were carefully filled with composite resin (Filtek Z250 XT, 3M ESPE, St Paul, USA; shade A2) and light cured for 20 s. At least two cylinders of composite resin were built in each specimen, one over the enamel surrounding carious cavity (as close as possible to the outer surface) and one over the sound enamel (at the opposite side of the carious cavity). A single trained operator performed all adhesive and restorative procedures at room temperature.

Table 1. Materials used in the study.

Material	Manufacturer	Lot. No.	Classification	Composition	Application mode
Single Bond Universal*	3M/ESPE (St. Paul, MN, USA)	1508500365	Self-etch	MDP phosphate monomer, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	1. Apply the adhesive to the enamel with a microbrush and rub it in for 20 s 2. Gentle air for 5 s 3. Light-cure for 10 s
			Etch-and-rinse	37% phosphoric acid (DFL, Rio de Janeiro, RJ, Brazil) MDP phosphate monomer, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	1. Apply etchant for 15 s 2. Rinse for 15 s 3. Air dry to remove excess of water 4. Apply the adhesive using the self-etch mode
Adper Single Bond 2**	3M/ESPE (St. Paul, MN, USA)	1501300547	Two-step etch-and-rinse adhesive	37% phosphoric acid (DFL, Rio de Janeiro, RJ, Brazil) BIS-GMA, HEMA, dimethacrylate, amines, methacrylic copolymer of polyacrylic and polyitaconic acids, ethanol, water, photoinitiator	1. Apply etchant for 15 s 2. Rinse for 15 s 3. Air dry to remove excess of water 4. Apply 2 consecutive coats of adhesive for 15 s with gentle agitation 5. Gently air for 5s to evaporate the solvent 6. Light-cure for 10 s
Clearfil SE Bond	Kuraray Noritake, Tokyo, Japan	51550	Two-step self-etching adhesive	Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, di-canphoroquinone, aromatic tert-amine, water Bond: 10-MDP, bis-GMA, HEMA, hydrophilic dimethacrylate, photoinitiator, aromatic tert-amine, silanized colloidal silica	1. Apply primer to enamel and leave in place for 20s 2. Blow dry for 20s at a distance of 20 cm 3. Apply bond for 20s 4. Gently air dry for 5s 5. Light-cure for 10s

*Scotchbond Universal Adhesive (United States and Europe); **Adper Single Bond Plus (United States and Europe); According to manufacturers' information. Abbreviations: bis-GMA: bisphenol A glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate.

Microshear Bond Strength (μSBS) Test

The specimens were stored in distilled water at 37°C for 24 h. After this period, the starch tubes were gently removed using air/water spray and a probe. Cylinders of composite resin were examined under a stereomicroscope (Stereo Discovery V20, Carl Zeiss do Brazil Ltda., Rio de Janeiro, Brazil) at 10× magnification to verify the presence of bubbles and gaps in the interface or other defects. When defects were found, all the specimens from the tooth were excluded and the tooth was replaced.

Each specimen was individually attached to a universal testing machine (Emic, São José dos Pinhais, Brazil), equipped with a 100 N load cell (10 kgf), with 0.01 N reading resolution and minimum sensitivity of 2 N. A stainless steel wire (0.20 mm diameter) was looped around the composite resin cylinder as closer as possible to the resin/enamel interface. A shear load was applied at a crosshead speed of 1.0 mm/min until failure. To avoid bias, a single and blinded operator carried out bond strength measurement procedures.

Table 2. The μSBS (MPa) means and standard deviations [number of tested cylinders/pretest failures] for all experimental groups.

Adhesive system (etching mode)	Enamel location	Mean (SD)	Composite Resin cylinder
Single Bond Universal (ER)	Surrounding carious cavities	11 (1.6)	21/2
	Sound enamel	14 (2.4)	23/3
Single Bond Universal (SE)	Surrounding carious cavities	9.9 (1.6)	19/2
	Sound enamel	11 (2.4)	25/2
Adper Single Bond 2	Surrounding carious cavities	7.8 (2.1)	19/1
	Sound enamel	8.4 (1.3)	21/2
Clearfil SE Bond	Surrounding carious cavities	8.3 (2.6)	17/3
	Sound enamel	9.0 (3.3)	221

Failure mode

All debonded specimens were observed under stereomicroscope at 40× magnification by a trained and blinded examiner to determine failure mode: cohesive failure within enamel or resin and interfacial failure.[17] Only specimens with interfacial fractures were considered in the calculation of bond strength values.

Four representative specimens from each experimental group were prepared for failure mode evaluation under scanning electron microscope (SEM). Specimens were dehydrated in ascending degrees of ethanol (50, 75, and 90% for 5 min each, and 100% for 3 h)[30] and kept in vacuum for 24 h. Subsequently, they were gold sputter-coated for SEM observation.

Statistical analysis

The tooth was considered the experimental unit. Thus, the μSBS values of all composite cylinders from the same tooth, according the enamel location, were averaged for statistical purposes. The sample size of 7 teeth per group was estimated previously considering an 80% power, a coefficient of variation of 20%, and assuming a two-sided 5% significance level for comparisons. Pretest failures (PTFs) that occurred during specimens' testing preparation were not included in the statistical analysis because were equally distributed in all groups.

The normal distribution of the data and equality of variances were confirmed with Kolmogorov-Smirnov and Cochran tests, respectively. The μSBS means were submitted to two-way ANOVA with pairwise comparisons (enamel location *vs.* adhesive system) and Tukey's *post hoc* tests at a significance level of 5%. All statistical analyses were performed using the Minitab software (Minitab Inc., State College, USA).

Table 3. The μSBS (MPa) means and standard deviations [number of tested cylinders/premature failures] considering the factors "adhesive system" and "enamel location".

Adhesive System (etching mode)	MPa
Single Bond Universal (ER)	12.3 ± 1.8^{a} [44/5]
Single Bond Universal (SE)	10.5 ± 0.8^{ab} [44/4]
Adper Single Bond 2	8.1 ± 0.4^{c} [40/3]
Clearfil SE Bond	8.7 ± 0.5^{bc} [39/4]
Enamel location	**MPa**
Surrounding carious cavities	9.3 + 1.5^{a} [76/8]
Sound enamel	10.5 + 2.3^{b} [91/8]

*Different superscript lowercase letters indicate statistically significant differences between rows ($p < 0.05$).

Results

Descriptive statistics, including means, standard deviations, and the number of tested specimens per group are presented in Table 2. The cross-interaction product "enamel location *vs.* adhesive system" was not significant ($p = 0.62$). However, the main factors "enamel location" ($p = 0.03$) and "adhesive system" ($p < 0.00$) significantly influenced the μSBS values. Bond strength of adhesive system to enamel surrounding carious cavities, assumed as demineralized enamel, was approximately 10% lower than that obtained to sound enamel. SBU presented similar values in ER (12.3 ± 1.8) and SE (10.5 ± 0.8) strategies, and higher values than those obtained with ASB (8.1 ± 0.4). CSE (8.7 ± 0.5) showed similar μSBS values to ASB and the SBU in the SE mode. PTFs occurred in all groups (Table 3).

All specimens showed interfacial failures, regardless of the enamel location (Figure 2).

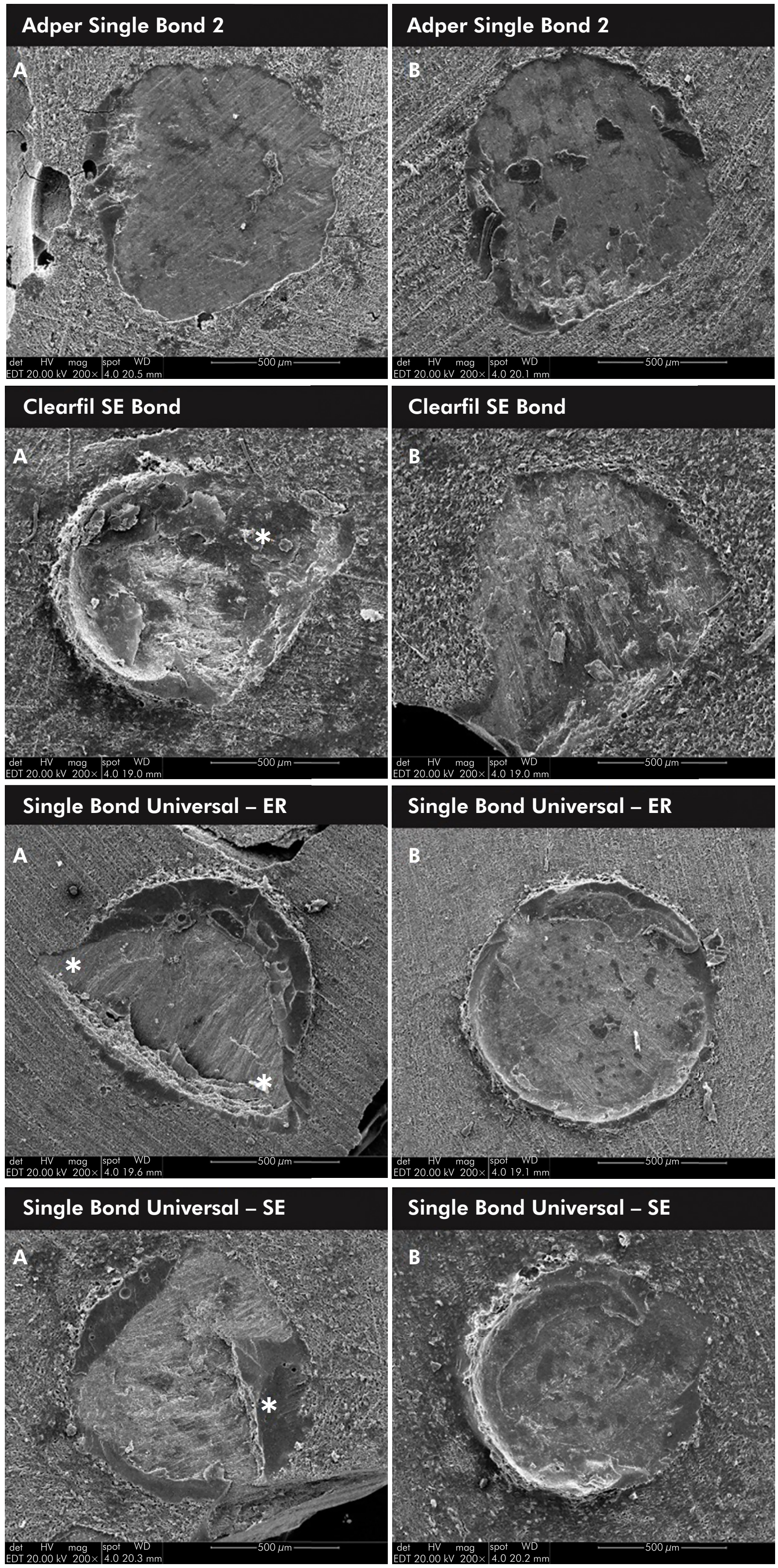

Figure 2. Representative SEM images of specimens with interfacial failures. All the adhesive systems evaluated showed interfacial failures when bonded to assumed-demineralized enamel (surrounding the carious lesion) (A) and sound enamel (B). * represents areas with enamel substrate involvement in the interfacial failures.

Discussion

The current investigation has demonstrated that bond strength of adhesive systems to enamel assumed demineralized was lower than those obtained to sound enamel. Thus, the proximity to the carious lesions can influence negatively the bond strength values to enamel. Moreover, since the use of artificial lesions decreased external validity,[31] the evaluation of the performance of adhesive systems to enamel surrounding real-life carious cavities, consequence of natural caries process, increases the external validity of our findings, besides being original and clinically relevant.

The histological structure of demineralized enamel is different from sound enamel,[23,24,25] which should be related to reduced bond strength due to unsatisfactory conditioning pattern and infiltration of resin monomers.[22] This fact may justify the lower bond strength of adhesive systems to enamel adjacent to dentin carious lesions. This is in line with previous studies that compared sound and demineralized enamel substrates, exhibiting significant differences in bond strength values.[12,22] Considering that the enamel condition may compromise adhesion, it may be suggested changes in the treatment of enamel surrounding cavities subjected to non-selective and selective removal approaches in teeth with 'open' cavities (ICDAS 5 or 6). Since the mineral loss suffered by enamel is not always clinically visible,[32] the enamel suspected of mineral loss and unintentionally left after dentin carious removal could be carefully prepared prior the adhesive procedures with rotary instruments at high speed.

The enamel surrounding carious cavities requires more attention in non-selective and selective excavation technique. Despite that the enamel surrounding open cavities is less demineralized than that in closed lesions,[33] it is still very demineralized and this condition could jeopardize the adhesion, as previously seen in laboratory studies.[12,22] Besides that, the presence of enamel margins is relevant, because dentinal margins showed more marginal imperfections, gaps, and microleakage.[31]

The inner enamel surface was used in the present study, since the cut of slices was transversal. Moreover, the enamel surface was ground with 600-grit silicon carbide paper, which affected the nature of enamel smear layer, turning the substrate more receptive to bonding with self-etch systems.[34,35] Thereby, the performance of Single Bond Universal in both strategies was similar, in agreement with previous studies that evaluated universal adhesives.[10,36,37] However, recent studies evaluating the performance of Single Bond Universal to enamel[11,13,13,14,15,38] stated that the enamel bond strength values are higher when used with the etching strategy.

The results of the two-step self-etch adhesive system (Clearfil SE Bond) did not differ from bond strength values of the Single Bond Universal in the self-etch mode. Both adhesives contain 10-MPD (10-methacryloxydecyl dihydrogen phosphate) as acidic monomer. This monomer contains phosphate groups able to produce ionic chemical bonds with calcium in hydroxyapatite, which may explain the similar performance. Previous studies also found similar bond strength to these adhesive systems.[11,12] Besides that, the way the enamel prisms were exposed in axial longitudinal sections may have influenced the bond strength results,[39] favouring the Single Bond Universal, which has the additional chemical interaction of 10-MDP and hydroxyapatite, compared to Adper Single Bond 2.[11,40] However, both the two-step self-etch system (Clearfil SE Bond) and the etch-and-rinse adhesive (Adper Single Bond 2) presented similar performance on permanent enamel, in accordance with previous studies.[22,29,34] They are considered as control adhesives (gold standards) to self-etch and etch-and-rinse strategies, respectively, and frequently used in bond strength studies.[41,42,43,44,45,46]

The failure mode analysis found interfacial failures in all experimental groups. Moreover, in general, the failures occurred within the limits of the composite cylinder, excepted in specimens from demineralized enamel groups, which presented larger areas of substrate involvement in the fracture. This was expected, since the microshear bond strength test used in this study is associated to few cohesive failures in tooth substrate or composite.[47,48] The frequency of pretest failure was very low and it was not prevalent in a specific group. The use of starch tubes to build the specimens may be the reason for the low occurrence of premature failure. The procedure of starch tube removal is very easy, does not require a blade or other cutting instrument, and does not cause pressure or stress in the interface of resin composite cylinder, which is a great advantage.[29]

This study has limitations, such as the lack of an initial quantitative assessment of enamel surrounding cavities to validate the enamel condition (sound or demineralized), and the use of only visual inspection to assess the enamel condition. Moreover, the enamel surrounding carious cavities was assumed demineralized, because of the suspicion that it presented mineral loss before the start of the carious process. However, future studies should confirm this assumption.

Within the limitations of an *in vitro* study, the results obtained suggest that more attention should be given to the treatment of enamel surrounding carious cavities, since the presence of undetected demineralized enamel might be the cause of failures in adhesive restorations performed after selective caries removal. Further studies evaluating the enamel adjacent to cavities submitted to selective excavation approach are required.

Conclusions

The bond strength of adhesive systems to enamel assumed demineralized is lower than that to sound enamel. The proximity to carious cavities with little or no enamel preparation may jeopardize the bonding of adhesives to enamel.

The bonding strategy does not influence the enamel bond strength of universal adhesive system.

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