Microtensile Bond Strength of Contemporary Adhesives to Primary Enamel and Dentin

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The purpose of this study was to assess bond strength of three self-etching and two total-etch adhesive systems bonded to primary tooth enamel and dentin. ***Materials and methods:*** *Forty extracted primary human molars were selected and abraded in order to create flat buccal enamel and occlusal dentin surfaces. Teeth were assigned to one of the adhesive systems: Adper Scotch Bond Multi Purpose, Adper Single Bond 2, Adper Prompt L-Pop, Clearfil SE Bond and AdheSE. Imediately to adhesive aplication, a composite resin (Filtek Z250) block was built up. After 3 months of water storage, each sample was sequentially sectioned in order to obtain sticks with a square cross-sectional area of about 0.72 mm^2. The specimens were fixed lengthways to a microtensile device and tested using a universal testing machine with a 50-N load cell at a crosshead speed of 0.5 mm/min. Microtensile bond strength values were recorded in MPa and compared by Analysis of Variance and the post hoc Tukey test (a=0.05).* ***Results:*** *In enamel, Clearfil SE Bond presented the highest values, followed by Adper Single Bond 2, AdheSE and Adper Scotch Bond Multi Purpose, without significant difference. The highest values in dentin were obtained with Adper Scotch Bond Multi Purpose and all other adhesives did not present significant different values from that, except Adper Prompt L-Pop that achieved the lowest bond strength in both substrates. Adper Scotch Bond Multi Purpose and Adper Single Bond 2 presented significantly lower values in enamel than in dentin although all other adhesives presented similar results in both substrates.* ***Conclusions:*** *contemporary adhesive systems present similar behaviors when bonded to primary teeth, with the exception of the one-step self-etching system; and self-etching systems can achieve bond strength values as good in enamel as in dentin of primary teeth.*

Keywords: *dentin bonding agents, primary dentition, self-etch adhesives, total-etch adhesives.*
J Clin Pediatr Dent 32(2): 127–132, 2007

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INTRODUCTION

Although there are substantial microstructural differences between permanent and primary dentin, knowledge about permanent teeth is usually transferred to primary teeth, which have greater tubule density and diameter, resulting in a reduced area of intertubular dentin available for bonding.[1] Chemically, primary dentin seems to be more reactive to acidic conditioners,[2,3] which could be explained by the reduced degree of mineralization observed in primary dental hard tissues.[4,5]

The majority of protocols for dentin bonding require acid etching that removes the smear layer, smear plugs and decalcifies the underlying dentinal structures. Peritubular dentin is partially removed and the dentinal tubules acquire a funnel-shaped appearance. Concomitantly, intertubular dentin is etched exposing a collagen-based organic matrix.[6] The application of the resin monomers results in the formation of an hybrid layer,[7] also called the interdiffusion zone.[8] However, it has been shown that if the adhesive does not penetrate the exposed matrix as deeply as acid conditioners do, it can leave exposed collagen at the dentin-adhesive interface[2,9,10]

This incomplete penetration of the monomers results in nanoleakage creating zones susceptible to hydrolytic degradation,[11] and could be greater in primary teeth because its above mentioned dentin characteristics. Because of that, a decrease in acid conditioning time for primary teeth has been suggested[12] or even the use of a weaker acid.[2]

Self-etching adhesive systems have been developed in an attempt to prevent discrepancies between depth of demineralized dentin by acid and primer/adhesive ability to penetrate this layer. Self-etch adhesives are also referred to simplify the adhesive protocol, reducing operative time and making the technique less sensitive.[13] Although adhesion to enamel has undergone few alterations in the past years, the simplification of the technique caused by self-etching adhesives turned its use in primary teeth an interesting alternative. Thus, the behavior of contemporary total-etch and self-etch adhesives in both enamel and dentin of primary teeth claims to be investigated. Therefore, the aim of this study was to assess bond strength of three self-etching, and two total-etch adhesive systems bonded to primary tooth enamel and dentin by the microtensile test.

MATERIALS AND METHODS

Forty sound extracted or exfoliated human primary molars were gathered after obtaining informed consent and approval from the Medical Ethics Commission of the Pontifical Catholic University of Rio Grande do Sul.

Two groups of teeth were randomly formed: One group included 20 teeth to test the enamel substrate (E). On the buccal surfaces of these teeth, enamel was ground with 320-grit sandpaper in order to create a flat surface without exposing the underlying dentin. Another 20 teeth, for testing the dentin substrate (D), had their entire occlusal enamel cut away with a water-cooled diamond saw, producing a middle dentin surface for adhesion. With the intention of standardizing the smear layer formed on the substrate, the surfaces were polished with progressively finer sandpapers of 320µ, 400µ, and 600µ-grit for about 30s with each paper, then washed with water and air-dried.

The prepared enamel and dentin samples were then randomly divided into five subgroups according to the bonding system to be applied (four teeth per adhesive): a total-etch three-step system *Adper Scotch Bond Multi Purpose* (SMP) – 3M ESPE, Dental Products, St. Paul, MN, USA; a total-etch two-step system *Adper Single Bond 2* (SB) – 3M ESPE, Dental Products, St. Paul, MN, USA; a self-etch one-step system *Adper Prompt L-Pop* (PRO) – 3M ESPE, Dental Products, St. Paul, MN, USA; a self-etch two-step system *Clearfil SE Bond* (CL) – Kuraray Medical Inc., Tokyo, Japan; and another self-etch two-step system *AdheSE* (AD) – Ivoclar Vivadent, Schaan Liechtenstein. All adhesive systems were manipulated in accordance with the manufacturers' instructions.

A 6 mm-height composite resin block (*Filtek Z250* – 3M ESPE, Dental Products, St. Paul, MN, USA*)* was built up on the hybridized area, by 3 sequential 2 mm increments of material, each one light cured for 20s with XL3000 (3M ESPE, Dental Products, St Paul, MN, USA) with 500 mW/cm^2 intensity monitored by radiometer (Demetron, Kerr, Orange, CA, USA).

After 3 months of storage in water at 37°C, each sample was sequentially sectioned with a water-cooled diamond disc (Buehler Series 15LC Diamonds) at 500 rpm (Labcut 1010 machine Extec Corp.,London, UK) perpendicularly to adhesive interface, along the mesio-distal and vestibulo-lingual axis, each one 1 mm apart, in order to obtain sticks with a square cross-sectional area of about 0.72 mm^2.

It was estimated that each tooth would yield about 6 sticks, but some sticks presented cracks and visual defects and were excluded, others were lost during cutting or fixing to the microtensile device and were included as null bond strength values in the final calculation of mean bond strength, which resulted in 20 specimens per group eligible for microtensile testing. The specimens were fixed lengthways to the microtensile device using a cyanoacrylate adhesive (Super Bonder Gel, Loctite Brasil Ltd.) and a catalyst (Zip Kicker, Pacer, Rancho Cucamonga, CA, USA). The microtensile strength test was performed using a universal testing machine DL-2000 EMIC (São José dos Pinhais, Paraná, Brazil) with a 50-N load cell at a crosshead speed of 0.5 mm/min. Data on microtensile bond strength values were collected in MPa using a specific computer software *Mtest* (EMIC, São José dos Pinhais, PR, Brazil) connected to the universal machine. After checking the normal distribution (Kolmogorov-Smirnov test, $p>0.05$), the comparisons in mean bond strength values among the 10 groups were statistically analyzed by two-way Analysis of Variance and the *post hoc* Tukey's test (a= 0.05).

Next, scanning electron microscopy (SEM) was used to determine the failure mode in the fractured area of the specimens. The protocol applied was as follows: the fractured tooth-side of the sticks were immersed in 2.5% glutaraldehyde for 12 hours at 4ºC for fixation, washed with 20 mL buffer solution of sodium cacodylate 0.2 M with pH 7.4 for 1 hour, and washed in distilled water three times for 1 minute. For dehydration, the specimens were sequentially immersed in ethyl alcohol (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, and 95% for 20 minutes), then transferred to a critical-point dryer for 30 minutes. The prepared specimens were gold-sputtered at 10mA for 1 minute and observed in SEM (LEO 440, Stereo Scam; Leica Cambridge, En). The failure mode was evaluated by 2 examiners reaching a consensus and classified as: adhesive, partially adhesive associated with cohesive failure in dentin or resin (mixed), and cohesive in dentin or resin.

RESULTS

The mean bond strength values and standard deviations for each bonding material and substrate are presented in Table 1.

Bond strengths were affected by substrate type ($F=14.9$; $p<0.001$) and adhesive system ($F=3.7$, $p=0.005$) although, interaction between both factors was not significant ($F=2.05$, $p=0.09$).

In enamel, Clearfil SE Bond presented the highest values,

Table 1. Mean microtensile bond strengths (MPa) ±SD for each substrate ans different adhesives.

Adhesive	ENAMEL				DENTIN			
	N	Premature failures	Mean (MPa)*	SD	N	Premature failures	Mean (MPa)*	SD
SMP	20	5	15,22 A,B,b	9,57	20	2	32,80 A,a	15,09
CL	20	0	25,66 A,a	13,44	20	5	27,68 A,B,a	17,61
SB	20	1	18,54, A,B,b	13,48	20	0	27,68 A,B,a	13,26
AD	20	1	18,16 A,B,a	10,99	20	3	23,71 A,B,a	16,41
PRO	20	5	13,71 B,a	13,37	20	5	17,05 B,a	12,77

For each vertical column: values with different caption letters indicate statistically significant difference ($p<0.05$). Adhesives are compared within the same substrate. For each horizontal row: values with different lower case letters indicate statistically significant difference between substrates when maintained the same adhesive ($p<0.05$). Specimens with premature failure during specimen preparation were included as null bond strengths in the calculation of mean bond strength.

followed by Adper Single Bond 2, AdheSE and Adper Scotch Bond Multi Purpose, without significant difference, and Adper Prompt L-Pop provided the significantly lowest values.

The highest values in dentin were obtained with Adper Scotch Bond Multi Purpose and all other adhesives did not present significant different values from that, except Adper Prompt L-Pop that achieved the lowest values.

Adper Scotch Bond Multi Purpose and Adper Single Bond 2 presented significantly lower values in enamel than in dentin, although self-etch adhesives presented similar results in both substrates.

Concerning failure mode, the great majority of specimens presented partial adhesive failure between bonding material and substrate associated with a partial cohesive failure in substrate (Figure 1). Figures 2 and 3 show representative specimens of enamel and dentin substrates with mixed failure mode.

DISCUSSION

Despite the differences between primary and permanent teeth, the same protocols have been indicated for both substrates. Some studies have compared primary to permanent teeth, but have found conflicting results on bond strength values recorded,[14-19] and others investigated bond strength using microtensile test in one primary substrate, dentin or enamel.[20-25] This study focused in comparing microtensile bond strength in primary enamel and dentin.

The microtensile test induces stress to concentrate in the

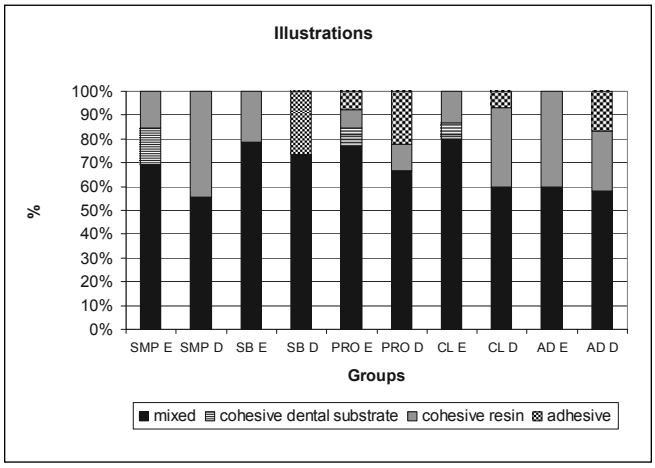

Figure 1. Failure mode after microtensile test percent distribution according to groups

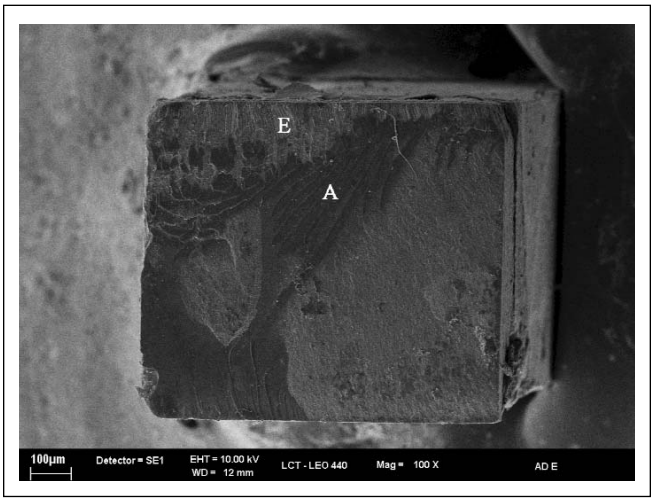

Figure 2. Group AD E (AdheSE on enamel) sample presenting mixed failure pattern (original magnification x100). E, Enamel; A, Adhesive.

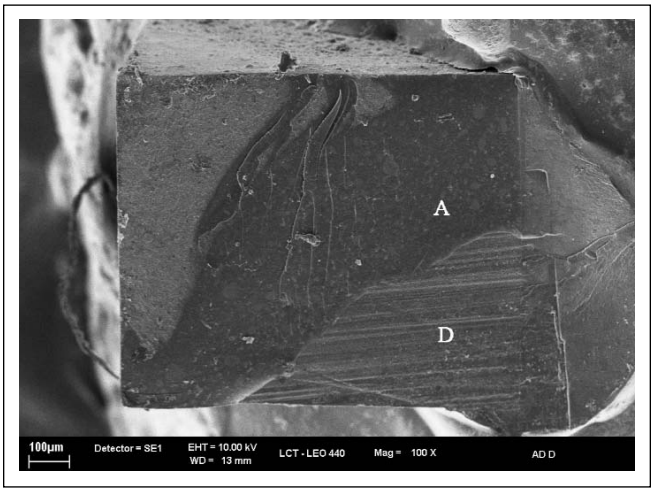

Figure 3. Group AD D (AdheSE on dentin) sample presenting mixed failure pattern (original magnification x100). D, Dentin; A, Adhesive.

bonded area and then displays more reliable results than the conventional tensile test. Specimens for microtensile testing can be prepared in hour-glass or stick shape.[26,27] Sticks result from a sequence of parallel serial sections perpendicular o the bond interface, but it can induce stress at the adhesive interface leading to some cohesive failures within dental substrate. In primary tooth substrates this difficulty is aggravated from its smaller dimensions and physiological resorption that causes loss of dental structure and creates a fragile specimen that is more prone to fracture.[20] During the experimental phase of this study, many specimens were lost due to cohesive failures in substrate. Despite the above-mentioned difficulties, sticks with premature failure during specimen preparation were included as null bond strengths in the final calculation of mean bond strength as suggested by Shono *et al.*[28] This caused a decrease in mean values and an increase in standard deviation, but reflects reality concerning the fragility of the interface or the substrate.

With regard to the substrate, a SEM analysis of the interface demonstrated the presence of more structural defects in enamel than in dentin specimens, which could influence the bond strength when specimens are under load. It was speculated that the brittleness and low elasticity of enamel, especially at the reduced thicknesses of microtensile sticks, may render them more prone to premature failure,[29] which could explain the overall lower bond strength values recorded for enamel in comparison with dentin in the present study, in accordance with Cardoso *et al.*[30] results when evaluating Clearfil SE Bond. These results differ from conventional tensile bond strength test results, thus it has been suggested that the inherent fragility of enamel in the small cross-sections of microtensile specimens was responsible for their failure under relatively low load levels in comparison with dentin.[27] In the present investigation all tested self-etching systems achieved bond strength values as good in enamel as in dentin of primary teeth, while conventional total-etch systems presented lower bond strength in enamel than in dentin.

The null hypothesis was rejected because adhesive systems significantly differ each other to microtensile bond strength when applied to enamel and dentin. The few studies that assessed the adhesive interface in primary enamel aimed to verify adhesive systems bond strength to unground occlusal enamel surfaces in situations simulating fissure sealing.[20,23] Although it has been suggested that microtensile testing allows bonds to irregular surfaces to be tested, because a very small area is used,[31] in the present study the surface was flattened and polished, which provided a situation more similar to clinical conditions. Therefore, a direct comparison with the results of the present study is limited, since the use of unground enamel results in a less homogenous etching pattern, due to the presence of an aprismatic layer. Despite this, the same tendency was observed, one-bottle total-etch systems and self-etching primers present similar bond strengths.[23] Phosphoric acid creates a different etching pattern to that of self-etching primers, particularly the less acid ones, although this morphological differences do not seems to be directly associated with bond strength values.[32]

Adhesion to dentin has always been a controversial matter, and many different results have been found concerning the influence of the substrate and adhesive system. In this study the bond strength values were similar for total-etch and self-etching adhesives in primary dentin, although it was not valid for the one-step self-etching adhesive Prompt L-Pop, which has also presented low performance in other studies.[33,34] The three-step total-etch system, Adper Scotch Bond Multi Purpose achieved the highest bond strength values similar to Clearfill SE Bond, in agreement with the outcomes of Casagrande *et al.*[24] The good performance of Adper Scotch Bond Multi Purpose has been demonstrated in clinical[35] and laboratory studies,[21,23,33] and could be considered a gold standard as regards adhesion. The simplified two-step total-etch system, Single Bond, showed lower bond strength values but not significant in relation to the classic total-etch (Adper Scotch Bond Multi Purpose), inconsistent with the theory that any kind of simplification in the clinical application procedure results in loss of bonding effectiveness.[36,37] It was also found no difference between Single Bond and Clearfil SE Bond corroborating Soares *et al.*[18] and Rocha *et al*.[21] studies. Although Nakornchai *et al.*[25] verified lower bond strengths for Single Bond in comparison with Clearfill SE Bond on sound primary dentin.

In conventional total-etch systems, it has been shown that inadequate adhesive penetration into the exposed matrix, that is, as deeply as acidic conditioners penetrate, can leave exposed collagen at the dentin-adhesive interface,[2,9,10] which is prone to degradation. In an attempt to reduce this phenomena Sardella *et al.*[12] investigated the effect of shortening the etching time on the bond strength of a conventional and a self-etching primer adhesive system used in primary tooth dentin and found higher microtensile bond strengths for Single Bond in primary dentin than for Clearfill SE Bond. Shortening of acid etching time by 50% improved bond strength only for Single Bond, while no detrimental effect on bond strength for both systems was observed. The key concept of self-etching primers is demineralization and primer/adhesive diffusion into the collagen network happening at the same time and at the same depth, in an attempt to prevent discrepancies between depth of demineralized dentin by acid and primer/adhesive ability to penetrate this layer. Although, a zone of partially etched but uninfiltrated dentin has been identified beneath the hybrid layers in some milder self-etch adhesives, occasionally in Clear?l SE Bond, and was absent in the more aggressive ones like AdheSE.[41] These zones are believed to create potential sites for degradation.[40] The good results found in our investigation for Clearfill SE Bond and AdheSE are in accordance with this, since teeth were kept in water at 37°C for three months before sectioning and testing, they probably presented little degradation.

Based on Griffith's theory microtensile testing has been believed to reduce cohesive failures of the substrate due to

the smaller cross-sectional area and consequently uniform stress distribution.[39] Sardella *et al*.[12] observed a predominance of adhesive failures reflecting the peculiar characteristic of the microtensile test which induces stress to concentrate in the bonded area. Although, as mentioned before, enamel brittleness and low elasticity added by the disc cutting method make this substrate more prone to failure. Therefore, in the present study it was verified a high percentage of partial adhesive failure between bonding material and substrate associated with a partial cohesive failure in substrate (Figure 1), which corroborates the findings of Rocha *et al.,*[21] who verified 95.9% of mixed failures. Nevertheless, Suwatviroj *et al.*[22] found a majority of partial adhesive failure between bonding material and substrate associated with partial cohesive failure in bonding resin. Considering total-etch adhesives and the possibility of a demineralized dentin zone in the hybrid layer, Casagrande *et al.*[24] reported a greater number of failures at the bottom of the hybrid layer when Adper Scotch Bond Multi Purpose was applied, while more failures at the top of the hybrid layer were observed for Clearfill SE Bond. Sardella *et al.*[12] also observed a greater percentage of cohesive failures in dentin when the total-etch system was applied following a 15s acid etching.

Based on the results of this *in vitro* study, it can be concluded that contemporary adhesive systems present similar behaviors when bonded to primary teeth, with the exception of the one-step self-etching system, which provide the lowest bond strength value. All self-etching systems can achieve bond strength values as good in enamel as in dentin of primary teeth.

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In Memoriam:

Professor Célia Rodrigues passed away during the preparation of this manuscript. She will be missed.