



**Mestrado Integrado em Medicina Dentária**  
**Faculdade de Medicina da Universidade de Coimbra**

**Microtensile bond strength evaluation of self-etch adhesives to  
primary dentin**

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## **Microtensile bond strength evaluation of self-etch adhesives to primary dentin**

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## Abstract

**Introduction:** A considerable research of adhesive systems is responsible for many publications about the behavior of these materials. While the dentin bond strength of permanent teeth is assessed quite often, a significantly smaller number of researches was carried on aiming to test the dentin bond strength of primary teeth. Although there are substantial microstructural differences between permanent and primary dentin, knowledge about permanent teeth is usually and merely adapted to primary teeth. Current self-etch adhesive systems may represent an attractive addition in pediatric dentistry because, besides their efficacy, they simplify the adhesive protocol reducing operative time and making the technique less sensitive.

**Purpose:** The aim of this study was to evaluate the adhesive interfaces of four adhesive systems to primary dentin.

**Materials and methods:** Sixteen sound human primary molars were ground flat to expose dentin randomly divided into four experimental groups, according to the different adhesive materials evaluated: Clearfil™ Protect Bond, Prime&Bond® NT, Clearfil™ S<sup>3</sup> Bond Plus and Futurabond® U. The adhesives were applied under manufacturer's instructions and the crowns restored with a composite resin. Then, the restored teeth were cross-sectioned to obtain sticks (1,2mm x 1,2mm x 9 mm). Each stick was evaluated by a microtensile test in a universal testing machine, with a crosshead speed of 0,5mm/minute. The fracture modes were examined and classified with an optical microscope (40x magnification). Primary dentin disks were additionally obtained aiming samples preparation for interface's morphology characterization by SEM (scanning electron microscopy). The bond strengths data were analyzed using one-way ANOVA and Tukey HSD test. All statistical tests were applied at a confidence level of 95%.

**Results:** Clearfil™ S<sup>3</sup> Bond Plus presented the highest values (47,28MPa), followed by Prime&Bond® NT (43,11MPa), Clearfil™ Protect Bond (40,33MPa) and Futurabond® U (35,16MPa). Clearfil™ S<sup>3</sup> Bond Plus, Prime&Bond® NT and Clearfil™ Protect Bond showed statistically comparable results. Futurabond® U bond strengths was statistically similar to Clearfil™ Protect Bond but significantly lower from Prime&Bond® NT and Clearfil™ S<sup>3</sup> Bond Plus.

SEM evaluation of hybrid layer showed that Clearfil™ S<sup>3</sup> Bond Plus and Futurabond® U (one-step adhesive systems) have produced a thinner hybrid layer compared to Prime&Bond® NT and Clearfil™ Protect Bond (two-step adhesive systems).

**Conclusions:** Concerning the limitations of this in vitro study, self-etch adhesives may be considered as a suitable alternative to etch-rinse adhesives in Pediatric Dentistry.

**Keywords:** “Bond strength”; “Adhesives”; “Self-etch”; “Deciduous teeth”; “Microtensile”

## Introduction

In modern restorative dentistry every focus is diverted to conserve tooth structure using restorative materials, which adhere to tooth by minimal intervention. The clinical success of composite restorations depends on the adhesive system, which provides a durable bonding of the composite to dentin and enamel<sup>1,2</sup>. Strong adhesion between the tooth and the restorative material would prevent the formation of marginal gaps occurring due to polymerization stress, which favors leakage, bacterial penetration, recurrent caries and postoperative sensitiveness<sup>3,4</sup>.

The first meaningful proof of tooth adhesion was reported in 1955 when Michael Buonocore stated that acids could be used to change the surface of enamel to render it more receptive to adhesion<sup>5</sup>. Since that time, the dental adhesive systems have evolved through several “generations” with changes in chemistries, mechanisms, number of bottles, application techniques and clinical effectiveness<sup>6,7</sup>. Although adhesion of composite resins to enamel has become a routine and reliable aspect of modern restorative dentistry, bonding of restorative materials to dentin is generally more difficult and less predictable<sup>5</sup>.

Currently, there are two different ways in which adhesive systems obtain acceptable micromechanical retention between resin and enamel/dentin: etch-and-rinse and self-etching<sup>6,7</sup>. One of the most recent innovations in adhesive technology involves the introduction of “all-in-one” adhesive systems, which combine the etching, priming and bonding procedures into one solution applied in one<sup>7</sup>. This approach is less technique sensitive and reduces the time required for the bonding<sup>7</sup>.

The increasing demand for aesthetic restorations in pediatric dentistry has sparked interest by adhesive systems with sufficient bonding ability to enamel and dentine and with fewer bonding steps<sup>7</sup>. Current self-etch systems may represent an attractive addition to the day-to-day dental practice due to their shortened application protocol, particularly significant in pediatric dentistry<sup>8-12</sup>.

Traditionally, knowledge acquired by *in vivo* or *in vitro* studies using permanent teeth has been extrapolated to primary teeth. Regardless of eventual chemical and morphological peculiarities, the same protocols have been recommended for bonding to primary and permanent teeth<sup>13</sup>. Concerning morphological differences, there is evidence suggesting that the density and diameter of dentinal tubules is higher in primary than in permanent dentin, resulting in a reduced area of intertubular dentin available for bonding<sup>14</sup>. Also, the higher prevalence of microchannels in primary teeth would further reduce bond strength<sup>15</sup>. Moreover, the dentin from central areas of crowns of permanent teeth is harder than dentin

from same area of primary teeth<sup>14,16</sup>. Chemically, the concentration of calcium and phosphate in peritubular and intertubular dentin is lower in primary teeth than in permanent teeth, which increases the reactivity of primary dentin to acidic solutions, resulting in the formation of thicker hybrid layers compared with permanent teeth<sup>13,14,17-19</sup>. All these differences between primary and permanent dentin may influence adhesive performance, leading to lower bond strength for primary dentin<sup>15,16</sup>.

The aim of this study was to evaluate the adhesive interfaces of four adhesive systems to primary dentin.

The null hypothesis was that “there are no significant differences in the bond strength between the different adhesive systems evaluated”.

## Materials and methods

Eighteen sound human primary molars were used in this study, sixteen for microtensile bond strength ( $\mu$ TBS) test and two for ultra morphological evaluation. The teeth exfoliated normally or had been extracted for orthodontic reasons. In cases of physiologic exfoliation, the inclusion criterion was that the teeth had at least 2mm of remaining dentin thickness. The teeth were stored in distilled water for twelve weeks, at room temperature.

### Specimen preparation

Whenever necessary, roots were sectioned 2 mm below the amelocemental junction. The pulp tissue of each tooth was gently removed with an excavator and the pulp chamber was filled with a dual-cure composite resin (ParaCore® white, Coltène/Whaledent AG, Switzerland) bonded with ParaBond® adhesive system (Coltène/Whaledent AG, Switzerland). Similarly, the teeth were fixed and included in a cylinder of ParaCore® (Coltène/Whaledent AG, Switzerland), in continuity with teeth's crowns to simulate the roots ended by a dish of self-cure acrylic resin, Orthocryl® (Dentaurum, Germany) (Figure 1a). These additional procedures were performed to reinforce the tooth structure and fix it during sectioning.

The occlusal surfaces of the teeth were cut just below the dentino-enamel junction to expose a flat area of dentin using an Accutom 5 machine (Accutom 5, Struers, Ballerup, Denmark) under water refrigeration (Figures 1b and 1c). The exposed dentin surfaces were further wet polished with 240-, 400- and 600-grit silicon-carbide sandpaper in a circular motion, 60 seconds each, to create a uniform smear layer. The dentin prepared surfaces were observed under an optical microscope (M 300, Leica, Switzerland) to ensure the absence of residual enamel.

### Bonding and restorative procedures

Teeth were randomly assigned into four groups, according to the bonding procedures: (1) Clearfil™ Protect Bond (Kuraray Medical Inc., Tokyo, Japan); (2) Prime&Bond® NT (Dentsply DeTrey, Konstanz, Germany); (3) Clearfil™ S<sup>3</sup> Bond Plus (Kuraray Medical Inc., Tokyo, Japan); and (4) Futurabond® U (Voco, Cuxhaven, Germany) (Table I). In the present study, Prime&Bond® NT was considered the control group because it is an etch-rinse adhesive extensively studied and clinically used. The adhesives were applied according to the manufacturer's instructions (Table II) and photopolymerization performed using a LED device (Bluephase®, Ivoclar Vivadent, Lichtenstein). After applying the adhesive system, a composite resin – Synergy® D6, A1/B1 (Coltène/Whaledent AG, Switzerland) was built up using increments approximately 1,5mm thick; the first increment was light-activated for 10

seconds with the same light-unit and the next increments for 20 seconds, complemented by a final polymerization time of 40 seconds (Figure 1d).

A single operator, at room temperature, carried out all the bonding procedures.

**Table I** Materials and characteristics

	Material	Composition	pH	Lot
<i>Adhesive System</i>				
Group 1	<b>Clearfil™ Protect Bond</b> (2-step self-etch adhesive)	<b>Primer:</b> MDPB, MDP, HEMA, Hydrophilic dimethacrylate, PI, water <b>Adhesive:</b> MDP, BIS-GMA, HEMA, Hydrophobic dimethacrylate, PI (dl-Camphorquinone), N,N-Diethanol-p-toluidine, Silanated colloidal silica, NaF	2,0	041243
Group 2	<b>Prime&amp;Bond® NT</b> (2-Step Etch & Rinse Adhesive)	<b>Etchant:</b> 36% H <sub>3</sub> PO <sub>4</sub> <b>Adhesive:</b> Di- and Trimethacrylate resins, PENTA, Nanofillers, Photoinitiators, Stabilizers, Cetylaminehydrofluoride, Acetone		1112001212
Group 3	<b>Clearfil™ S<sup>3</sup> Bond Plus</b> (1-step / 1-bottle, self-etch adhesive)	MDP, BIS-GMA, HEMA, Hydrophilic aliphatic dimethacrylate, Hydrophobic aliphatic methacrylate, Colloidal silica, PI, Accelerators, Initiators, NaF, Ethanol	2,3	0031AA
Group 4	<b>Futurabond® U</b> (1-step / 2-bottles, self-etch adhesive)	<b>Liquid 1:</b> HEMA, BIS-GMA, HEDMA, acidic adhesive monomer, urethanedimethacrylate, catalyst <b>Liquid 2:</b> Ethanol, initiator, catalyst	2,3	1313495
<i>Restorative Material</i>				
	<b>Synergy® D6</b>	Methacrylates, Barium glass, silanized Amorphous silica, hydrophobed		0224694

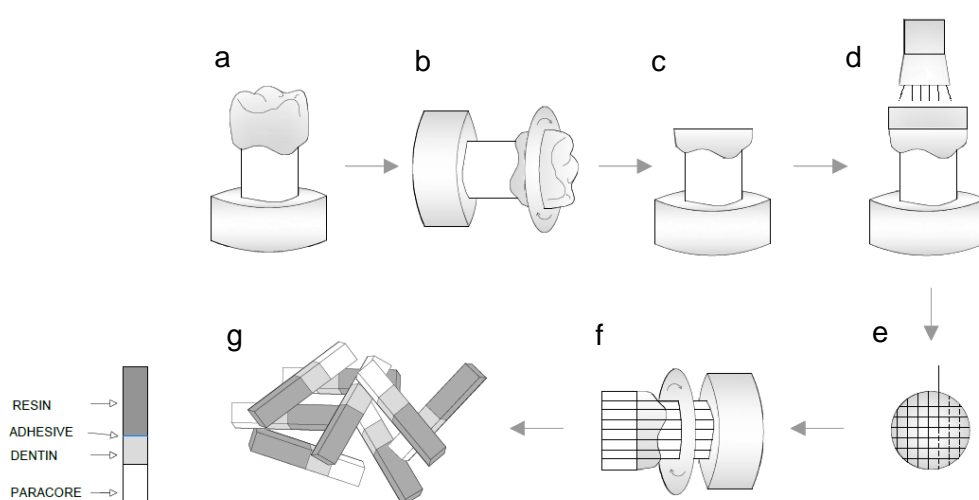
MDPB: 12-methacryloyloxydodecylpyridinium bromide; MDP: 10-methacryloyloxydecyl dihydrogenphosphate; HEMA: 2-hydroxyethyl methacrylate; MF: multifunctional methacrylate; PI: photoinitiator; NaF: sodium fluoride; PENTA: dipenta-erythritol penta acrylate monophosphate; BIS-GMA: Bisphenol A-diglycidyl methacrylate; H<sub>3</sub>PO<sub>4</sub>: phosphoric acid

**Table II** Adhesive application procedures according to the manufacturer's instructions

Group	Application procedure
<b>1 - Clearfil™ Protect Bond</b>	Apply primer and leave for 20 sec; dry with gentle air flow; apply bond; air flow gently; light-cure for 10 sec.
<b>2 - Prime&amp;Bond® NT</b>	Apply 36% phosphoric acid for 15sec; spray and rinse with water for 15sec; blot dry conditioned areas; apply adhesive and leave the surface wet for 20 sec; gently dry for at least 5 sec; polymerize for 10 sec; apply a second layer of adhesive in similar way.
<b>3 - Clearfil™ S<sup>3</sup> Bond Plus</b>	Apply bond for 10 sec; dry with mild pressure air flow for 5 sec; light-cure for 10 sec.
<b>4 - Futurabond® U</b>	Apply bond for 20 sec; dry for at least 5 sec; light-cure for 10 sec.

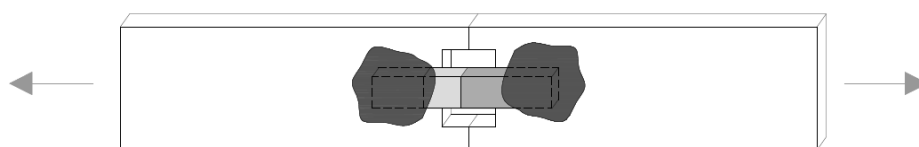
### Microtensile test

After storage in distilled water at 37°C for one week the bonded samples were cross-sectioned perpendicular to the adhesive interface into quadrangular bonded sticks using an Accutom 5 machine (Accutom 5, Struers, Ballerup, Denmark) under water refrigeration at 300 rpm (Figures 1e and 1f). Resultant sticks presented approximately 1.2x1.2 mm of square section (Figure 1g) were measured with a digital caliper rule, and then examined with an optical microscope at 40x magnification. Those presenting enamel or defects like bubbles, lack of material or irregular area in adhesive interface were discarded. Finally, 123 sticks were obtained (31 for Clearfil™ Protect Bond; 38 for Prime&Bond® NT; 30 for Clearfil™ S3 Bond Plus; and 24 for Futurabond® U).



**Figure 1** Schematic drawing of the sample preparation for microtensile bond strength test

Afterwards, the specimens were individually attached to the jig of microtensile testing with a cyanoacrylate adhesive (Permabond® 735, Permabond International Co, Englewood, NJ) (Figure 2). The microtensile bond strength was evaluated using a universal testing machine (Model AG-I, Shimadzu Corporation, Kyoto, Japan) at a crosshead speed of 0.5mm/min until failure occurred. Bond strength values were achieved by the software Trapezium (Shimadzu Corporation, Kyoto, Japan) and calculated in MegaPascal (MPa), with applied force (N) being divided by the stick cross-sectional area (mm<sup>2</sup>).



**Figure 2** Drawing illustrative of placing sticks in the microtensile sample holder

### **Failure analysis**

Fractured surfaces were inspected with an optical microscope (M 300, Leica, Switzerland) at a magnification of 40x to characterize the failure modes, which were classified as: a) adhesive (failure at resin/dentin interface); b) cohesive in dentin (failure exclusively in dentin); c) cohesive in composite (failure exclusively in composite resin); or d) mixed (failure partial at the resin/dentin interface including some cohesive pattern on the neighboring substrates). Two examiners crosschecked this observation and confirmed the different findings.

### **Ultra-morphological evaluation**

Two primary teeth were used for interfacial ultra-morphological characterization by scanning electron microscopy (SEM; Hitachi S-4100, Japan). Approximately 1mm-thick dentin disks were obtained from the teeth using Accutom 5 machine (Accutom 5, Struers, Ballerup, Denmark) under water refrigeration. The first dentin disk was used to observe the morphology created by the different dentin conditioning procedures, and the other one to evaluate the resin-dentin interfacial ultra-morphology. The disks were fixed in 2,5% glutaraldehyde for 24h. The first disk was only subjected to some dentin pre-treatment procedures in order to observe the interaction with smear-layer; the second disk was split in four parts. Each part was prepared according the complete application of the four adhesive systems evaluated. All specimens were soaked in 6Mol/L HCL for 30s to dissolve the mineral component of the dentin and then immersed in 5% sodium hypochlorite for 10 minutes to remove collagen. After that, they were sequentially dehydrated in increasing concentrations of ethanol (50% - 75% - 95% - 100%), immersed in hexamethyldisilazane (HMDS) to evaporate, and completely air-dried. Finally the specimens were mounted in aluminum stubs which were then placed in vacuum chamber and sputter-coated with gold-palladium layer and observed in SEM (Hitachi S-4100, Japan).

### **Statistical analysis**

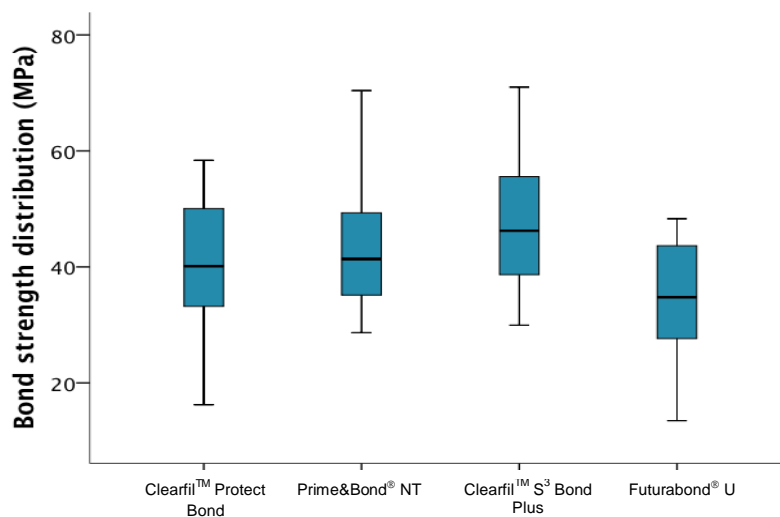
Once bond strength data were normally distributed (Shapiro-Wilk test) and homogeneous in variances (Levene's test), a one-way ANOVA test was performed to examine the effect of different adhesive systems. Post-hoc multiple comparisons were performed using Tukey HSD test. The fracture modes were compared using the  $\chi^2$ -test. All statistical tests were applied at a confidence level of 95%.

## Results

Table III and Figure 3 show the results of the microtensile bond strength of the four adhesive systems tested.

**Table III** Mean microtensile bond strength (MPa $\pm$ SD)

Adhesive system	Mean $\pm$ (SD)
1 - Clearfil™ Protect Bond	40,33 $\pm$ 12,09
2 - Prime &Bond® NT	43,11 $\pm$ 9,86
3 - Clearfil™ S <sup>3</sup> Bond Plus	47,28 $\pm$ 9,82
4 - Futurabond® U	35,16 $\pm$ 9,92



**Figure 3** Bond Strength distribution (MPa)

ANOVA reported statistically significant differences in  $\mu$ TBS values among the groups [F(3,119)=6.355,  $p<0.01$ ].

Further statistical analysis using Turkey HSD found statistically significant differences for Futurabond® U and Clearfil™ S<sup>3</sup> Bond Plus (M=-12.12, SE=2.87,  $p<0.01$ ) and Futurabond® U and Prime&Bond® NT (M=-7.94, SE=2.73,  $p=0.022$ ). Post-hoc multiple comparisons are summarized in table IV.

**Table IV** Post-hoc multiple comparisons

(I) Group		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Tukey HSD	Prime&Bond® NT	-2,77559	2,53373	,693	-9,3777	3,8266
	Clearfil™ S <sup>3</sup> Bond Plus	-6,95166	2,68122	,052	-13,9381	,0348
	Futurabond® U	5,16634	2,84645	,271	-2,2507	12,5834
	Clearfil™ S <sup>3</sup> Bond Plus	-4,17607	2,55688	,364	-10,8386	2,4864
	Futurabond® U	7,94193	2,72965	,022	,8293	15,0546
	Futurabond® U	12,11800	2,86708	,000	4,6472	19,5888

Clearfil™ S<sup>3</sup> Bond Plus exhibited the highest mean  $\mu$ TBS value, which was not significantly different from the mean  $\mu$ TBS value obtained for Prime&Bond® NT and Clearfil™ Protect Bond. Conversely, the lowest  $\mu$ TBS was obtained from the Futurabond® U, which was statistically different from Prime&Bond® NT and Clearfil™ S<sup>3</sup> Bond Plus. Thus, the null hypothesis is rejected.

### Distribution of the failure mode

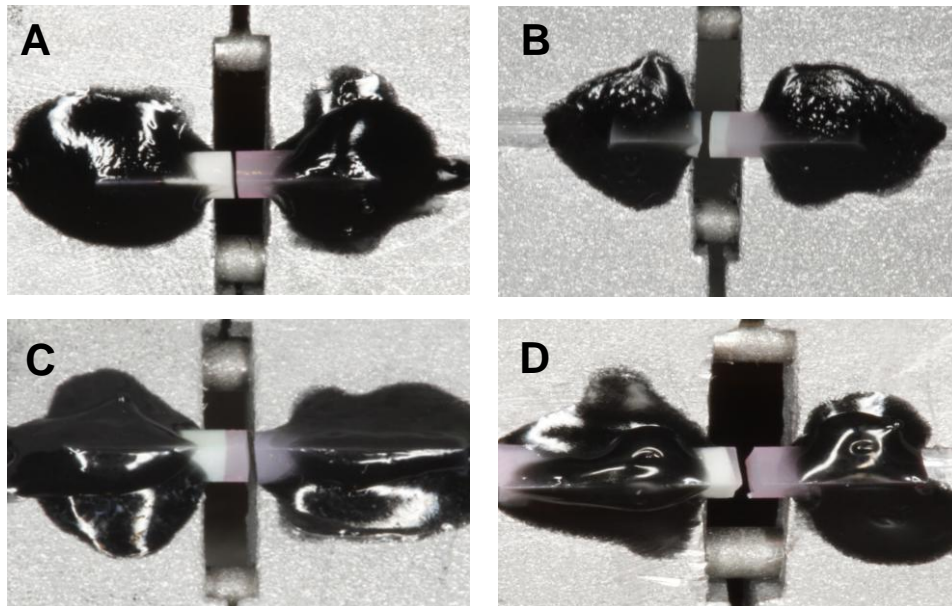
Distribution of the failure/fracture mode is summarized in Table V. Representative images of the fracture patterns are showed in Figure 4.

**Table V** Distribution of the failure mode for each group ( $\chi^2$ -test)

			Clearfill™ Protect Bond	Prime&Bond® NT	Clearfil™ S³ Bond Plus	Futurabond® U	Total
Fracture mode	AD	Count	8 <sub>a, b</sub>	18 <sub>b</sub>	3 <sub>a</sub>	5 <sub>a, b</sub>	34
		% within fracture mode	23,5%	52,9%	8,8%	14,7%	100,0%
	CC	Count	13 <sub>a, b</sub>	7 <sub>b, c</sub>	15 <sub>a</sub>	0 <sub>c</sub>	35
		% within fracture mode	37,1%	20,0%	42,9%	0,0%	100,0%
	M	Count	5 <sub>a</sub>	9 <sub>a, b</sub>	8 <sub>a, b</sub>	12 <sub>b</sub>	34
		% within fracture mode	14,7%	26,5%	23,5%	35,3%	100,0%
	CD	Count	5 <sub>a</sub>	4 <sub>a</sub>	4 <sub>a</sub>	7 <sub>a</sub>	20
		% within fracture mode	25,0%	20,0%	20,0%	35,0%	100,0%
Total		Count	31	38	30	24	123

AD: adhesive failure; CC: cohesive failure in composite resin; M: mixed failure; CD: cohesive failure in dentin

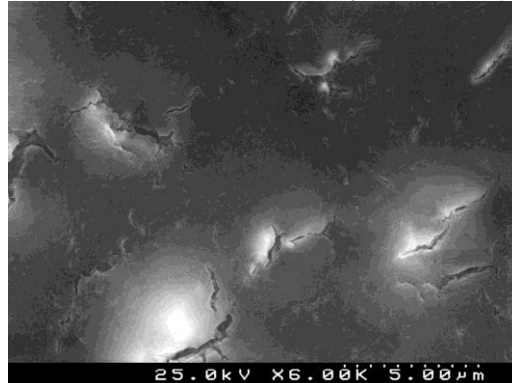
Adhesive failures occurred mostly with Prime&Bond® NT than with Clearfil™ S<sup>3</sup> Bond Plus. Clearfil™ S<sup>3</sup> Bond Plus and Clearfil™ Protect Bond showed higher percentage of cohesive failures in composite resin than Futurabond® U. Regarding dentin cohesive fractures there were no differences between the groups.



**Figure 4** Representative images of the different failure patterns: A) adhesive, B) cohesive failure in dentin, C) cohesive failure in composite resin, D) mixed

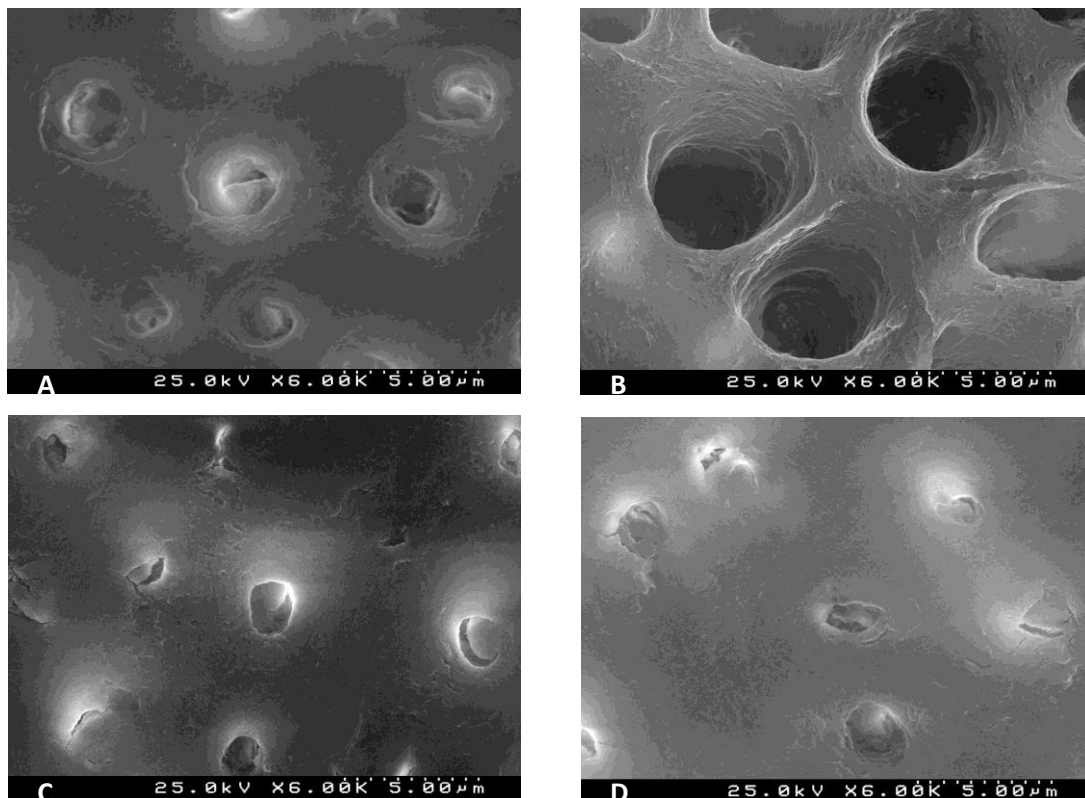
### SEM observations

Figure 5 is a representative SEM image of the smear layer adhered to dentin surface and almost occluding the dentinal tubules before any adhesive procedure.



**Figure 5** SEM representative image illustrating the smear layer covered dentin (6000x)

Analysis of the specimens in cross-sections showed a varied morphology associated to different dentin conditioning procedures (Figure 6).



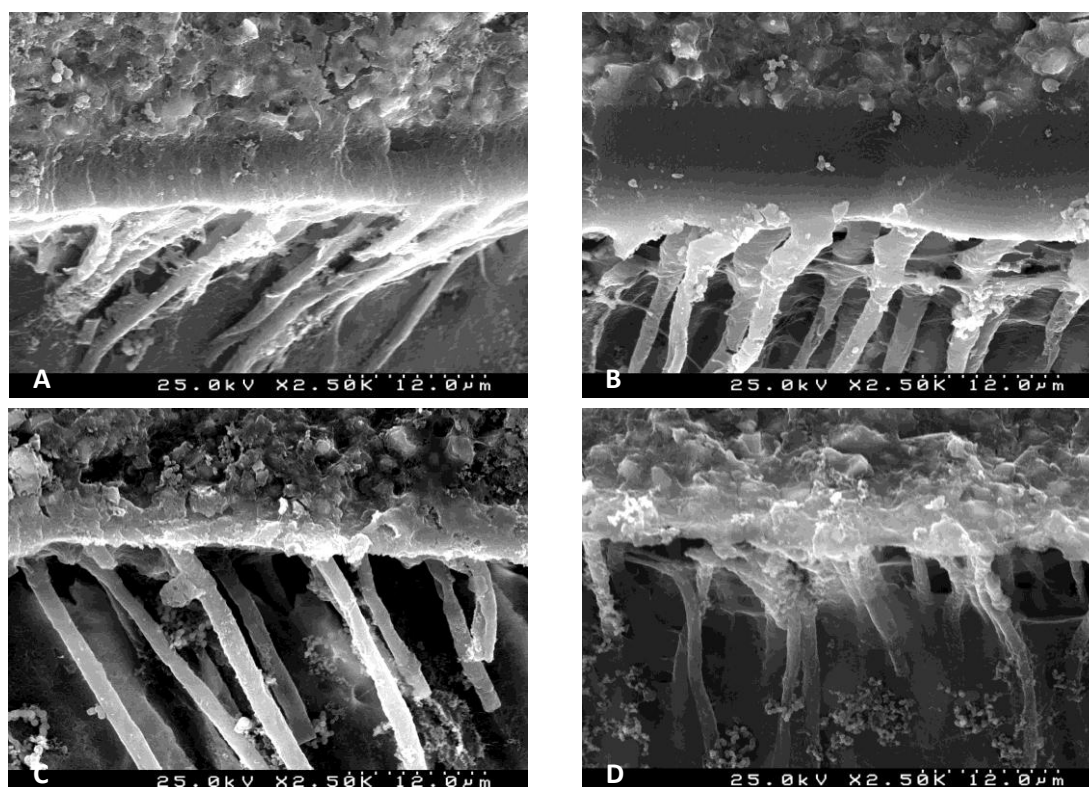
**Figure 6** SEM representative images illustrating a cross-section of the “conditioned” dentin surfaces after treatment with each adhesive system (6000x): (A) Clearfil™ Protect Bond primer; (B) 36% phosphoric acid; (C) Clearfil™ S<sup>3</sup> Bond Plus; (D) Futurabond® U

The self-etch primer of Clearfil™ Protect Bond removed various smear plugs, and partially demineralizes the peritubular dentin collar. Remnants of the dissolved smear layer can be seen over the intertubular dentine surface.

When the dentin surface was treated with 36% phosphoric acid, the smear layer and plugs were removed, and dentinal tubules were totally opened and enlarged.

Clearfil™ S<sup>3</sup> Bond Plus and Futurabond U® do not remove the smear layer or smear plugs, not sufficient to open the dentinal tubules. The demineralization was superficial and did not show that noticeable difference between the intertubular or peritubular dentin collar around the tubules lumen.

Analysis of the specimens in longitudinal sections revealed the difference in resin/dentin interfaces between the groups (Figure 7).



**Figure 7** SEM images illustrating the dentin/resin interface of the different adhesive systems (2500x): (A) Clearfil™ Protect Bond; (B) Prime&Bond® NT; (C) Clearfil™ S<sup>3</sup> Bond Plus; (D) Futurabond® U

Despite the limitations of the SEM images, the thickness of the hybrid layer produced by Clearfil™ Protect Bond and Prime &Bond® NT is similar and it is larger than that produced by Clearfil™ S<sup>3</sup> Bond Plus and Futurabond® U.

Clearfil™ S<sup>3</sup> Bond Plus and Futurabond® U exhibited cylindrical resin tags with scarce lateral branches. The funnel-shaped configuration of the resin tags is evident mainly in Prime&Bond® NT, which also exhibits numerous lateral branches, and partially in Clearfil™ Protect Bond.

## Discussion

Most newly developed adhesives have been introduced in the market with little supportive clinical data. A clinical trial is the most valid way to evaluate the quality and efficacy of adhesive systems; however, this is somewhat difficult to conduct<sup>4</sup>. Therefore, *in vitro* tests are useful and important since it provides initial predictions for the success of a material<sup>20</sup>. Bond strength measurement is one of the most effective and used method for characterizing dentin bonding, which is generally tested in tension or in shear mode<sup>3,20,21</sup>.

In the present study microtensile bond strength test was used to measure bond strength of four adhesives to primary dentin. This kind of test, developed in 1994 by Sano *et al.* uses a small bonding interfaces, in the order of 1mm<sup>2</sup>, and is commonly applied to compare the adhesive abilities of different materials<sup>22</sup>. One advantage of this method is the possibility to obtain multiple specimens from a single tooth, which is considered particularly useful in cases where teeth are difficult to obtain<sup>23</sup>. Each microtensile specimen is interpreted as a separate experimental unit, regardless of whether it is obtained from the same or different teeth<sup>23</sup>. The use of small samples also allows a better stress distribution at the resin/dentin interface, increasing the incidence of adhesive failures when compared with other methods<sup>21,23,24</sup>. In the other hand, the number of cohesive fractures in dentin is significantly reduced when a microtensile bond test is performed. However, in the present study, the incidence of mixed and cohesive failures was high, which can be interpreted as showing that the adhesive bonding to the dentin was stronger than the cohesive strength of dentin and resin.

Microtensile tests have many advantages, although they are difficult to carry out and require quite a long time in the preparation of samples. This fact could be especially pertinent in primary tooth<sup>24</sup>. Preparation of the  $\mu$ TBS specimens involves sectioning the specimens several times after the bonding procedure is completed, which may lead to pretesting failure due to the vibrations created during sectioning<sup>20</sup>. In primary teeth this limitation is more pronounced due to its smaller dimensions and physiological resorption that frequently causes less dentin availability, creating a fragile specimen, more prone to fracture<sup>10</sup>. In order to prevent this, a silicone impression material was used after performing the first directional cut, filling the spaces between slices in order to reducing the vibration applied during the second cross-sectional cut. This procedure could be relevant to prevent premature pre-testing fractures during sample preparation.

Nowadays, in order to keep up the clinical demand, simplified versions of adhesive systems, such as self-etch, are continuously being developed; however, most of them still

need to be effectively evaluated in temporary teeth. The tendency of simplifying and shortening the adhesive application protocol is frequently associated with loss of bonding efficiency and/or durability, albeit the literature is not consensual about it<sup>1</sup>. Some studies have reported low adhesion values for self-etch adhesive systems, as opposed to others who claim that their use is advantageous, particularly in Pediatric Dentistry<sup>8,12,17,25-29</sup>.

In this study, concerning adhesion mean values, the all-in-one system, Clearfil™ S<sup>3</sup> Bond Plus exhibited the highest bond strength values and statistical similar effectiveness when compared to Prime&Bond® NT (two-step, etch-rinse) and Clearfil™ Protect Bond (two-step, self-etch). One probable reason for immediate high values obtained by Clearfil™ S<sup>3</sup> Bond Plus could be the acidity of the adhesive that determines the depth to which resin monomers can penetrate into dentin<sup>30</sup>. In accordance with its etching aggressiveness, Tay, in 2001, subdivided self-etching primers into mild, moderate and aggressive<sup>31</sup>. Under to this classification Clearfil™ S<sup>3</sup> Bond Plus (pH=2,3) should be considered as a mild self-etching adhesive once it provides dentine demineralization only to a depth of 1µm<sup>31</sup>. Moreover, this demineralization occurs only partially, leaving a substantial amount of residual hydroxyapatite still attached to the collagen<sup>7</sup>. The preservation of hydroxyapatite within the submicron hybrid layer may serve as a receptor for additional chemical bonding and hybrid layer stabilization<sup>32</sup>. Along with the pH, the kind of adhesive monomers within bonding agents significantly influences bond durability<sup>7,32</sup>. Researchers have pointed out that some functional monomers in self-etch adhesives, such as 10- MDP present in the Clearfil™ S<sup>3</sup> Bond Plus, has a chemical bonding potential to the calcium in the residual hydroxyapatite<sup>32,33</sup>. This additional chemical interaction has been associated with better resistance towards degradation by prevention of micro- and nanoleakage, which seems to be valuable in terms of bond durability<sup>33</sup>. The combination of micromechanical and chemical adhesion is probably responsible for the high bond strengths obtained with Clearfil™ S<sup>3</sup> Bond Plus. Another feasible reason for high values obtained could be the application time of this adhesive system. Osorio *et al.* evaluate the effect of shortening the application time of an one-step self-etch adhesive (One-Up® Bond) compared with the time recommended by the manufacturer and concluded that half application time of One-Up® Bond improved bond strength to primary dentin<sup>34</sup>. Similarly, Clearfil™ S<sup>3</sup> Bond Plus have a recommended application time of 10 seconds, which is significantly reduced comparing the most part of self-etch adhesives.

In agreement with other studies, would be expected that Prime&Bond® NT exhibited better results than the self-etch adhesives tested<sup>10,25</sup>. However, our study found that the Clearfil™ S<sup>3</sup> Bond Plus (all-in-one adhesive system) exhibited higher microtensile bond

strength, but statistically significant, than Prime&Bond<sup>®</sup> NT. The etching time can influence the values obtained by Prime&Bond<sup>®</sup> NT. Several studies compared the bond strengths in primary teeth depending on the etching time<sup>18,34,35</sup>. All of them concluded that a reduction in etching time might produce an increase in microtensile bond strength. According to the manufacturer's instructions, 36% phosphoric acid should be applied at least 15 seconds, before Prime&Bond<sup>®</sup> NT application; although this is the recommended etching time for permanent teeth, the more reactive characteristic of primary dentin to acidic conditioners means that an eventual reduction in the etching time can prevent the formation of a non-impregnated demineralized dentin, which compromise the bonding efficacy<sup>13</sup>. Osorio *et al.*, 2010 evaluated the effect of shortening the etching time on bond strength of Single Bond (etch-rinse) and concluded that reducing the etching time of phosphoric acid to one half of the manufacturer's recommended (15 to 7 seconds), promoted a significant increase in microtensile bond strength (29,38MPa to 42MPa)<sup>34</sup>. Thus, we can speculate that shortening the phosphoric acid etching time for Prime & Bond<sup>®</sup> NT could be advantageous in primary dentin and deserves specific research.

Clearfil<sup>™</sup> Protect Bond (two-step self-etch) exhibited similar bond strength to Clearfil<sup>™</sup> S<sup>3</sup> Bond Plus (one-step self-etch). This result is in discordance with other studies, which report that two-step self-etch adhesive system exhibit a superior *in vitro* performance in comparison to one-step self-etch systems<sup>30,36,37</sup>. The influence of introduction of an antibacterial monomer (MDPB) in Clearfil<sup>™</sup> Protect Bond is controversial. One study report that incorporation of the antibacterial monomer MDPB to the self-etching primer causes a decrease in bond strength to primary teeth dentin, whereas other studies mention that introduction of MDPB not influence the bond strength of adhesive systems<sup>38-40</sup>. In theory, the introduction of MDPB can influence the bond strengths in water-based adhesives. Once this monomer is quite hydrophobic, ethanol and acetone may be the most suitable solvents for it<sup>32</sup>. Another important factor to be considered is the HEMA concentration in Clearfil<sup>™</sup> Protect Bond (25-45%). High amounts of HEMA in the adhesive composition result in flexible polymers with inferior qualities, and the potential to reduce bond strength due to the attraction of water, which may contribute to monomers dilution and reduction on polymerization degree<sup>32,41</sup>.

The worst microtensile bond strength was obtained with Futurabond<sup>®</sup> U, which was statistically difference to Clearfil<sup>™</sup> S<sup>3</sup> Bond Plus and Prime&Bond<sup>®</sup> NT. Futurabond<sup>®</sup> U is a one-step self-etch adhesive characterized by a relatively mild pH (2,3) and high HEMA concentrations. The lack of technical and scientific data for this adhesive system applied in primary dentin difficult the results interpretation and discussion.

Some studies that used adhesive systems in primary dentin (similar to those employed in this study) did observe lower bond strengths<sup>38,42,43</sup>. Yildirim *et al.*, 2008 evaluated the microtensile bond strength of antibacterial bonding system to primary dentin (Clearfil™ Protect Bond) obtained 30,69MPa, lower to those obtained in this study (39,38MPa)<sup>38</sup>. Krifka *et al.*, 2008 also obtained lower values for Clearfil™ Protect Bond (29.9 MPa)<sup>42</sup>. Burrow *et al.*, 2002 compared microtensile bond strengths of several dentin bonding systems to primary and permanent dentin; Prime&Bond® NT also obtained lower bond strength (18,1MPa) than in this study (43,11MPa)<sup>43</sup>. This wide variation could be attributed to differences between the methods employed, as well as factors related to the tooth and material used<sup>44-46</sup>.

Other studies evaluated the performance of adhesive systems not contemplated in this study. Uekusa *et al.*, 2006 examined the microtensile bond strength of one-step self-etch systems (Clearfil™ S<sup>3</sup> Bond and One-Up Bond F Plus) to primary and permanent dentin; in the case of primary dentin the microtensile bond strengths (44,7MPa for Clearfil™ S<sup>3</sup> Bond and 40,6MPa for One-Up Bond F Plus) were similar than those obtained by one-step self-etch in this study<sup>47</sup>. Marquezan *et al.*, 2008 and Miranda *et al.*, 2010 evaluated the microtensile bond strengths of some adhesive systems, including Clearfil™ SE Bond (the precursor of Clearfil™ Protect Bond); these results (27,68MPa and 18,94MPa, respectively) were low compared with those obtained by Clearfil™ Protect Bond in this study (40,32MPa)<sup>10,36</sup>.

The present study did not evaluate dentin bond strength on permanent teeth and, in this regard, most studies concluded that the bond strengths are higher in permanent teeth<sup>12,29,36,37,47,48</sup>. Differences in chemical composition, tubular density, intrinsic moisture and dentinal permeability between primary and permanent teeth may be responsible for the lower bond strength values obtained in primary dentin<sup>13,15</sup>. In 2006, Shinohara *et al.*, conducted a study with a laboratory protocol similar to the present work, which evaluated the influence of antibacterial and fluoride-releasing adhesive system (Clearfil™ Protect Bond) on permanent dentin; the results for the Clearfil™ Protect Bond in permanent teeth (47,64MPa) were higher in comparison to those obtained in this study in primary teeth (39,38MPa)<sup>39</sup>. Mazur *et al.*, 2009 also evaluated other adhesive system tested in our study, Prime&Bond® NT, and obtained 21,35MPa in permanent teeth which was lower than that obtained for the same adhesive system in our study, in primary teeth (43,11MPa)<sup>49</sup>. As mentioned previously, these conflicting results cannot be compared, due to variations in the protocol.

SEM evaluation of hybrid layer showed that adhesive with best results - Clearfil™ S<sup>3</sup> Bond Plus - has produced a thinner hybrid layer compared to Prime&Bond® NT and Clearfil™

Protect Bond. Actually, no study has successfully established a positive correlation between the thickness of the resin infiltrated layer and bond strength in primary dentin, suggesting that the quality, rather than the thickness of the resin-infiltrated layer, is more important for bond strength measurements.

The present findings should be interpreted with caution, as the results were obtained under laboratory conditions. Bond strength values can be used as a comparison of the effectiveness of bonding systems; however *in vitro* high values do not necessarily indicate good clinical performance. Furthermore, only one property of the adhesive systems was evaluated in the current study. So, researches evaluating other bonding properties and different adhesive bonding approaches should be targeted. It is advisable standardize the research test conditions, involve more than one operator, apply different type of tests for the same materials and perform aging studies complemented by clinical trials.

## Conclusions

Based, and within the limitations of this in vitro study, it can be concluded that:

1. Some self-etching systems can achieve high bond strength values in primary dentin, as good as etch-rinse tested adhesive;
2. Clearfil™ S<sup>3</sup> Bond Plus and Prime&Bond® NT showed the highest microtensile bond strength;
3. Clearfil™ S<sup>3</sup> Bond Plus permits easier and quicker application, so its use may be an interesting alternative in Pediatric Dentistry.

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