Microtensile Bond Strength of Different Adhesive Systems to Primary and Permanent Dentin

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Abstract

Purpose: The aims of this in vitro study were to: (1) compare bond strength of different adhesive systems to primary and permanent dentin using microtensile test; and (2) evaluate the interaction of these materials to primary and permanent dentin by means of scanning electron microscopy (SEM).

Methods: Middle-coronal dentin surfaces of 18 exfoliated primary and 18 extracted permanent molars were exposed and teeth were randomly divided, according to their adhesive system, into 3 groups (N=6 per group): (1) Clearfil SE Bond (SE); (2) One Up Bond F (OU); and (3) Single Bond (SB). Then, 5-mm high composite blocks were constructed. After bonding procedures, the teeth were stored in distilled water at 37°C for 24 hours prior to the specimens' preparation. For the microtensile test, teeth (N=5 per group) were longitudinally sectioned into 2 axes rendering beam-specimens that were glued to special devices, which were mounted in a Universal Testing Machine to be loaded under a cross-head speed of 1 mm/min until fracture. One tooth of each group was prepared for SEM.

Results: Microtensile bond strength mean values (MPa) to primary/permanent dentin were: (1) SE=60.0/61.4; (2) OU=54.5/53.3; and (3) SB=70.1/64.9. Two-way analysis of variance (ANOVA) showed no significant differences ($P>.05$) for the bond strength values among primary and permanent dentin groups, neither among groups SE×SB and SE×OU. SEM images of SE and SB showed a well-defined, uniform, and continuous hybrid layer. A continuous hybrid layer, however, was not found for OU.

Conclusions: Bond strength and micromorphologic characteristics of the adhesive systems evaluated were not influenced by the substrate. OU achieved worse results. (Pediatr Dent 2005;27:457-462)

KEYWORDS: DENTAL BONDING, DENTIN, TENSILE STRENGTH, TOOTH, PRIMARY

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Adhesive systems are widely used in daily clinical practice, and new products are constantly developed that improve on their predecessors[1] in the areas of: (1) technical simplification[2]; (2) reduced application time; and (3) minimized procedure errors.[3] These simplified materials can be classified in 2 main categories:

1. total-etch single bottle systems that require a previous etching procedure, generally with phosphoric acid, and that include primer and bond in only one bottle;
2. self-etching systems, which do not require separately etched procedures, since the etching step is combined with the acidic primer application.

In the second case, demineralization and resin monomer infiltration occur simultaneously,[4] thereby reducing possible discrepancies and gap formation.[5] Acidic monomers can be combined with the primer, requiring a separate bond application (self-etching primers). Acidic primer and bond can be combined in a single clinical step (self-etching adhesives or all-in-ones), thus achieving an important advantage in technique simplification.[6-8]

Despite these advantages, clinical indication depends on bonding performance. Bond strength to enamel or dentin is an important indicator of an adhesive systems' effectiveness, since the bonding layer must support not only composite shrinkage stress but also occlusal forces—mainly in restorations in stress-bearing areas—to avoid gap formation[9] that can lead to microleakage, secondary caries, and postoperative sensitivity.[10]

A great number of studies have already evaluated the bond strength of simplified adhesive systems to permanent

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dentin.[11-15] Only limited information, however, is available in the literature regarding their performance on primary teeth.[16,17] Moreover, measurement of bond strength to primary dentin is controversial, since some studies showed lower bond strength to this substrate compared to permanent dentin,[18-20] while others demonstrated similar[21-23] or higher values for primary dentin.[16] Differences in the percentage of mineral components—in terms of tubular density, diameter, and intrinsic moisture to primary and permanent teeth—may influence adhesive system performance.[16,24-26] Nör et al[27] demonstrated that primary dentin is more reactive to acid etching than permanent dentin, and they suggested different application protocols for primary teeth. Contrary results have been reported concerning hybrid layer formation,[17,18] indicating that primary dentin is less permeable than permanent dentin.[24] Moreover, a direct relationship does not appear to exist between dentin appearance after acid conditioning as verified in micromorphologic analyses and bond strength values.[18] Thus, it is important to clarify the effectiveness of simplified adhesive systems to primary and permanent dentin.

Besides bond strength measurements, another important tool used to evaluate adhesive system performance is dentin/adhesive interface observation by means of scanning electron microscopy (SEM).[28-31] Hybrid layer and resin tag formation and the intimate contact with underlining dentin are significant occurrences that can be observed under SEM, clarifying the interaction between adhesive systems and dentin.

Therefore, the aims of this in vitro study were to: (1) compare bond strength of different adhesive systems to primary and permanent dentin using a microtensile test; and (2) evaluate the interaction of these materials to primary and permanent dentin by means of SEM. The null hypotheses tested were:

1. There are no differences in primary and permanent bond strength and interfacial micromorphology.
2. There are no differences in bond strength and interfacial micromorphology achieved by different tested adhesive systems.

Methods

This study's protocol was reviewed and approved by the local ethical committee (Comitê de Ética em Pesquisa da Faculdade de Odontologia da Universidade de São Paulo, protocol No. 190/02).

Eighteen sound exfoliated primary first and second molars and 18 sound third molars extracted for orthodontic reasons were collected, stored in 0.5% chloramine T at 4°C, and used within 3 months after retrieval. The occlusal enamel was removed with a water-cooled diamond disc in a cutting machine (Labcut 1010, Extec Co, Enfield, Conn) to obtain flat dentin surfaces. Surrounding enamel was also removed with a diamond bur in a high-speed handpiece with water spray. Exposed occlusal dentin surfaces were then polished with 600-grit silicon carbide paper under running water to create a standardized smear layer.[17]

Primary and permanent teeth were randomly divided, according to the adhesive system tested, into 3 groups (N=6 per group): (1) Clearfil SE Bond (SE [Kuraray Medical Inc, Osaka, Japan]); (2) One Up Bond F (OU [Tokuyama Corp, Tokyo, Japan]); and (3) Single Bond (SB [3M ESPE St. Paul, Minn]). All materials were applied on dentin surfaces according to the manufacturers' instructions. A fresh drop of each adhesive system was used for each tooth, applied in the same way to primary and permanent dentin. For SB, after rinsing the phosphoric acid, a moist dentin surface was created with a 5-second stream of oil-free air 20 cm away from the tooth. Care was taken to not desiccate the dentin with this air stream.

After bonding procedures, resin composite blocks were constructed (Z100, 3M ESPE, St. Paul, Minn) in increments of 2 mm, individually light cured for 40 seconds (Optilux 401, Demetron/Kerr, Orange, Calif; 500 nW/cm^2), until it was 6-mm high. Teeth were stored in distilled water at 37°C for 24 hours prior to preparation for the microtensile test or SEM evaluation.

Five teeth of each subgroup were randomly selected and longitudinally sectioned into 2 axes with a water-cooled disc (Extec 12205, Extec Co, Enfield, Conn) mounted in a cutting machine (Labcut 1010, Extec Co, Enfield, Conn). This was done to obtain beam-shaped specimens with a cross-sectional area of 0.4 mm^2 for microtensile tests. Each of the beams was glued with Super Bonder gel (Henkel Loctite, Itapevi, Brazil) to specially designed microtensile grips mounted on a universal testing machine (Kratos Dinamometros, Embu, Brazil). When the loading machine was activated under tension at a crosshead speed of 1 mm/min, purely tensile forces were applied to the microtensile beams until the specimens fractured. Fractured specimens were then examined under a stereomicroscope (×25 magnification) to analyze fracture mode, which was classified as adhesive or cohesive failure. Only specimens with adhesive failures were used to calculate bond strength means.

Microtensile bond strength data were first analyzed with a regression analysis to verify the influence of tooth of origin in the measured strengths. Since it was statistically significant, microtensile data were analyzed by tooth (N=5 per group) and not by beams using 2-way ANOVA with subsequent Tukey test at $P<.05$. Also, a chi-square test was performed to analyze the fracture mode proportions among the various materials and substrate.

For SEM analysis, 1 tooth in each experimental group was cut in half perpendicularly to the bonded interface with a water-cooled diamond disc. Each half was polished with 1,200- and 2,000-grit sandpaper under refrigeration. The final polish was obtained with increasingly fine diamond pastes (1 μm and 0.25 μm, METAD I and II, respectively, Buehler, Lake Bluff, Ill). Debris was ultrasonically removed (Cavitron 3000, LD Caulk, New York, NY) for 10 minutes between each polishing procedure. After obtaining specular brightness, specimens were stored at room temperature in 2.5% buffered glutaraldehyde solution (pH=7.4) and ultrasonically cleaned for 10 minutes before being exposed to a 50% phosphoric acid solution for 5 seconds and to a 1% sodium hypochlorite for 10 minutes. This technique demineralized any dentin that was not infiltrated with resin so that the dentin could be dehydrated with silica for 24 hours.[32,33] Specimens were gold sputtered and observed under a Philips XL30 scanning electron microscope (Philips, Eindhoven, The Netherlands).

Results

Microtensile bond strength mean values for the adhesive systems tested are summarized in Table 1. Regarding substrate factor, primary and permanent dentin showed similar bond strength values, with no significant differences (P>.05). On the other hand, materials were statistically different (P<.05). SB showed bond strength values significantly higher that OU, whereas SE showed intermediate bond strength values similar to the other 2 systems (P>.05).

Table 2 shows failure mode results. A chi-square test confirmed that there were no statistically significant differences in fracture mode proportion for primary and permanent dentin or among the various materials. Even though it was not statistically significant, SB showed a higher number of cohesive fractures regardless of the substrate.

SEM images of SE and SB showed good adaptation and established formation of a uniform hybrid layer to primary dentin as well as to permanent dentin. For SB, the hybrid layer was about 2-μm thick (Figure 1), which is almost twice as thick as those observed for SE (Figure 2). OU SEM images (Figure 3), however, showed an even thinner hybrid layer of approximately 0.5μm. Moreover, nonprotected or noninfiltrated collagen fibrils were seen (Figures 3c and 3d), which were not observed for the other materials.

Table 1. Bond Strength Mean Values (MPa) and Standard Deviation for the Adhesive Systems Tested, Both on Primary and Permanent Dentin

Adhesive systems	Means±SD*	
	Primary dentin	Permanent dentin
Clearfil SE Bond	60.0±8.9[a,b]	61.4±6.4[a,b]
One Up Bond F	54.5±2.4[b]	53.3±3.7[b]
Single Bond	70.1±3.8[a]	64.9±10.0[a]

*Means with the same letters are not significantly different (P>.05).

Table 2. Fracture Mode for Experimental Groups: Chi-square Results of the Fracture Mode Proportions Among Various Materials

Adhesive systems	Adhesive/cohesive failures*			
	Primary dentin	Chi-square	Permanent dentin	Chi-square
SE	57/3	4.06 (NS)	88/2	0.78 (NS)
OU	47/2	↓	54/2	↓
SB	53/8		82/4	

*NS=not significant.

Discussion

Since the development of early adhesive systems, an effort has been made to improve bond quality to dental substrate—mainly to dentin, which is more complex and less predictable than enamel.[6] Despite the great number of studies that used permanent teeth, there are only a few studies that evaluated bond strength to primary dentin. Even fewer studies utilized the microtensile test,[17,34] which is now considered the most reliable adhesion testing technique that is capable of assessing the "true' interfacial strength between an adhesive and the bonding substrate, since pure tensile load is applied to reduced sections with a uniform stress distribution.[35,36] As already demonstrated, in the microtensile test the specimen's bonded area is inversely proportional to bond strength values recorded.[11,14,37] The high bond strength values obtained in this study are related to the small bonded area and to the specimen configuration used. A cross-sectioned area of approximately 0.4 mm^2 was employed to accommodate the reduced dimensions of primary teeth and to obtain a higher number of specimens per teeth, thereby reducing the intertooth variation.

The results found in the literature regarding bond strength to primary and permanent teeth show wide variation.[19,20,23,38] The present study showed that the adhesive systems evaluated performed equally well on primary and permanent dentin, since neither bond strength values nor SEM micrographs showed significant differences. Similar results were already found in previous studies,[21-23] despite the fact that they employed tensile and shear bond strength tests and that morphological and structural differences existed between the substrates.

In addition, the primary teeth employed had undergone exfoliation. Hence, the dentin characteristics are expected to be different from those of younger impacted permanent teeth, which are generally used in this kind of study. However, the influence of the type of tooth on bond quality seems to be insignificant.[39] In the same way, SEM analysis revealed similar performances for primary and permanent dentin, emphasizing that the kind of dentition does not influence the results.

With their simplified technique, self-etching adhesives are particularly valuable in pediatric dentistry, particularly when it comes to saving time. Regardless of their easier application, however, self-etching adhesives must also be efficient. The results

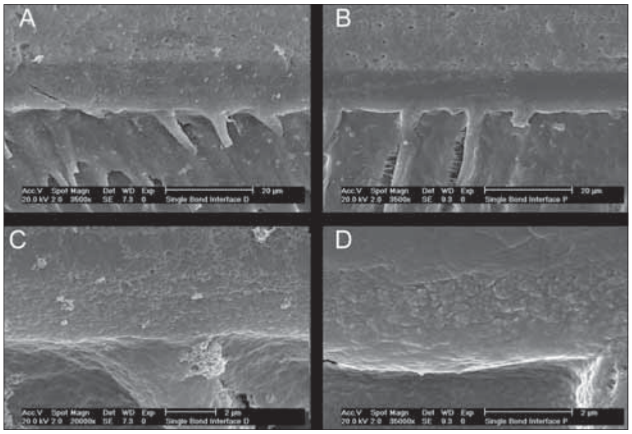

Figure 1. Scanning electron microscope image of Single Bond specimens showing a uniform hybrid layer and resin tags on (a) primary dentin and on (b) permanent dentin. At a higher magnification (×20,000), details of the hybrid layer on (c) primary dentin and on (d) permanent dentin.

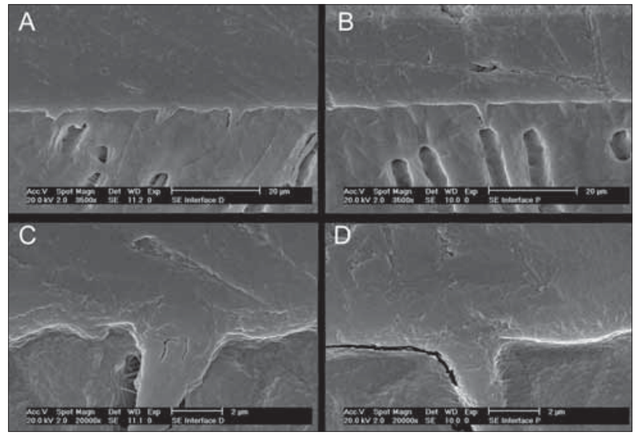

Figure 2. Scanning electron microscope image of Clearfil SE Bond specimens showing a uniform hybrid layer and resin tags on (a) primary dentin and on (b) permanent dentin. At a higher magnification (×20,000), details of the hybrid layer on (c) primary dentin and on (d) permanent dentin.

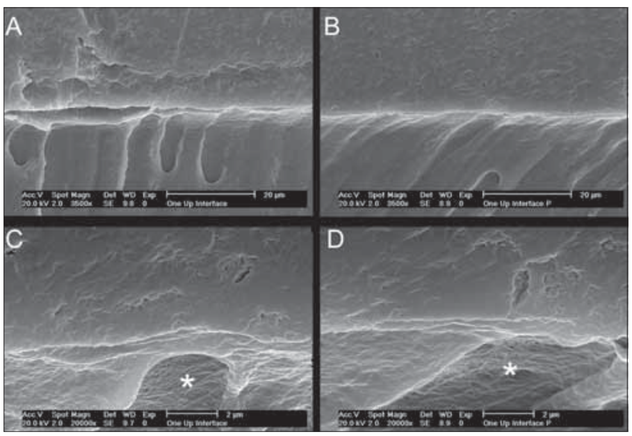

Figure 3. Scanning electron microscope image of One Up Bond F specimens demonstrating a thin hybrid layer and poor resins tags infiltration (a) and also a discontinuity area on primary dentin and on (b) permanent dentin. At a higher magnification (×20,000), details of the nonuniform hybrid layer and exposed collagen fibrils not infiltrated by monomers (*) on (c) primary dentin and on (d) permanent dentin.

found in the present study corroborate those from literature,[1,13,21,35,40] confirming that 2-step self-etch systems are comparable to conventional total-etching systems in terms of bond strength and morphological characteristics. In fact, primary and permanent dentin bond strength values and morphologic characteristics observed with Clearfil SE Bond were similar to those obtained with Single Bond (P>.05; Figures 1 and 2). These results are also supported, regardless of the testing technique, by those obtained from Senawongse et al,[18] who evaluated the performance of these materials in primary teeth by means of a microshear test.

Previous studies[26,27] based on morphological analyses have suggested different application protocols for primary and permanent teeth based on the more intense action of conditioners in primary dentin. Nevertheless, in the present study, adhesive system applications were identical for the 2 substrates. As previously demonstrated,[41] this choice was based on the concept that there was not a relationship between bond strength and hybrid layer thickness. A hybrid layer with identical thickness was observed in both primary and permanent dentin, which agreed with previous studies[17,19] despite differences in mineral concentration between these 2 substrates.[42] These results confirm, once again, that hybrid layer thickness does not influence bond strength values. More relevant than hybrid layer thickness is its favorable and uniform interaction with the adjacent tooth substrate.

The 1-step self-etching system (One Up Bond F) showed the lowest bond strength among the 2 substrates compared to the total-etch system. The simultaneous etching, primer, and bond application can limit the permeation of the monomers through the substrate during demineralization. This promotes poor infiltration, which can result in areas without dentin hybridization.[6,43] In Figure 3, a very thin and nonuniform hybrid layer can be observed, which becomes more obvious when compared to that formed by Single Bond (Figure 1). Moreover, it is possible to notice discontinuous areas in certain regions, characteristic of adhesion failure, which possibly explains the low bond strength values for this adhesive system.

During SEM specimens' preparation, samples were demineralized for 5 seconds with phosphoric acid, exposing the bottom portion of the hybrid layer and improving its visualization. For One Up Bond F, in higher magnifications (areas marked with * in Figures 3c and 3d), a collagen network is visible at the base of the hybrid layer. This is characteristic of poor monomer infiltration instead of the smooth hybrid layer surface observed for the other systems (Figures 1 and 2). This morphology pattern was observed both in primary and permanent dentin.

The methodological limitations of this in vitro study do not permit a direct extrapolation to the clinical situation. This study's results, however, suggest that the same protocol of adhesive system application can be employed with primary and permanent teeth. The micromorphologic characteristics of the hybrid layer formed with One Up Bond F suggest careful clinical use until further research corroborates its effectiveness.

Conclusions

Within the limitations of this in vitro study, the following conclusions could be made:

1. The adhesive systems evaluated performed similarly on primary and permanent dentin, considering their bond strength and scanning electron miscroscopy evaluation.
2. Well-defined hybrid layers were observed for Single Bond and Clearfil SE Bond with different thickness. These adhesive systems also produced similar bond strengths.
3. One Up Bond F produced a very thin and nonuniform hybrid layer showing noncontinuous regions. Bond strength values for this system were similar to Clearfil SE Bond and lower than Single Bond.

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Abstract of the Scientific Literature

Fingernails and Toenails as Biomarkers of Subchronic Exposure to Fluoride

The aim of this article was to evaluate the use of fingernails and toenails as biomarkers of subchronic fluoride exposure from fluoride dentifrices in 2- to 3-year-old children. Ten children living in a fluoridated community were instructed to brush their teeth with: (1) a placebo dentifrice for 28 days; (2) a fluoride dentifrice (1,570 ppm) for another 28 days; and (3) a placebo dentifrice for the last 28 days. Fingernails and toenails were clipped at the beginning of the experiment and every 2 weeks for 34 weeks and analyzed for fluoride content with an electrode following hexamethyldisiloxane-facilitated diffusion. Nail fluoride concentrations ranged from 1.26 to 17.42 μg/g, with 2 peaks seen 4 and 16 weeks after starting the use of fluoride dentifrice. A significant positive correlation was found between the fluoride concentration of fingernails and toenails. In conclusion, this study demonstrates that nails can be used as biomarkers of subchronic exposure to fluoride from dentifrices in small children.

Comments: It is important to objectively determine the levels of exposure to fluoride. Fingernails and toenails can be good alternatives to use as biomarkers to determine subchronic fluoride exposure. JLC

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