Bond Strength of Two-Step Etch-and-Rinse Adhesive Systems to the Dentin of Primary and Permanent Teeth

Hérica Adad Ricci * / Mariane Emi Sanabe ** / Carlos Alberto de Souza Costa *** / Josimeri Hebling

Objective: *The purpose of this study was to compare the immediate microtensile bond strength (μTBS) of two-step etch-and-rinse adhesive systems to the dentin of primary and permanent teeth.* ***Study Design:*** *Non-carious human teeth (12 primary molars and 12 premolars) were assigned to 3 groups according to the adhesive system. The adhesive systems were applied to flat superficial coronal dentin surfaces etched with phosphoric acid and composite resin blocks were built up. The teeth were sectioned to produce beam-shaped specimens with 0.81 mm² cross-sectional area subjected to μTBS testing. μTBS data were analyzed statistically by ANOVA and Tukey's test (a= 0.05).* ***Results:*** *The adhesive systems produced statistically similar mean μTBS to each other (p> 0.05) and no significant differences (p> 0.05) were found when the same material was applied to primary or permanent tooth dentin. The mean μTBS values (MPa) obtained were: Prime & Bond NT: 41.7±14.4 (permanent) and 40.8±13.4 (primary); Single Bond: 42.9±8.6 (permanent) and 41.4±11.9 (primary); Excite DSC: 46.3±11.3 (permanent teeth) and 43.4±12.0 (primary).* ***Conclusion:*** *There was no difference in the immediate μTBS of two-step etch-and-rinse adhesive systems when applied to the dentin of primary and permanent teeth.*

Keywords: *Dentin-Bonding Agents; Dentin; Tensile Strength; Tooth, Deciduous; Dentition, Permanent*
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INTRODUCTION

Despite the evolution in the chemistry of adhesive systems, adhesion to dentin is still considered less predictable and more critical than to enamel.[1,2] The challenge imposed by the dentin substrate is due to its intrinsically moist nature and its structural and compositional heterogeneity,[3] which varies regionally within the same tooth. These characteristics are even more accentuated when different types of dentin substrates are compared, such as sound vs. carious dentin or primary vs. permanent tooth dentin.[4]

Micromorphological differences have been identified between primary and permanent dentin. Primary teeth have a smaller area of intertubular dentin than permanent teeth due to their greater density and larger diameter of dentinal tubules.[5] It has also been shown that the peritubular dentin of primary teeth can be two to five times thicker than that of permanent teeth.[6] The dentin of primary teeth has less mineral content,[7] which could reduce its buffering capacity and increase its reactivity to acidic solutions. The fact that primary dentin seems to be more reactive to acidic conditioners than permanent dentin has been accepted to explain the formation of hybrid layers nearly 25-30% thicker in primary teeth when the dentin is etched for the time recommended for permanent teeth.[8] However, there are no research-based data to support this assumption.

The results of studies comparing the bond strength to primary and permanent tooth dentin are not consensual and divergences are found regarding the adhesive performance of different systems to these substrates. While lower values have been observed for primary dentin in some studies,[9-11] others reported similar bond strengths for both substrates[12–16] or even higher bond strength to primary dentin.[17] Considering the need to establish an adequate bonding protocol for primary teeth, the purpose of this study was to compare the immediate microtensile bond strength (μTBS) of two-step etch-and-rinse adhesive systems to the dentin of primary and permanent teeth. The tested null hypothesis was that there is no difference in the bond strength of the adhesive systems when applied to both substrates.

* Hérica Adad Ricci, DDS, MS, PhD, Student in Pediatric Dentistry, Department of Orthodontics and Pediatric Dentistry, Araraquara School of Dentistry, UNESP – Univ. Estadual Paulista, Araraquara, SP, Brazil.

** Mariane Emi Sanabe, DDS, MS, PhD, Student in Pediatric Dentistry, Department of Orthodontics and Pediatric Dentistry, Araraquara School of Dentistry, UNESP – Univ. Estadual Paulista, Araraquara, SP, Brazil.

*** Carlos Alberto de Souza Costa, DDS, MS, PhD, Associate Professor, Department of Physiology and Pathology, Araraquara School of Dentistry, UNESP – Univ. Estadual Paulista, Araraquara, SP, Brazil

**** Josimeri Hebling, DDS, MS, PhD, Associate Professor, Department of Orthodontics and Pediatric Dentistry, Araraquara School of Dentistry, UNESP – Univ. Estadual Paulista, Araraquara, SP, Brazil

Send all correspondence to: Dra. Josimeri Hebling, Faculdade de Odontologia de Araraquara – UNESP, Rua Humaitá, 1680, 14801-903 Araraquara, SP, Brasil

Phone: +55-16-3301-6334.
Fax: +55-16-3301-6329

Email:jhebling@foar.unesp.br

MATERIALS AND METHOD

Tooth Preparation

Twenty-four noncarious human teeth (12 primary molars and 12 premolars) were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the Research Ethics Committee of the Dental School of Araraquara, São Paulo State University, Brazil (Protocol # 18/06).

Both primary and permanent teeth were randomly assigned to 3 groups (n= 4) according to the adhesive system: Adper Single Bond (3M/ESPE, St. Paul, MN, USA), Prime & Bond NT (Dentsply, Milford, DE, USA) and Excite DSC (Ivoclar Vivadent, Schaan Liechtenstein). Table 1 presents the principal components and batch numbers of the materials used in the study. Because the primary teeth were in an advanced stage of physiological root resorption, the coronal pulp chamber was filled with a first increment of flowable composite resin (Filtek Flow; 3M/ESPE) pigmented with a small amount of rhodamine B and additional increments of a hybrid composite resin without that pigment (Filtek Z250; 3M/ESPE) were added to reproduce the coronal root third.

The occlusal surface of all teeth was ground with 320-grit silicon carbide paper under copious water cooling in a polishing machine (DP 10; Panambra Industrial e Técnica Ltda, São Paulo, SP, Brazil) at 500 rpm until the enamel layer had been completely removed and a flat superficial coronal dentin was obtained. The specimens were carefully examined with a stereomicroscope at ×30 magnification to confirm the absence of enamel islets. Each dentin surface was further hand polished during 30 s with wet 320-grit silicon carbide paper[18] to produce a standardized smear layer.

Bonding Procedures

In both primary and permanent teeth, the smear-covered flat dentin surfaces were etched with 35% phosphoric acid (Scotchbond, 3M/ESPE) during 15 s, rinsed thoroughly with distilled water for 10 s and gently blotted with absorbent paper to obtain a moist surface.

The adhesive systems were applied according to the manufacturers' instructions. After acid etching, two coats of Adper Single Bond were applied on the entire dentin surface, air-thinned with mild oil-free air streams for 5 s at 10 cm distance to facilitate solvent evaporation, and were light cured for 10 s. The same protocol was followed for Excite DSC. For Prime & Bond NT, a first coat was applied to the acid-etched dentin and left undisturbed for 20 s; then a second coat was applied, gently air-dried and light cured for 10 s.

Next, a 3-mm-high composite resin (Z250; 3M/ESPE) block was built up incrementally on the treated dentin surface of each tooth with 1-mm-thick increments of material being individually light cured for 20 s. All photoactivation procedures were performed with the same light-curing unit (Optilux 50; Demetron Research Co., Danbury, CT, USA) with mean irradiance of 480 ± 10 mW/cm^2 as measured with a curing radiometer (Optilux Radiometer model 100; Demetron Research Co.). The restored teeth were maintained in 100% humidity at 37ºC for 24 h.

Microtensile Bond Testing

In a high-precision cutting machine (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA) with a water-cooled diamond saw, serial sections were done in a mesiodistal direction longitudinally to the tooth crown and perpendicular to the adhesive interface, maintaining a distance of 0.9 mm between sections. The teeth were rotated 90 degrees and a new series of 0.9-mm-wide sections were done, obtaining beam-shaped specimens with a cross-sectional area of approximately 0.81 mm^2. The beams were carefully examined with a light microscope (model SZX 7; Olympus, São Paulo, SP, Brazil) at ×30 magnification and those with defects at the resin-dentin interface were discarded. Each

Table 1. Materials used in the study.

Commercial brand	Principal components	Batch
Adper Single Bond	Bis-GMA, HEMA, dimethacrylates, acid polyalkenoic, initiators, ethanol and water	4 KE
Prime & Bond NT	Di and trimethacrylate resins, colloidal silica, PENTA, photoinitiators, stabilizers, cetylamine hydrofluoride, acetone	0503000963
Excite DSC	*Adhesive*: HEMA, dimethacrylates, phosphonic acid acrylate, silicon dioxide, catalysts, stabilizers, ethanol; *Microbrush*: initiators	J04042
Filtek-Z250	Bis-GMA, UDMA, Bis-EMA resins, zirconium, silica	4 BB
Scotchbond Etchant	35% phosphoric acid	5 EN

Bis-GMA: bisphenol glycidyl dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; PENTA: dipentaerythritol pentacrylate phosphoric acid ester; UDMA: urethane dimethacrylate; Bis-EMA: bisphenol-ethyl-dimethacrylate.

beam was individually fixed to a custom-made testing jig with cyanoacrylate glue (Super Bonder Gel e Ativador 7456; Henkel Loctile Ltda., São Paulo, SP, Brazil) and subjected to microtensile strength in a mechanical testing machine (Material Test System, MTS 810; Minneapolis, MN, USA) set with a load cell with maximum capacity of 1 kN and running at a crosshead speed of 0.5 mm/min until failure. Immediately after testing, the debonded halves of each microtensile beam were stored in closed receptacles at room temperature until the analysis of the fracture pattern with a stereomicroscope at approximately ×30 magnification. The failure modes were classified as cohesive in resin or dentin, adhesive or mixed.

Statistical Analysis

A two-way ANOVA was applied to the μTBS data (in MPa) to analyze the factors *adhesive system* (Adper Single Bond vs. Prime & Bond NT vs. Excite DSC) and *type of dentin substrate* (permanent vs. primary). Two-by-two comparisons were done by Tukey's test. Significance level was set at a=0.05. The statistical unit was beams, not teeth.

Scanning Electron Microscopy

In each group, two additional teeth were prepared as previously described and sectioned in a buccolingual direction to obtain 1-mm-thick slices for analysis of the resin-dentin interface. These specimens were embedded in chemically activated epoxy resin (Epoxicure; Buehler Ltd.) and were sequentially hand polished with silicon carbide paper of decreasing abrasiveness (600- 1200- and 2000-grit). The teeth were then treated with HCl 6 N for 15 s[19] and 1% NaOCl for 10 min and were dehydrated in a series of ethanol solutions of increasing concentration (30%, 50%, 95% for 30 min and 100% for 60 min). After the last ethanol solution, the specimens were dried by immersion in hexamethyldisilazane (HMDS; Ted Pella, Redding, CA, USA) for 30 min and maintained in a vacuum desiccator for 24 h. The specimens were mounted on stubs and were sputter-coated with gold for the analysis of the adhesive-dentin interfaces in a scanning electron microscope (LEO 435 VP; Carl Zeiss, Cambridge, England).

RESULTS

Microtensile Bond Strength

The mean immediate μTBSs (in MPa) of the tested two-step etch-and-rinse adhesive systems to primary and permanent dentin are presented in Table 2. The two-way ANOVA revealed that neither the tested factors (*adhesive system* and *type of dentin substrate*) nor their interaction had a statistically significant effect (p>0.05) on the mTBS to primary or permanent tooth dentin. The adhesive systems produced statistically similar mean μTBS values to each other (p>0.05) and no significant differences (p>0.05) were found when the same material was applied to primary or permanent tooth dentin.

Failure Mode Distribution

Table 3 shows the failure mode distribution of the tested two-step etch-and-rinse adhesive systems in primary and permanent dentin. There was a predominance of adhesive failures regardless of the adhesive system and type of dentin substrate. No fracture distribution pattern was observed.

Table 2. Microtensile bond strengths (MPa) to the dentin of primary and permanent teeth according to the adhesive system.

Substrate	Adhesive Systems		
	Prime&Bond NT	Single Bond	Excite DSC
Permanent	41.7±14.4 [30]* a**	42.9±8.6 [18] a	46.3±11.3 [17] a
Primary	40.8±13.4 [26] a	41.4±11.9 [23] a	43.4±12.0 [29] a

*The values represent: mean ± standard deviation [number of specimens]. **Means followed by same lowercase letters do not differ significantly (Tukey's test, p>0.05).

Table 3. Failure mode distribution according to the adhesive system and type of dentin substrate (permanent teeth or primary).

	Substrate					
	Permanent dentin			Primary dentin		
Failure Mode	P & B NT	Single Bond	Excite DSC	P & B NT	Single Bond	Excite DSC
Adhesive	26 (86.7%)	15 (83.3%)	14 (70.0%)	23 (88.5%)	15 (83.3%)	17 (58.6%)
Mixed	-	-	-	1 (7.7%)	2 (8.7%)	1 (3.4%)
Cohesive in dentin	1 (3.3%)	1 (5.6%)	3 (15.0%)	2 (3.8%)	5 (21.7%)	9 (31.0%)
Cohesive in resin	3 (10.0%)	2 (11.1%)	-	-	1 (4.3%)	2 (6.9%)
Specimens *per* group	n=30	n=18	n=17	n=26	n=23	n=29

Values expressed as absolute frequencies (percentage relative to the total number of specimens in each group)

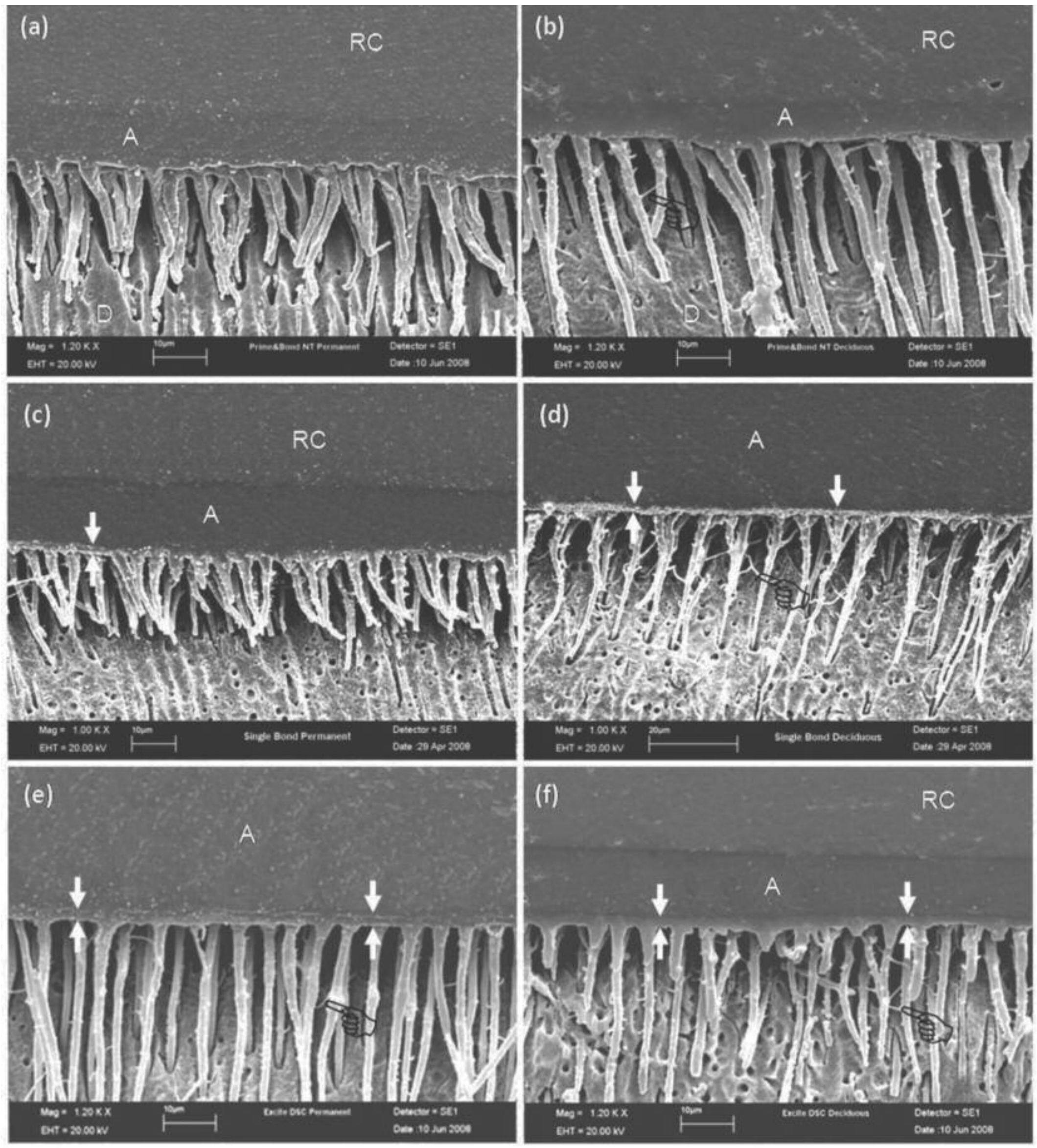

Figure 1. SEM micrographs representative of the resin-dentin interface produced in permanent teeth (left) and primary teeth (right) using Prime & Bond NT (a and b), Single Bond (c and d) and Excite DSC (e and f). There was great variation in the thickness of the adhesive layer (A), while hybrid layer formation was clearly visible in only few specimens (between arrows). Overall, numerous resin tags with lateral branches (Pointers) were seen in all groups. CR: composite resin; D: dentin.

Scanning Electron Microscopy

A panel of SEM micrographs representative of the resin-dentin interface produced by the tested adhesive systems in primary and permanent teeth is illustrated in Figure 1. Variations in the thickness of the adhesive layer as well as in resin tag number and length were observed, but these morphological features were not representative of a specific group. It was not possible to determine the existence of differences in hybrid layer formation because this structure was not clearly visible in all specimens. However, regardless of the adhesive system and type of dentin substrate, long resin tags with lateral branches were clearly visible and many of them were fractured due to the loss of mineral support after treatment of the specimens with HCl/NaClO during preparation for SEM analysis.

DISCUSSION

Two-step etch-and-rinse adhesive systems reduce the clinical operative time because they associate two of the three cardinal steps for establishment of the union of polymeric materials to dentin substrate: (1) creation of diffusion pathways (dentin etching), (2) surface wetting (primer) and (3) monomer infiltration (adhesive). In these systems, the primer and bonding agent components are applied as a single step since they are combined in the same bottle. In spite of this simplification, two-step etch-and-rinse adhesive systems have been shown to produce bond strengths comparable to those of three-step systems.[20] Especially in Pediatric Dentistry, simplification of the operative steps for adhesive system application allows for shortening the clinical chair-time for restorative procedures, which justifies the interest of the present study in investigating the bond strength of these adhesives. However, it should be stressed that technique simplification cannot be detrimental to functional and biological quality of the resin-dentin interface produced by the adhesive systems.

To date there is no consensus in the literature about the performance of adhesive systems applied to the dentin of primary and permanent teeth.[9-17] Based on the outcomes of the present study it could be stated that the adhesive systems performed equally well in both primary and permanent dentin in terms of bond strength which is in agreement with previous studies.[14-16] In addition, SEM micrographs showed no significant morphological differences when comparing resin-dentin bonds produced by the same adhesive system in primary and permanent dentin (Figure 1) as previously demonstrated.[16]

Differences in the chemical composition and microstructure of primary and permanent tooth dentin are generally considered the main factors accounting for the inferior performance of adhesive systems in primary dentin reported by some studies.[9-11,13] The lower mineral content of primary dentin may result in a lower buffering capacity when in contact with acidic substances.[8,19,21] It has been reported that hybrid layers produced in primary teeth were approximately 25–30% thicker than those produced by the same adhesive system in permanent teeth and concluded that primary dentin is more reactive to the acid etchants than permanent dentin.[8,14] These results are supported by the assumption that primary dentin are more reactive to phosphoric acid because of its lower mineral content, which leads to a deeper demineralization of this substrate using the same etching time as that used for permanent teeth.[14] Unfortunately, due to technique related limitations, the hybrid layer was clearly visible in only few specimens evaluated under SEM, impeding any conclusion about the comparison of primary and permanent teeth produced interfaces based on the thickness of that layer.

Still regarding to the mineral content, the present study compared dentin surfaces obtained from exfoliated primary molars (old teeth) and premolars extracted from 12-15-year-old patients due to orthodontic reasons (young teeth). Therefore, considering the gradual deposition of minerals in the dental tissues over time,[22,23] it may be speculated that the difference in the mineral content between primary and permanent dentin and the probable effect of this characteristic on bond strength was not significant, justifying the comparable bond strengths observed for both substrates.[16]

Concerning the dentin microstructure, greater tubule density and diameter for primary teeth in comparison to permanent teeth, resulted in a reduced area of intertubular dentin available for bonding.[5] This characteristic could affect adversely the bonding efficacy of adhesive systems, as demonstrated in studies investigating the influence of dentin depth on the bond strength of these materials.[24,25] However, in the present study, flat superficial coronal dentin was obtained from primary and permanent teeth. It has been demonstrated that dentin depth plays an important role in the bond strength of a two-step etch-and-rinse adhesive system only when superficial and deep dentin are compared, while no significant difference was found between superficial and mid dentin.[25] Therefore, it may be suggested that minor changes in the superficial region of the intertubular dentin were not significant enough to influence the performance of the adhesive systems in such a way to be detected by the microtensile testing. Additionally, superficial dentin has a large area of intertubular dentin to form the hybrid layer what could also justify the lack of difference in bond strength seen in the present study between primary and permanent dentin.

Based on the results of the present study, there would be no need to differentiate the bonding protocol for primary and permanent dentin considering the immediate adhesive performance of two-step etch-and-rinse adhesive systems applied to older primary molars. The results demonstrated that etching the primary dentin for the same time used for permanent dentin (15 seconds) did not adversely affect the immediate bond strength of the two-step etch-and-rinse adhesive systems. However, shortening the acid etching time of dentin has been considered in order to improve the long-term[27] durability of the adhesive interfaces produced in primary teeth. Further studies are still required to elucidate unclear issues regarding primary dentin morphology and reactivity to acidic conditioners before changes in the bonding protocols for primary and permanent teeth can be recommended. Likewise, clinical studies evaluating the longevity of resin-primary dentin bonds created using a reduced etching time are still necessary to consolidate its effectiveness.

CONCLUSION

There was no difference in the immediate μTBS of two-step etch-and-rinse adhesive systems when applied to the dentin of primary and permanent teeth.

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