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Effect of Cyclic Loading on the Microtensile Bond Strengths of Total-etch and Self-etch Adhesives

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Clinical Relevance

Resin-dentin bonds, which may have an influence on the long-term success of restorations, are prone to deterioration after cyclic loading. The tested one-step self-etching system (Etch&Prime 3.0) provided the least reliable dentin adhesion. After acid etching of dentin, alcohol/based adhesives performed better than those containing acetone as solvent.

SUMMARY

***Objective*: To evaluate the effect of mechanical loading on the microtensile bond strength (MTBS) of five adhesive systems to dentin. *Methods*: Flat dentin surfaces from human molars were divided into five groups and bonded with total-etch self-priming adhesives (Single Bond, Prime&Bond NT and Prime&Bond XP), two-step self-etching primer (Clearfil SE Bond) and an all-in-one adhesive (Etch&Prime 3.0), according to the manufacturers' instructions. Composite build-ups were constructed incrementally with Tetric Ceram. After 24 hours of water storage, half the specimens were load cycled (5000 cycles, 90 N). The teeth were then sectioned into beams of 1.0 mm² cross-sectional area. Each beam was tested in tension in an Instron machine at 0.5 mm/minute. Data were analyzed by two-way ANOVA and Student Newman Keuls multiple comparisons tests (p<0.05). *Results*: Clearfil SE Bond and Single Bond attained higher MTBS than the other three adhesives. Prime&Bond NT and Prime&Bond XP performed equally, and Etch&Prime 3.0 resulted in the lowest MTBS. After mechanical loading, MTBS decreased in all groups except Prime&Bond XP. Clearfil SE Bond, Single Bond and Prime&Bond XP obtained higher MTBS than Prime&Bond NT. Specimens bonded with Etch&Prime 3.0 resulted in premature failures and MTBS could not be measured. *Clinical Relevance*: When using Etch&Prime 3.0, bond structures did not withstand mechanical loading, which may have an influence on the long-term success of restorations. If dentin is acid-etched, alcohol-based adhesive systems showed higher bond strength after mechanical loading.**

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INTRODUCTION

Dentin bonding systems have been simplified and improved in order to provide increased long-term strength and promote the durability and reliability of adhesive restorations (Nikaido & others, 2002a). Different strategies are used to create dentin bonding: total-etch bonding systems work by removing the smear layer with phosphoric acid, followed by the application of a primer and adhesive in two different steps or even in the same step (total etch self-priming systems). With these two systems, incomplete expansion of the demineralized collagen matrix may impair resin infiltration and compromise bonding (Van Meerbeek & others, 1994; Pashley & others, 2003) and the self-etching approach, in which increased concentrations of acidic monomers enable the primer or adhesive to etch and prime the dentin simultaneously. Two different self-etch systems may be encountered; they may be applied in one (1-step self-etch systems) or two steps (2-step self-etch systems).

In the clinical situation, dentin-resin bonds are not only subjected to immediate stresses, which may disrupt the developing bonds, but also to cyclic loading during mastication, which will induce a generation of cracks and subsequent crack growth, which challenges the long-term survival of these bonds. It has been shown that changes in the bonded interfaces *in vivo* may occur under occlusal stresses, resulting in mechanical degradation of the bonds between the restoration and dentin (Sano & others, 1999). Teeth are continuously subjected to stresses during mastication, swallowing and parafunctional habits. Maximum biting force, recorded on first molars, is approximately 40-90 Kg. Although masticatory loads recorded on a single molar are lower (ca 11-27 Kg) (Bates, Stafford & Harrison, 1975; Anderson, 1956), they may still represent a challenge to the long-term durability of resin-dentin bonds.

Static bond strength tests cannot adequately demonstrate the potential detrimental effects that porosities and other internal defects within the adhesive layer may have on bonding durability (Givan & others, 1995). After cyclic loading, the effect of these interfacial defects on long-term bonding may be more readily apparent. It is anticipated that the combined use of mechanical loading with microtensile bond strength (MTBS) testing permits evaluation of the *in vitro* durability of resin-dentin bonds under more clinically relevant conditions than are usually employed in static bond strength testing techniques.

Thus, this study compared the results of mechanical loading vs static bond strength evaluation on the MTBS of five total-etch and self-etch adhesives to human dentin. The null hypothesis tested was that the incorporation of mechanical loading prior to bond strength evaluation has no effect on the MTBS of the adhesives to dentin.

METHODS AND MATERIALS

Forty caries-free extracted human third molars were stored in 0.5% chloramine T at 4°C and used within one month of extraction. The specimens were sectioned below the dentinoenamel junction and ground flat with 180-grit silicon carbide abrasive papers under running water to provide uniform and smear layer covered surfaces. Three total-etch self-priming adhesives (Single Bond, Prime&Bond NT and the experimental adhesive system Prime&Bond XP), a two-step self-etching primer (Clearfil SE Bond) and an all-in-one self-etch adhesive (Etch&Prime 3.0) were examined. Table 1 shows the mode of application, components and manufacturers of these adhesives. They were bonded to the dentin surfaces according to the manufacturers' instructions.

After bonding, composite build-up, each 6 mm in height, was constructed incrementally (1.5 mm) with a light-cured microhybrid resin composite (Tetric Ceram, Ivoclar-Vivadent, Schaan, Liechtenstein. Lot n° F60099). Each layer of the composite was light activated for 40 seconds with a Translux EC halogen light-curing unit (Heraeus-Kulzer GmbH, Hanau, Germany). Light intensity output, monitored with a Demetron Curing Radiometer (Model 100 Demetron Research Corporation, Danbury, CT, USA), was found to be at least 600 mW/cm^2.

The bonded specimens were stored in distilled water for 24 hours at 37°C. For each experimental group, half of the specimens were mounted in plastic rings with dental stone for load cycling under 90 N (5000 cycles, 3 cycles/seconds) with force applied longitudinally along the center of the tooth. This compressive load was applied to the flat resin composite build-ups using a spherical stainless steel plunger 5 mm in diameter that was attached to a cyclic loading machine (S-MMT-250NB; Shimadzu, Tokyo, Japan). The rest of the specimens from each group were not subjected to cyclic loading and were stored in water until load cycling for the other teeth was completed. Each tooth was then sectioned into beams with an approximate cross-sectional area 1 mm^2 following the method described by Shono and others (1999). This resulted in the generation of 35 to 40 beams for each experimental subgroup.

Each beam was tested for MTBS by attaching it to modified Bencor Multi-T testing apparatus (Danville Engineering Co, Danville, CA, USA) with a cyanoacrylate adhesive (Zapit, Dental Venture of America Inc, Corona, CA, USA). The beams were stressed to failure in tension using a universal testing machine (Instron 4411, Instron Corporation, Canton, MA, USA) at a crosshead speed of 0.5 mm/minute. The fractured

Table 1: *Mode of Application, Compositions and Manufacturers of Tested Adhesives*

Materials	Components	Mode/Steps of Application	Manufacturer
Single Bond Total-etch Self-priming	2-Hydroxyethylmethacrylate; water; ethanol; Bis-GMA; dimethacrylates; amines; methacrylate-functional; copolymer of polyacrylic and polyitaconic acids.	Etch for 15 seconds. Rinse with water spray for 10 seconds leaving tooth moist. Apply two consecutive coats of the adhesive with a fully saturated brush tip. Dry gently for 2 to 5 seconds. Light cure for 10 seconds.	3M, St Paul, MN, USA Lot #4242
Prime & Bond NT Total-etch Self-priming	PENTA; UDMA resin; Resin R5-62-1; T-resin; D-resin; nanofiller; initiators; stabilizer; cetylamine hydrofluoride; acetone.	Etch for 15 seconds. Rinse with water spray for 15 seconds and remove water with a soft blow of air. Leave a moist surface. Apply ample amounts of the adhesive to saturate the surface, reapply if necessary. Leave the surface undisturbed for 20 seconds. Remove solvent by blowing gently with air for at least 5 seconds. Light cure for 10 seconds.	Dentsply/De Trey GmbH, Konstanz, Germany Lot #0209000918
Prime & Bond XP Total-etch Self-priming Experimental	TCBresin; PENTA; UDMA; TEGDMA; BHT; camphorquinone; functionalized amorphous silica; ethyl-4-dimethylaminobenzoate; t-butanol.	Etch for 15 seconds. Rinse with water spray for 15 seconds and remove water with a soft blow of air. Leave a moist surface. Dispense directly into a disposable brush. Apply ample amounts of the adhesive to saturate the surface, reapply if it is necessary. Leave the surface undisturbed for 20 seconds. Remove solvent by blowing gently with air for at least 5 seconds. Light cure for 10 seconds.	Dentsply/De Trey GmbH, Konstanz, Germany Lot #0304000987
Clearfil SE Bond 2-step	Primer: 10-methacryloyloxydecyl dihydrogen phosphate; 2-hydroxyethyl methacrylate; Hydrophilic dimethacrylate; di-camphorquinone; N,N-diethanol-p-toudine, water. Bond: 10-methacryloyloxydecyldihydrogen phosphate; N,N-diethanol-p-toludine; 2-hydroxyethylmethacrylate; Bis-phenol A diglycidylmethacrylate; silanated colloidal silica; hydrophobic dimethacrylate; di-camphorquinone.	Apply Primer for 20 seconds. Mild air stream. Apply Bond. Gentle air stream. Light cure for 10 seconds.	Kuraray Co, Osaka, Japan Lot #390
Etch & Prime 3.0 1-step Self-etch	Universal: 2 hydroxyethylmethacrylate; Water; ethanol Catalyst: Tetramethacrylocyethylpyrophophate.	Mix Etch & Prime 3.0 Universal and Catalyst. Apply for 30 seconds. Air blow gently. Light cure for 10 seconds. Repeat the above mentioned steps.	Degussa AG, Hanau, Germany Lot #019920

PENTA= penta-acrylate ester; TEGDMA= triethylene glycol-dimethacrylate; Bis-GMA= bysphenyl glycidyl methacrylate, UDMA= urethane dimethacrylate; BHT= butylated hydroxyl toluene; TCB resin = carboxylic acid modified dimethacrylate.

beams were carefully removed from the apparatus and the cross-sectional area at the site of failure was measured to the nearest 0.01 mm with a pair of digital calipers (Sylvae Ultra-Call, Li, USA). The bond strength values were calculated in MPa and analyzed by two-way ANOVA and Student Newman Keuls multiple comparison tests at $\alpha=0.05$ to examine the contribution of the two factors: adhesive type and cyclic loading and their interactions to the bond strength results. Fractured specimens were examined with a stereomicroscope (Olympus SZ-CTV, Olympus, Tokyo, Japan) at 40x magnification to determine the mode of failure. Failure modes were classified as adhesive or mixed.

Representative fractured specimens from each of the 10 subgroups were dehydrated for 48 hours in a desiccator (Sample Dry Keeper Simulate Corp, Japan), then mounted on aluminum stubs with carbon cement. They were then coated with gold by means of a sputter-coating unit (E500; Polaron Equipment Ltd, Watford, England) and observed with a scanning electron microscope (SEM) (Zeiss DSM-950, Karl-Zeiss, Germany) at an accelerating voltage of 20 kV to examine the morphology of the debonded interfaces.

RESULTS

Table 2 shows the mean MTBS values and failure modes obtained for the different groups. Both the type of adhesive ($F=25.02$; $p<0.0001$) and use of mechanical loading ($F=41.91$; $p<0.0001$) influenced MTBS to dentin. No interaction existed between these two factors ($F=2.07$; $p=0.11$). The power of the statistical analysis for MTBS was 0.78.

Multiple comparison tests further revealed that Clearfil SE Bond and Single Bond exhibited greater MTBS to dentin than the other three adhesives. Prime&Bond NT and Prime&Bond XP performed simi-

Table 2: *MTBS Values and Distribution of Failure Modes (A: Adhesive; M: Mixed) Obtained with the Different Adhesive Systems With and Without Cyclic Loading (T-E: Total-etch systems; S-E: Self-etching systems).*

		Without Load Cycling			**Load Cycling**		
		Mean (SD)	**A**	**M**	**Mean (SD)**	**A**	**M**
T-E	Single Bond	43.34 (11.1) **A**	27.9%	72.1%	28.29 (8.4) **a***	40%	60%
	Prime&Bond NT	29.08 (4.7) **B**	34%	66%	11.73 (3.2) **b***	77.8%	22.2%
	Prime&Bond XP	29.79 (4.5) **B**	20%	80%	25.15 (7.9) **a**	27.9%	72.1%
S-E	Clearfil SE Bond	46.07 (12.1) **A**	34.5%	65.5%	30.61 (5.3) **a***	44.5%	55.5%
	Etch&Prime 3.0	16.99 (7.7) **C**	72.7%	27.3%	XX	XX	XX

Values are means (standard deviation) in MPa, with the number of beams chosen as the statistical unit (n=35-40) and α=0.05.
Within the same column, groups with the same letter are not statistically significant.
**Indicates significant differences between groups that received or not load cycling.*
XX: No MTBS data could be obtained due to premature failure of all the specimens during beam preparation.

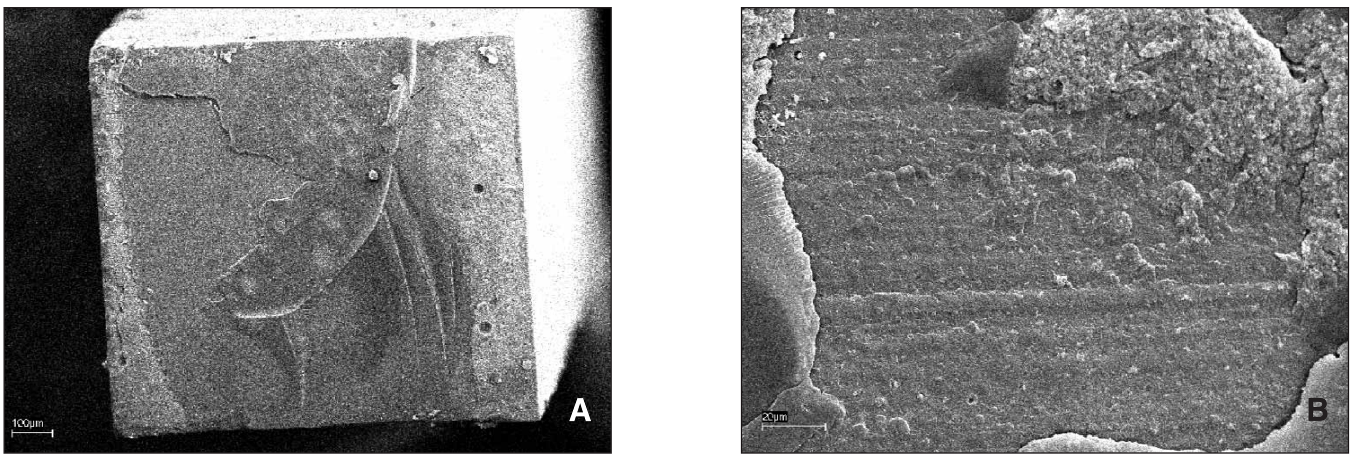

Figure 1. *SEM images of the fractured dentin surface of a specimen bonded with Single Bond after cyclic loading. Figure 1A) A mixed failure may be observed, with resin composite present at the right and left margins and adhesive in the central area. Figure 1B) A higher magnification view of the failure that occurred at the top of the hybrid layer.*

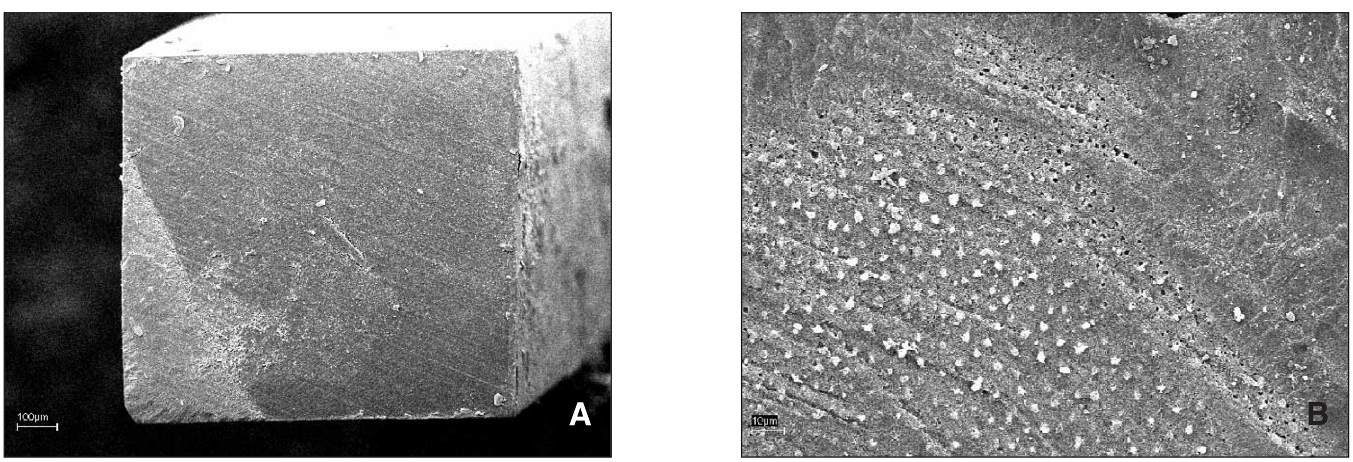

Figure 2. *SEM observations of the fractured surface along the dentin side of a specimen bonded with Prime&Bond NT after cyclic loading. Figure 2A) An adhesive failure, mainly at the top of the hybrid layer, is observed, but a small area (left and inferior corner) failed at the bottom of the hybrid layer. Figure 2B) At a higher magnification, resin-filled dentinal tubules are shown; non-infiltrated dentin and porosity within the hybrid layer are also shown.*

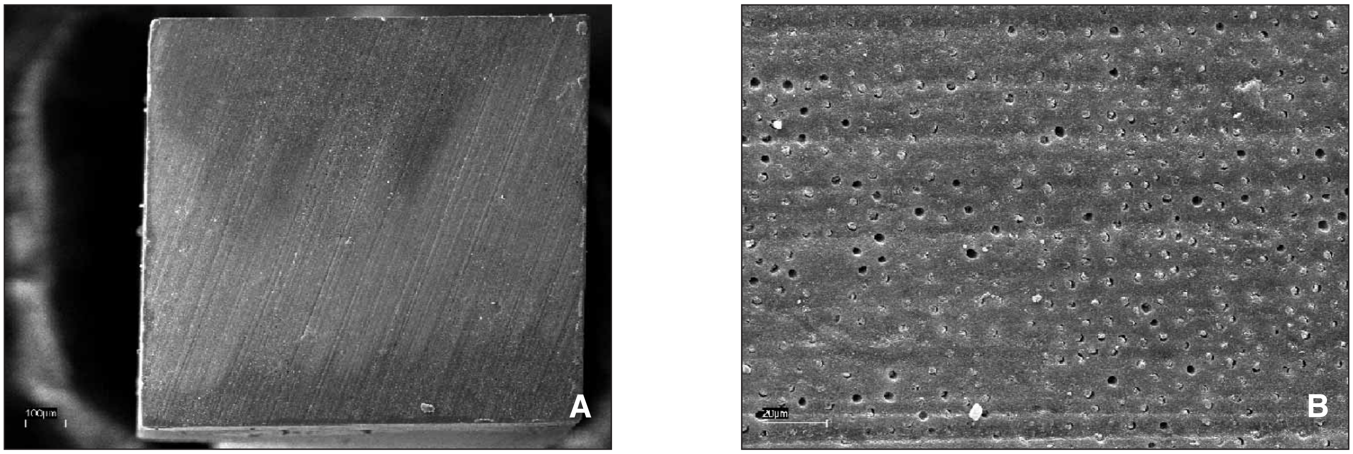

Figure 3. *SEM observations of the fractured surface along the dentin side of a specimen bonded with Etch&Prime 3.0 before cyclic loading. Figure 3A) A general image of a typical adhesive failure. Scratches, which remain from preparation of the bonding dentin surface with silicon carbide papers, confirmed that the interface failed adhesively at the level between dentin and the adhesive. Figure 3B) The enlarged entrances of the dentinal tubules may be observed and only some were occluded by resin tags.*

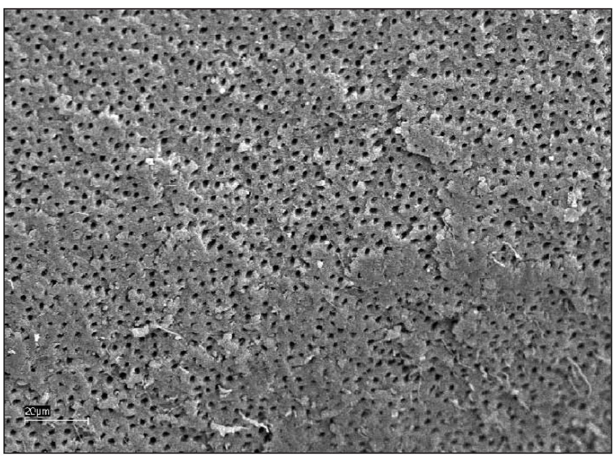

Figure 4. *SEM image of a specimen bonded with Clearfil SE Bond showing cohesive fractures of the dentin just below the hybrid layer.*

larly and Etch&Prime 3.0 resulted in the lowest MTBS. When specimens were subjected to mechanical loading, decreases in MTBS were observed for all groups except for Prime&Bond XP. Clearfil SE Bond, Single Bond and Prime&Bond XP attained higher MTBS than Prime&Bond NT. All the specimens bonded with Etch&Prime 3.0 failed prematurely during laboratory beam preparation and MTBS could not be obtained.

Most of the observed modes of failure were mixed except for specimens bonded with Etch&Prime 3.0 and those bonded with Prime&Bond NT after mechanical loading, in which the failure modes were predominantly adhesive. Adhesive failures were associated with lower bond strengths. No cohesive failure of dentin or resin composite was observed in any specimen.

Fractured dentin surfaces after MTBS testing are shown in Figures 1 through 4. Mixed fracture modes showed partially cohesive failures within the adhesive resin in all groups (Figures 1A and 2A). For the simplified total-etch adhesives (Single Bond, Prime&Bond NT, Prime&Bond XP), failures were frequently observed at either the top or base of the hybrid layer (Figures 1B and 2B). Partial cohesive fractures of demineralized dentin just below the hybrid layer were sometimes observed. Specimens bonded with Etch&Prime 3.0 failed adhesively between both the tooth substrate and bonding layer (Figures 3A and 3B). Images from the Clearfil SE Bond specimens showed cohesive failures in both resin composite and adhesive, with some fractures seen at the base of the hybrid layer (Figure 4) or within the underlying dentin.

DISCUSSION

Optimal dentin bonding is not always obtained in clinical practice, as normal daily functioning, malocclusion and parafunctional habits such as bruxism impose additional stresses upon the tooth and restorative system, which may adversely affect the adhesive bond (Nikaido & others, 2002a). A load of 90 N was used in this study, as it was considered to be within normal functional range (Anderson, 1956). In most of the studies, 1,000 to 8,000 cycles are used, with 5,000 cycles being the median value (Abdalla & Davidson, 1996).

The one-step self-etching adhesive Etch&Prime 3.0 exhibited the lowest MTBS results and frequent adhesive failures (Figure 3A). Consensus exists in the literature that supports the *in vitro* low bond strength results of some all-in-one adhesives (Fritz & Finger, 1999; Inoue & others, 2000; Toledano & others, 2001; Toledano & others, 2003; Osorio & others 2003; De Munck & others, 2003a), although they were able to completely dissolve the smear layer and form a relatively thick hybridized complex (Haller, 2000; Cardoso, Placido & Moura, 2002; Toledano & others, 2003; Osorio & others 2003; Fritz & Finger, 1999) that incorporated the smear layer (Santini, Plasschaert & Mitchell, 2001). Several reasons have been advocated to account for the suboptimal performance of these adhesive systems: (1) the combination of acidic hydrophilic and hydrophobic monomers into a single step may compromise polymerization of the adhesive (De Munck & others, 2003a), (2) the stronger etching process may destabilize the collagen, leading to a decrease in bond strength (Yoshiyama & others, 1995), (3) the inherent weak strength of the adhesive polymer (Fritz & Finger, 1999; Haller, 2000; Inoue & others, 2000) and (4) the lower degree of polymerization of the resin monomer due to a major solvent/oxygen inhibition effect in the photo-polymerization of these adhesives (Nunes & others, 2005). The lack of adequate polymerization may also account for the specimens inability to withstand the occlusal loading forces, so that all specimens failed prematurely before testing.

Prime&Bond NT and the new experimental version of this simplified total-etch adhesive, Prime&Bond XP, showed similar initial MTBS values. Both adhesive systems have similar compositions that contain PENTA, an acidic phosphonated monomer which may have some kind of interaction with the calcium ions left on the dentin surface or even with the underlying dentin (Inai & others, 1998). After load cycling, the MTBS values for Prime&Bond NT decreased, while those from Prime&Bond XP did not. Three main differences between these adhesives may account for these results: 1) Prime&Bond XP contains TEGDMA, which lowers the initial viscosity of the monomer mixture, enhancing its diffusion into the demineralized collagen matrix, increasing flexibility of the hybridized dentin and improving the rate of polymerization of the adhesive (Morgan & others, 2000; Nunes, Swift & Perdigão, 2001; Nunes & others, 2005). 2) Camphorquinone is included as a photosensitizer, increasing polymerization of the monomers and bond strength to dentin (Miyazaki & others, 1995). 3) Prime&Bond XP contains t-butanol as a solvent (instead of acetone in Prime&Bond NT). After demineralization, the collagen fibrils adhere to one another via intrafibrillar hydrogen bonding. A solvent with a solubility parameter for hydrogen bonding approximating the amino acid moieties of the collagen fibrils has a better capacity for breaking up these intrafibrillar hydrogen bonds and expanding the interfibrillar spaces to promote wetting and infiltration of the adhesive monomers (Pashley & others, 2003). It has been demonstrated that higher bond strengths were correlated with wider interfibrillar spaces, and such spaces should be properly infiltrated with resin (Eddleston & others, 2003). The application of acetone produces little solvation force, further affecting the infiltration of resin monomers, while alcohol produces progressively higher solvation pressures that develop at increasing rates (Pashley & others, 2002; Reis & others, 2003). The total-etch alcohol-based adhesive systems used in this investigation (Single Bond and Prime&Bond XP) are thought to maintain the collagen fibrils in an expanded condition after the evaporation of solvents, thus improving infiltration of the monomers (Tay & others, 1996; Perdigão & others, 1999). This may contribute to explaining the lower bond strengths of Prime&Bond NT after mechanical loading, because the decalcified non-infiltrated zone at the base of the hybrid layer is susceptible to degradation during aging (Hashimoto & others, 2002a,b; Pashley & others, 2002). Moreover, a low rate of polymerization of bonding resin within the hybrid layer has been shown for Prime&Bond NT (Hashimoto & others, 2002a); this may also lead to rapid degradation of the resin-dentin bonds.

Single Bond and Clearfil SE Bond obtained the highest MTBS to dentin. Single Bond is an adhesive based on a HEMA/alcohol mixture and has been shown to be very technique sensitive but able to obtain high bond strength values to dentin when compared to other total-etch adhesives (De Munck & others, 2003b). The results of Single Bond were also comparable to those of Clearfil SE Bond (Toledano & others, 2003). An MTBS decrease is observed after mechanical loading as well as previously reported after water degradation (De Munck & others, 2003b). Clearfil SE Bond is a two-step self-etching primer containing a highly hydrophilic 10-MDP monomer that is believed to improve wetting of the tooth surface (Van Meerbeek & others, 1994). Clearfil SE Bond causes minimal dissolution of the smear plugs and limited opening of the tubules, which reduces dentin permeability (Jackson & Söderholm, 2001) and facilitates penetration, impregnation, polymerization and entanglement of monomers with the underlying dentin to form a hybrid layer (Inoue & others, 2000; Toledano & others, 2003; Osorio & others, 2003). Moreover, 10-MDP has two hydroxyl groups that may chelate with calcium ions of dentin (Kubo & others, 2001; Nunes & others, 2003).

Within the limits of this study, the authors have to reject the null hypothesis, as cyclic loading lowered the resin-dentin bond strengths of all the total-etch or self-etching adhesive systems examined. Fatigue stress can expedite the degradation of bonds peripheral to the hybrid layer (Nikaido & others, 2002b; Sano & others, 1999; Qvist, 1983). When using Clearfil SE Bond, the loading stress seemed to be concentrated mostly at the interface between the adhesive and the hybrid layer and within the hybrid layer; whereas, specimens bonded using a total-etch approach (Single Bond, Prime&Bond NT and Prime&Bond XP) failed mostly at the top or beneath the hybrid layer where demineralized collagen fibrils were exposed and the adhesive failed to envelop the collagen network (Figures 1 and 2). Such factors have been perceived to be the weakest link in achieving durable long-term bonding (Nikaido & others, 2002b; Osorio & others, 2003; Pashley & others, 2002).

CONCLUSIONS

Although the results obtained from this study cannot be directly extrapolated from the clinical situation, they provide some information regarding the susceptibility of resin-dentin bonds deteriorating after cyclic loading. Long-term clinical data are still required to further evaluate the efficacy of these adhesives on dentin.

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