Differential effect of in vitro degradation on resin–dentin bonds produced by self-etch versus total-etch adhesives

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Received 16 December 2004; revised 12 July 2005; accepted 12 July 2005
Published online 3 January 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30656

Abstract: Objective: To evaluate the effect of an in vitro challenge (NaOCl immersion) on microtensile bond strength (MTBS) of five adhesive systems to dentin. Methods: Flat dentin surfaces from 40 molars were bonded with three total-etch adhesives (Single Bond, Prime&Bond NT and the experimental Prime&Bond XP), and two self-etching agents (Clearfil SE Bond and Etch&Prime 3.0). Composite build-ups were constructed with Tetric Ceram. Teeth were then sectioned into beams of 1.0 mm² cross-sectional area. Half of the beams were immersed in 10% NaOCl aqueous solution for 5 h. Each beam was tested in tension in an Instron machine at 0.5 mm/min. Data were analyzed by 2-way ANOVA and 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 resulted in the lowest MTBS. After NaOCl immersion, MTBS decreased in all groups. The highest MTBS values were obtained for Clearfil SE Bond and Prime&Bond XP. Scanning electron microscopy observation of debonded sticks evidenced dissolution and microstructural alterations of intertubular dentin, except when Clearfil SE Bond was used. Conclusions: Resin–dentin bonds are prone to chemical degradation. The extent of the resin degradation is adhesive system specific. Chemical degradation of the nonresin infiltrated collagen fibers does also exist in total-etch adhesives. Both processes may reduce long-term resin–dentin bond strength. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res 77A: 128–135, 2006

Key words: dentin; resin; degradation; bonding; sodium hypochlorite

INTRODUCTION

The durability of bonds between adhesive resins and dentin is of critical importance, and little is known regarding the stability of hybridized layers. In general, reports show that dentin bond strength decreased during water storage, over time, because of degradation of the resin and the collagen fibrils within the hybrid layer. Dentin bonding systems have been simplified and improved so as to provide increased long-term durability of adhesive restorations. Two main strategies are used to create dentin bonding: the total-etch (TE) bonding systems and the self-etching (SE) approach. TE bonding systems act by removing the smear layer with phosphoric acid, followed by the application of a primer and an adhesive in two different steps (or in the same one) (Single Bond, Prime&Bond NT, and Prime&Bond XP). When using this procedure, the demineralised collagen layer may impair resin infiltration and compromise bonding. In the SE systems, the acid and the primer are combined in one solution to form an acidic monomer, and a final bonding step is later applied (Clearfil SE Bond). SE all-in-one adhesives have also been introduced and contain all components in just one solution (Etch&Prime). For SE systems, no discrepancy is expected between the depth of demineralisation and the depth of resin infiltration.

It is possible to perform in vitro aging tests for challenging resin–dentin bonds. A reduction in specimen size (dentin–resin-bonded sticks of 1 mm² cross-sectional area) and immersion of the specimens in 10% sodium hypochlorite aqueous solution (NaOClaq) for a short experimental time period will permit evaluation of the ability of the resin monomers to protect the collagen matrix of dentin from proteolytic activity.

The null hypotheses to be tested are that there are no differences in dentin bond strength when using different adhesive systems, and that NaOCl immersion of specimens does not affect bond strength to dentin.
**TABLE I**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Components</th>
<th>Mode/Steps of Application</th>
<th>Manufacturer</th>
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</thead>
<tbody>
<tr>
<td>Single Bond</td>
<td>2-Hydroxyethylmethacrylate; water; ethanol; bis-GMA; dimethacrylates; amines; methacrylate-functional; copolymer of polyacrylic and polyitaconic acids.</td>
<td>Etch for 15 s. Rinse with water spray for 10 s, leaving tooth moist. Apply two consecutive coats of the adhesive with a fully saturated brush tip. Dry gently for 2–5 s. Light cure for 10 s.</td>
<td>3M, St.Paul, MN, USA. Lot. 4242.</td>
</tr>
<tr>
<td>Total-etch</td>
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<tr>
<td>Self-priming</td>
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<tr>
<td>Prime &amp; Bond NT</td>
<td>PENTA; UDMA resin; Resin RS–62-1; T-resin; D-resin; nanofiller; initiators; stabilizer; cetyleamine hydrofluoride; acetone.</td>
<td>Etch for 15 s. Rinse with water spray for 15 s 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 it is necessary. Leave the surface undisturbed for 20 s. Remove solvant by blowing gently with air for at least 5 s. Light cure for 10 s.</td>
<td>Dentsply/De Trey GmbH, Konstanz, Germany. Lot. 020900918.</td>
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<tr>
<td>Total-etch</td>
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<tr>
<td>Self-priming</td>
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<tr>
<td>Self-etch</td>
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<tr>
<td>2-steps</td>
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<tr>
<td>Self-etch</td>
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<tr>
<td>1-step</td>
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<tr>
<td>Prime &amp; Bond XP</td>
<td>TCBResin; PENTA; UDMA; TEGDMA; BHT; camphorquinone; functionalised amorphous silica ethyl-4-dimethylaminobenzoate; t-butanol.</td>
<td>Etch for 15 s. Rinse with water spray for 15 s 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 s. Remove solvant by blowing gently with air for at least 5 s. Light cure for 10 s.</td>
<td>Dentsply/De Trey GmbH, Konstanz, Germany. Lot. 0304000987.</td>
</tr>
<tr>
<td>Total-etch</td>
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<tr>
<td>Self-priming</td>
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PENTA: pentaacrylate ester; TEGDMA: triethylene glycol-dimethacrylate; Bis-GMA: bisphenyl glycidyl methacrylate, UDMA: urethane dimethacrylate; BHT: butylated hydroxyl toluene; TCB resin: carboxylic acid modified dimethacrylate.

**MATERIALS AND METHODS**

Forty caries-free extracted human third molars that were stored (4°C) in 0.5% chloramine T for up to 1 month were used. The specimens were sectioned below the dentin–enamel junction and ground flat with 180-grit silicon carbide abrasive papers under running water to provide uniform and smear-layer covered dentin surfaces. Single Bond, Prime&Bond NT, Prime&Bond XP, Clearfil SE Bond, and Etch&Prime adhesives were applied following manufacturers’ instructions. Table I displays mode of application, components, and manufacturers of the tested adhesives. Resin
build-ups, each 6 mm in height, were constructed incrementally (1.5 mm) with Tetric Ceram resin composite (Vivadent, Schaan, Liechtenstein). Each layer of the composite was light-activated for 40 s with a Translux EC halogen light-curing unit (Kulzer GmbH, Bereich Dental, Wehrheim, Germany). Light-intensity output was monitored with a Demetron Curing Radiometer (Model 100 Demetron Research Corporation, Danbury, CT) to be, at least, 600 mW/cm². The bond strength values were calculated in MPa and analyzed by ANOVA and Student Newman Keuls multiple comparisons. Mean MTBS values and modes of failures obtained with the different adhesive systems with and without NaOCl challenge are shown in Table II. The within the same column, groups with the same letter are not statistically significant.

### RESULTS

Mean MTBS values and modes of failures obtained for the different groups are shown in Table II. The adhesive system \( F = 25.06; p < 0.001 \) and \( \text{NaOCl}_{aq} \) immersion \( F = 150.79; p < 0.001 \) influenced MTBS to dentin. Interactions existed between both variables \( F = 6.68; p < 0.001 \). The power of the multiple ANOVA for MTBS was about 0.82.

Clearfil SE Bond (SE) and Single Bond (TE) give greater MTBS to dentin than the other three adhesives. Prime&Bond NT (TE) and Prime&Bond XP (TE) performed similarly, and Etch&Prime (SE) resulted in the lowest MTBS. When specimens were subjected to \( \text{NaOCl}_{aq} \) immersion, decreases in MTBS were observed for all groups. The SE system—Clearfil SE Bond—and the TE adhesive—Prime&Bond XP—attained higher MTBS than the rest of the adhesives. Specimens bonded with Etch&Prime (SE) produced pretesting failures and MTBS values could not be obtained.

Most of the observed modes of failure were mixed, except for specimens bonded with the one-step self-etch adhesive (Etch&Prime) that failed predominantly adhesively. Major rates of adhesive failures were associated with lower bond strengths. Specimens that undergo \( \text{NaOCl}_{aq} \) immersion failed predominantly mixed, except for Prime&Bond NT (TE) that presented mostly adhesive failures. No completely cohesive failure of dentin or resin composite was observed in any specimen.

SEM of debonded dentin surfaces after MTBS testing are shown in Figures 1–5. Mixed fracture modes were frequent in all groups except for Etch&Prime (SE) that failed predominantly adhesively at the top of the hybridized smear layer [Fig. 1(a)], and resin tags are observed occluding the enlarged tubule entrances [Fig. 1(b)]. In the case of the total-etch adhesives, failures were frequently mixed found at the base of the hybrid layer; partial cohesive fractures of demineralized dentin just below the hybrid layer were sometimes observed (not shown), usually associated with high bond strength data. Images from Clearfil SE Bond (SE) specimens showed mixed failures found at the top, at the base of the hybrid layer, or within the...
hibridized smear layer, and often both within the same section. After NaOCl\(_{aq}\) immersion, specimens showed partially cohesive failures within the adhesive resin [Figs. 2(a)–5(a)]. A gradual loss of adhesive from the periphery to the center portion of the bonding area is observed in all groups, and resin-remaining area is smaller for Prime&Bond NT than for the rest of the adhesives [Figs. 2(a)–5(a)]. For Single Bond and Prime&Bond NT (TE) groups, fractures were at the top of the hybrid layer (as scratching from dentin grinding are clearly observed) [Figs. 2(a) and 3(a)], and resin tags are maintained and protruding at the tubule entrances, intertubular dentin is clearly altered by NaOCl\(_{aq}\) [Figs. 2(b) and 3(b)]. When Prime&Bond XP (TE) specimens are examined, most of the fractures are located at the base of the hybrid layer and at the noninfiltrated underlying dentin [Fig. 4(a)], opened and enlarged dentin tubule entrances without resin tags were observed, so that, intertubular dentin had a typical pattern of etched and NaOCl-treated dentin [Fig. 4(b)]. Specimens bonded with Clearfil SE Bond (SE) showed most of the failures at the top of the hybrid layer [Fig. 5(a)], and small areas of fractures located at the base of the hybrid layer, exposing the underlying dentin, may also be observed [Fig. 5(b)]. Tubule entrances may be observed; they were not enlarged, but were opened and filled with resin tags, microstructural aspect of intertubular dentin was not modified by NaOCl\(_{aq}\) [Fig. 5(b)].

**Figure 1.** SEM observations of the fractured surface along the dentin side of a specimen bonded with Etch&Prime 3.0. (a) A general image of a typical adhesive failure. Existence of the scratches, produced by the preparation of the bonding dentin surface with carbide papers, confirmed that the interface failed adhesively at the top of the hybridized smear layer, and dentin remains covered by some adhesive resin. (b) Enlarged entrances of the dentinal tubules occluded by resin tags are observed.

**Figure 2.** SEM images of the fractured dentin surface of a specimen bonded with Single Bond and debonded after NaOCl\(_{aq}\) immersion. (a) A mixed failure may be observed, the main fracture occurred at the top of the hybrid layer (because of the loss of the adhesive resin). Some adhesive resin is remaining at the central area. (b) A higher magnification view of the failure that occurred at the top of the hybrid layer is observed, showing resin tags protruding at the tubule entrances. Intertubular dentin appearance is that of etched and NaOCl\(_{aq}\)-treated dentin, showing no collagen and enlarged tubules with many interconnecting canals.
DISCUSSION

The total-etch self-priming adhesives Prime&Bond NT and Prime&Bond XP showed similar initial MTBS values. Both adhesive systems contain PENTA, an acidic phosphonated monomer, which could have some kind of interaction with the calcium ions left on dentin surface, or even with the underlying dentin. The TE system Single Bond attained higher values; it is based on a HEMA/alcohol mixture that is able to better wet the etched dentin surface and maintain the collagen fibers in an expanded condition after the evaporation of solvents, improving the monomers infiltration, and has been shown to obtain high bond strength values to dentin, when compared to other total-etch adhesives.

Bond strength of the TE Single Bond to dentin was similar to that of the SE Clearfil SE Bond. Clearfil SE Bond contains a highly hydrophilic 10-MDP monomer, which is believed to improve the wetting of the tooth surface and chelate to calcium ions of dentin. This SE adhesive causes minimal dissolution of smear plugs and facilitates penetration, impregnation, polymerization, and entanglement of monomers with the underlying dentin to form a hybrid layer.

Etch&Prime is a 1-step SE adhesive system (all-in-one), obtaining the lowest MTBS values and frequent adhesive failures (Fig. 1). Consensus exists about the
low bond strength of most of these all-in-one adhesive systems.\textsuperscript{5,17,19–21} Even though, studies have shown that the bonding agent completely dissolved the smear layer and formed a relatively thick hybridized complex,\textsuperscript{17,20,22–24} incorporating the smear layer and tubule entrances were not only opened but also enlarged creating thick resin tags (Fig. 2).\textsuperscript{17,20} The poor bonding performance of these all-in-one adhesive systems could result from the low strength of the adhesive polymer\textsuperscript{19,22,24} and/or the low degree of polymerization of the resin monomer, probably due to a major solvent/oxygen inhibition effect in the photopolymerization of these adhesives.\textsuperscript{20,25}

After storage in NaOCl\textsubscript{aq}, the MTBS fell in all specimens. NaOCl\textsubscript{aq} is a nonspecific deproteinizing agent; in aqueous solution superoxide radicals—\textsuperscript{2}O\textsubscript{2}—are formed and induce oxidations that fragment long peptide chains of proteins.\textsuperscript{26} Chlorination of protein terminal groups is also produced as well as hypochlorous acid formation evidenced.\textsuperscript{27} Some of these amino acid-derived chloramines have also been shown to increase the proteolytic susceptibility of this modified collagen.\textsuperscript{28} The decline in bond strength is the result of both an hydrolytic process on the resin and the solubilization of unprotected collagen fibrils within the decalcified dentin.\textsuperscript{5,12,13,29–31}

Some studies have been conducted in order to evaluate the long-term durability of resin–dentin bonds. In vivo studies showed that the bond strength of a three-step total-etch adhesive may be reduced by 50–65\% after 2–3 years,\textsuperscript{32} or even by 60 and 77\% within 1 year when using a self-priming total-etch adhesive and a two-step self-etching adhesive respectively.\textsuperscript{33} However, in vitro studies, based on water storage of the specimens, attained smaller reductions in bond strength that are between 23 and 55\%,\textsuperscript{4–6,34} even when the dentin–resin interfaces were directly exposed to water up to 6 years.\textsuperscript{34} Observed reductions in bond strength in the present study, and those previously reported after NaOCl\textsubscript{aq} immersion (65–77\%)\textsuperscript{12,13} are similar to bond strength reductions obtained when in vivo degradation studies are performed.\textsuperscript{32,33} In vivo studies also reported that the microstructural aspect of the debonded surfaces suggested that the exposed collagen was digested by proteolytic enzymes, which may be released from leukocytes, salivary glands, and bacteria in plaque, thus explaining the attained very low bond strengths.\textsuperscript{32} Resinous materials seems to be degraded and extracted from the hybrid layer, increasing the porosities at the interface.\textsuperscript{2,33}

No bond strength reduction was expected after challenging resin–dentin bonds obtained with a SE system, because demineralized and nonresin infiltrated collagen fibers should not exist as the etching and the infiltrating processes are occurring together.\textsuperscript{8,9,12,13} However, a reduction in bond strength is observed. It may account for reported evidences supporting that some self-etching systems produce a continuous demineralization of dentin, after polymerization, creating an etched nonresin-infiltrated layer on the underlying dentin.\textsuperscript{11,35} It may also be that, when using such highly hydrophilic resins, the degradation of the resin and the loss in mechanical properties are the chief detrimental factors affecting long-term bonding effectiveness.\textsuperscript{13}

The higher water solubility of some hydrophilic resins and the existence of residual water within the hybrid layer may lead to: void formation, a lower degree of cure of the adhesive resin,\textsuperscript{36–38} and poorly
infiltrated collagen fibrils within the hybrid layer that might accelerate the degradation effect.\(^{38}\)

Fractographic analysis of debonded sticks showed a gradual loss of adhesive at the top of the hybrid layer or at the top of the hybridized smear layer (as grinding marks of polish may be observed on the exposed dentin surface) (Figs. 2, 3, and 5) with this loss of adhesive being from the periphery to the center of the debonded area. The TE Prime&Bond XP and the SE Clearfil SE Bond, which attained the better resistance to NaOCl\(_{aq}\) immersion, showed the smallest resin dissolution areas (Figs. 2–5). The better resistance of these resins to the chemical degradation may be the main reason of the lower reduction in bond strength of these adhesives. The lower susceptibility of these resins to hydrolysis is probably due to a higher degree of cure of the bonding resins. Prime&Bond XP contains TE-GMA, which brings down the initial viscosity of the monomer mixture, enhancing diffusion of reactive groups, increasing the flexibility, and the rates of polymerization of the resin.\(^{25,39}\) Camphorquinone is included in both adhesives systems (Prime&Bond XP and Clearfil SE Bond) as sensitizer. This activator is in charge to trigger the cascade curing reaction, producing free radicals that increase the rate of monomer conversion.\(^{37}\) Moreover, a low rate of polymerization of the bonding resin has been previously shown for Prime&Bond NT (Fig. 3),\(^{40}\) and for Etch&Prime\(^{25}\) leading to rapid degradation of the dentin bonds.

According to previous observations, the loss of resin was responsible for the loss of bond strength.\(^{12,13}\) The extent of resin dissolution is proportional to the bond strength decrease\(^{12,13}\) and it is material dependent. Moreover, the deterioration pattern of the intertubular dentin also depends on the adhesive system. The test medium acted more aggressively with the intertubular dentin of specimens bonded by a TE procedure compared with those bonded with the SE system Clearfil SE Bond. After phosphoric acid etching, collagen is highly susceptible to deproteinization processes.\(^{41}\) When bonding with TE adhesive systems, the NaOCl\(_{aq}\) can affect the resin–dentin bond structures following two pathways (1) the etched and noninfiltrated layer [Fig. 4(b)] and (2) the collagen that was not properly resin-infiltrated and later exposed because of the bonding resin dissolution by the NaOCl\(_{aq}\) [Figs. 2(b) and 3(b)]. SEM observations of the debonded surfaces that underwent a TE adhesive procedure showed collagen dissolution and microstructural alterations of the intertubular dentin [Figs. 2(b), 3(b), and 4(b)]. The resin tags that are protruding the altered dentin surface [Figs. 2(b) and 3(b)] suggest resin and/or collagen dissolution. Demineralized dentin with phosphoric acid is very unstable, and wet demineralized dentin is very difficult to be impregnated with resins.\(^{42}\) Improvement of monomer permeability is essential to the preparation of good hybridized den-

tin, providing a more stable bonding.\(^{43}\) As a result, when using TE systems, even with acceptable MTBS values, nanoleakage and secondary caries may be expected. In the case of Clearfil SE Bond, intertubular dentin appeared unaffected by NaOCl\(_{aq}\) and resin tags are not protruding. It may indicate the existence of a much more stable hybrid layer that may inhibit proteolytic degradation.\(^{10}\) This could result from the fact that the monomer of Clearfil SE Bond, 10-methacryloxydecyl dihydrogen phosphate (10-MDP), readily adhered to hydroxyapatite and formed a calcium salt with a very low dissolution rate.\(^{44}\)

Although the results obtained from this study may not be directly extrapolated to the clinical situation, they provide some information with regard to the performance of dentin treatment procedures. This approach can elucidate some specific factors that may be more detrimental to long-term bonding effectiveness, and future research can focus on improving it. Even so, long-term clinical data are still required to further evaluate the efficacy of these adhesives on dentin.

The null hypothesis has to be rejected as bond strength to dentin is different for the tested adhesives and formed resin–dentin bonds are prone to in vitro degradation after NaOCl\(_{aq}\) immersion.

**Clinical significance**

Resin–dentin bonds are prone to chemical degradation, and the tested all-in-one adhesive, E&P, provided the least durable bond strength. After the TE procedure, chemical degradation of the nonresin infiltrated collagen fibers does exist. Resin dissolution at the hybrid layer does also occur, leaving collagen unprotected. Both processes will help to reduce long-term resin–dentin bond strength. The rate of resin dissolution is adhesive system specific.

### References