Shear strength after ethylenediaminetetraacetic acid conditioning of dentin

Andreas Cederlund, Björn Jonsson and Johan Blomlöf

Department of Basic Oral Sciences, School of Dentistry, Karolinska Institutet, and Department of Woman and Child Health, Karolinska Institutet, Stockholm, Sweden

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On the basis of previous studies, it was hypothesized that a chelating agent such as ethylenediaminetetraacetic acid (EDTA) in a saturated aqueous solution (24%) can function as a dentin conditioning agent with exposure times comparable to that of phosphoric acid without compromising shear bond strength. Thirty caries-free human third molars, divided equally between two groups, were used. In group one, four experimental surfaces were prepared on each tooth, and cylindrical copper matrixes with a diameter of 5 mm were attached to the prepared surfaces. The experimental surfaces were then treated with a 24% EDTA gel for 30, 60, 120, or 240 sec, respectively. Dentin was bonded with All Bond 2, after which a flowable composite was added and light-cured. In group two, which served as control, two surfaces were prepared on each tooth. One surface was left unetched, whereas the other side was treated with 24% EDTA-gel for 30 sec. A shear bond strength test was performed at a crosshead speed of 1 mm/min until the composite debonded. There was no statistically significant difference ($P < 0.89$) between results of the shear bond strength test for the different EDTA conditioning times. The control group showed a significant difference in shear bond strength between untreated surfaces and surfaces conditioned for 30 sec with EDTA. Thus, the results indicate that the duration of EDTA gel conditioning of dentin surfaces need not exceed that of phosphoric acid in clinical practice to obtain an acceptable level of bond strength.

□ *Bonding; dentin; etching; ethylenediaminetetraacetic acid; shear strength*

Andreas Cederlund, Department of Basic Oral Sciences, School of Dentistry, Karolinska Institutet, P.O. Box 4064, SE-141 04 Huddinge, Sweden. Fax: +46 8-774-59-35, e-mail: andreas.cederlund@ofa.ki.se

Since first introduced, bonding of resins to dentin has become an indispensable therapy in clinical dentistry, and interest has been focused on development of stronger and more advanced bonding systems (1). In 1982 Nakabayashi et al. (2) proposed the concept of hybrid layer formation to increase dentin bond strength. The hybrid layer consists of a resin-infiltrated collagen network and resin-impregnated peri- and intratubular dentin. The concept of hybrid layer formation has since become generally accepted. The bonding mechanism of many bonding systems is based on the formation of a hybrid layer between hydrophilic resin components and partially demineralized dentin surfaces. Preparation of cavities with hand instruments or burrs always results in the formation of a smear layer (3). The smear layer, which is up to 10 μm thick, consists of hydroxyapatite and denatured collagen (4), and if caries is excavated, the smear contains bacterial remnants and carious dentin (5). To obtain a smear-free dentin surface with exposed collagen fibers, Nakabayashi and colleagues used a mixture of citric acid and ferric chloride. Many dentin-bonding systems based on the total-etch technique have used 32%–37% phosphoric acid to open up the entrance of the tubules and demineralize the dentin surface (6–8). However, phosphoric acid is a rather strong acid ($pK_{H_3PO_4}$ 2.12), active at pH 1, whereas ethylenediaminetetraacetic acid (EDTA) is a gentler chelating agent (pK_{EDTA} 6.1) working at neutral pH. Studies have shown that EDTA selectively removes the hydroxyapatite and preserves the structure of the collagen matrix (9, 10).

Recent experimental work has shown increased dentin shear bond strength with EDTA etching than with phosphoric acid etching, both used in combination with All Bond 2 (11). This study indicated that a 24% EDTA gel preparation might be used for etching for select bonding systems. However, the time used for etching (3 min) was based on results from experiments on root surface conditioning in periodontal treatment (9) and may not be clinically acceptable in restorative dentistry.

On the basis of previous studies, it is hypothesized that a chelating agent such as EDTA in a saturated aqueous solution (24%) can function as a dentin conditioning agent with exposure times comparable to that of phosphoric acid without compromising shear bond strength.

Materials and methods

Experimental material

Thirty intact and caries-free human third molars, extracted for hygienic reasons, were used in the present study. After extraction the teeth were rinsed in tap water, and attached soft tissue was removed. Before any experiments were performed the teeth were stored in sterile water for not less than 1 and not more than 2 months. During storage the water was changed periodically. To create good fixation of the roots, the teeth were mounted in brass rings, using plaster of Paris and leaving the crown accessible.

In the experiments All Bond 2 bonding system (Bisco Inc., Schaumburg, Ill., USA, lot 9900006295) was used. All Bond 2 is composed of a two-component primer and a bonding resin (Table 1).

Table 1. Composition of All Bond 2

	Primer A	Primer B	Dentin/enamel bonding resin
Primer/resin	NTG-GMA	BPDM	BIS-GMA, UDMA, HEMA
Solvent	Acetone, ethanol	Acetone, ethanol	—

All Bond 2 is composed of a two-component primer and a bonding resin. Primer A and primer B are mixed in equal amounts before use.

Experimental outline

The study consisted of two separate experiments. The first experiment was performed to assess the optimal EDTA etching time for dentin bonding. Experiment two served as control, in which the result of the optimal etching time used in experiment one was compared with a non-etched control surface.

Etching time

Fifteen teeth were used in this experiment. Four experimental surfaces were cut on each tooth (mesial, distal, buccal, and lingual) with a water-cooled diamond saw (Isomet 11-1180, Buehler Ltd, Lake Bluff, Ill., USA). The thickness of enamel and dentin that was removed was on average 2.1 ± 0.4 mm ($X \pm$ standard deviation (s)) when 0.3 mm was added to compensate for the diamond blade. The surfaces on each tooth were then randomized before etching and etched with a 24% EDTA gel preparation (Apoteksbolaget, Sweden) for 30, 60, 120, or 240 sec, respectively. This was followed by rinsing with tap water for 20 sec.

Control group

Fifteen teeth were used in this group. Dentin surfaces were cut on the mesial/distal or buccal/lingual aspects to create pairs. The thickness of enamel and dentin removed was on average 2.2 ± 0.3 mm ($X \pm s$) when 0.3 mm was added to compensate for the diamond blade. One of the surfaces on each tooth was etched with 24% EDTA gel for 30 sec, followed by rinsing with tap water for 20 sec, whereas the other was left untreated and served as control.

Bonding procedures

Before bonding, the teeth from the two groups were placed in a water thermostat at 37°C for 1 h. Wax was used to attach and seal the periphery of a 2-mm-high Dentatus size 1 copper ring with a diameter of 5.2 ± 0.06 mm ($X \pm s$) to each side. In a pilot study ground sections were made of test specimens produced with the same technique as in the present study. The results from the pilot study did not indicate any problems with wax seeping in under the copper rings. Dentin etching was performed by inserting the EDTA gel with a syringe into the copper ring for the time period described for each experimental group. Etching time was measured after completed gel placement. The surfaces were then rinsed in tap water for 20 sec, followed by gentle air drying, at an air pressure of 300 kPa and a distance of 4 cm from the surface, to remove excess water, leaving the surfaces moist. Dentin surfaces were subsequently bonded with All Bond 2 in accordance with the instructions from the manufacturer. One drop of Primer A was mixed with one drop of Primer B. The mixture was applied on the moist dentin surfaces in five consecutive coats and air-dried for 5 sec. If the surface did not show a glossy appearance, additional primer was applied. D/E Bonding Resin was applied and light-cured. Light curing was performed with an Apollo 95 E (DMDS, European Manufacturing, Fleury d'Aude, France) light-curing unit for 3 sec at a distance of 2–3 mm from the surface. The intensity of the Apollo 95 E was measured to be an average of 1.1 W/cm^2. After bonding, a flowable composite (Tetric Flow, Vivadent Ets, Liechtenstein, lot B00032) was inserted into the copper ring to a thickness of 2 mm and light-cured twice for periods of 3 sec. The bonded and filled teeth were stored in a water thermostat at 37°C for 24 h before testing.

Shear strength test

The wax, used to attach the copper rings, was removed before testing, but the copper rings were still in place. After storage for 24 h each tooth was mounted in an Alwetron universal testing machine (Alwetron TCT 50, Lorentzen & Wettre, Stockholm, Sweden), using a 0.04-mm steel matrix band (GAMA, Stockholm, Sweden) for alignment (Fig. 1). Shear strength test was performed by lowering a knife-edged chisel at a crosshead speed of 1 mm/min until the composites on the experimental surfaces debonded (Fig. 2).

Statistical evaluation of results

Shear strength results were analyzed in accordance with ISO protocol WP 11405 (12). Tests in accordance with Kolmogorov–Smirnov were performed to reject the hypotheses of non-normality of data. Consequently, the Levene test of homogeneity of variance could be applied (13). The strength of etching and bonding combinations was tested by using analysis of variance (ANOVA). Bivariate correlation analysis (Pearson) was used for analyses of bivariate correlation between shear strength and cutting depth. Multiple regression analysis using dummy variables was used in the multivariate analyses of influence from different surfaces and sawing depth on the result. When shear strength was compared using etching for 30 sec and no etching, the one-sample Student t test was used for differences of strength between pairs of surfaces (delta strength) from the same tooth. A validated statistical package was used for analysis (SPSS for Windows, V 10.0; SPSS Inc., Chicago, Ill., USA).

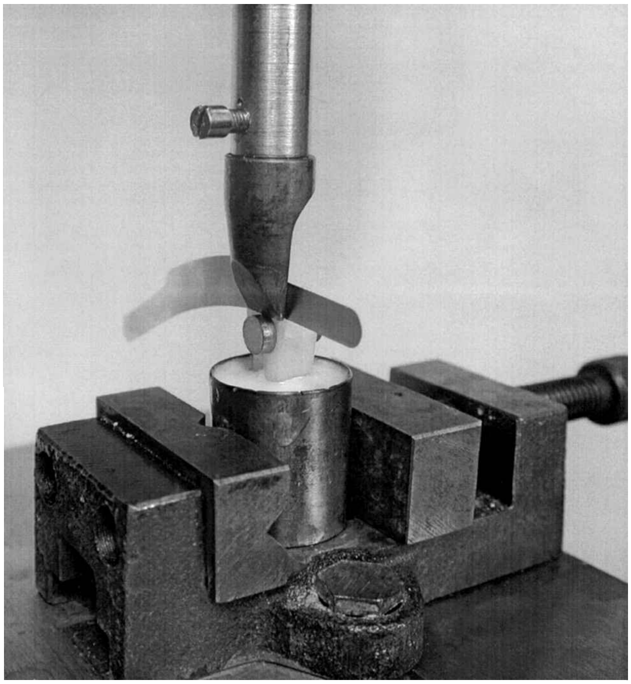

Fig. 1. Alignment of tooth surface for shear strength test. The brass ring holding the tooth was mounted in a vice with the tooth surface parallel to the 0.5-mm-wide knife-edged chisel used for the shear strength test. The matrix band used for alignment was detached before testing.

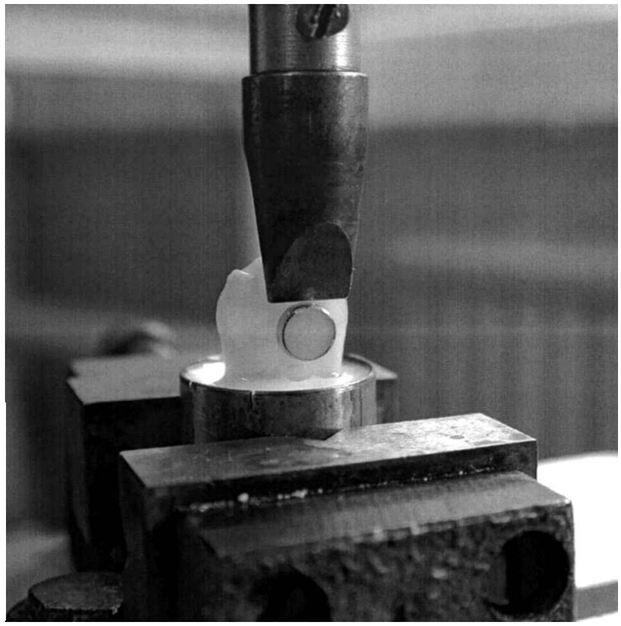

Fig. 2. Test specimen mounted in an Alwetron universal testing machine. Experimental cavity delineated by a 2-mm-high Dentatus size-1 copper ring. Top of image showing knife-edged chisel used to debond the experimental surface by shear force.

Results

Etching time

The results of varied dentin conditioning times on dentin shear strength are shown in Table 2. ANOVA yielded a non-significant result ($P < 0.89$), indicating that there were no statistically significant differences between groups of teeth with different etching time. Neither the bivariate correlation analysis nor the multivariate analyses could show any statistically significant correlations between shear strength, cutting depth, and type of surface used in the experiment. There were two cases of cohesive failures in the dentin. One occurred in the group etched for 60 sec, and one in the group etched for 120 sec. Since there were only two of 60 test samples and occurred in different groups, analysis of failure modes was not performed.

Etching versus no etching

Results of shear strength tests for the control group is summarized in Table 2. The one-sample *t* test did show a significant deviation ($P < 0.0001$) between etched surfaces and surfaces with no etching. There were no cohesive failures in these groups. There was no statistically significant correlation between cutting depth and shear strength.

Discussion

A previous study (11) has shown that dentin conditioning with a 24% EDTA gel may increase dentin shear bond strength compared with phosphoric acid etching. The etching time used in that study was 3 min and was based on studies of root surface conditioning for periodontal treatment (9). However, a shorter etching time (15–30 sec) is preferable in restorative dentistry. In the present study it was hypothesized that a chelating agent such as EDTA in a saturated aqueous solution (24%) can function as a dentin conditioning agent with exposure times comparable to that of phosphoric acid without compromising shear bond strength. The present results in the light of a previous

Table 2. Effects of ethylenediaminetetraacetic acid (EDTA) conditioning and application time on shear strength

Etching times (sec)	*n*	Mean shear bond strength (MPa)	*s*
30	15	15.3	4.3
60	15	16.0	3.2
120	15	15.5	3.1
240	15	16.3	4.0
Control			
Control 0	15	5.6	2.1
30	15	14.6	3.0

All of the etched groups used a 24% EDTA gel preparation applied with a syringe to the experimental surface. For dentin bonding All Bond 2 was used in all groups. *s* = standard deviation.

study (11) with phosphoric acid confirm the hypothesis. However, before results are discussed, some methodologic issues have to be addressed.

The method used for shear strength testing in the present study has frequently been used for testing of dental adhesives and is also described in ISO guideline WP 11405 (12). However, the details of the test protocol are left to the individual investigator. The use of metal molds to delineate the bonded surface has earlier been recommended because it creates conditions more 'clinical like' for light-cured composite materials (14). All specimens were placed in a water thermostat at 37°C before bonding, to more accurately mimic the clinical situation. Etching times were chosen on the basis of what is realistic in the clinical situation. Since it is known that EDTA etching is less effective on enamel than phosphoric acid, the clinical application of EDTA as a cavity etchant may be limited to dentin conditioning (10). In this setting it would be preferable and more effective to first insert the dentin conditioner in the cavity (EDTA gel), directly followed by application of the enamel etchant (phosphoric acid gel). Therefore, it would not be clinically realistic to have 15 sec as etching time for dentin.

In contrast to most other studies, which often only use the occlusal surface as bonding area, the present study used four sides on each tooth (mesial, distal, buccal, and lingual). This gives the opportunity to compare the results obtained within each tooth, minimizing the effect of irregularities in the tooth material between different teeth, as indicated by the high level of significance for this comparison in the present study. Studies using the same method as in the present study have shown various shear bond strengths, both higher and lower than those of the present study (15). A study of microtensile bond strength of All Bond 2 has shown numerical values as high as 46.8 MPa (16) compared with from 15.3 to 16.3 in the present study (Table 2). This indicates, as has previously been discussed, that the choice of method strongly influences the results and range of the bond strength (17, 18). Earlier results have shown that similarly etched and bonded specimens show different bond strengths when comparing shear and tensile strength testing methods (19). Therefore, it may be questioned whether it is appropriate to compare results from two principally different test methods.

It has been reported that a large dentin bonding area will result in lower dentin bond strength than a small bonding area (20). The reason for this may be due to more defects and stress induced at the bonding interface in a larger bonding area than in a smaller one. Another reason may be the uniformity of the material tested (21). The direction of the dentin tubules also varies more in a larger bonding area. In this study we used a relatively large experimental cavity, with a diameter of 5 mm, compared with other studies, which have often used a diameter of 2 mm. Test methods using small surface area samples have been described as very technique-sensitive (20). A method using a larger surface area may be preferred because it is easier to handle and enables better access when etchant, bonding agent, and composite material are inserted. It also better simulates the clinical situation, since a filling rarely has a diameter of 2 mm. This was established in a previous methodologic study (11).

In the present study there were two cases of cohesive failure, one in the group etched for 60 sec and one in the group etched for 120 sec. The role of cohesive failure has been debated. Some authors state that cohesive failure in dentin is due to superiority of the bond over dentin (22). Others have suggested that cohesive failure can be a result of the test method, with little correlation to bond strength (23).

Results from the present study showed that shear strength did not differ between dentin surfaces conditioned for periods varying from 30 to 240 sec. In clinical practice, a short conditioning time may be preferred, and, as indicated by the present study, 30 sec may be sufficient. However, when comparing etched and non-etched surfaces, a statistically significant difference in shear bond strength could be seen, showing that etching cannot be omitted. Thus, the results indicate that the duration of EDTA gel conditioning of dentin surfaces need not exceed that of phosphoric acid in clinical practice to obtain an acceptable level of bond strength.

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