Comparative study of a filled and an unfilled version of a dentin bonding system to dental hard tissues

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Abstract

Purpose: To evaluate the performance of a novel filled bonding system to enamel and dentin in comparison to its previous unfilled version.

Materials and Methods: For the microtensile test, 12 human extracted molars stored in a 0.5% chloramine T solution were used, providing 3 teeth per adhesive system, half of which were used for bond strength tests on enamel, and the other half for adhesion testing on dentin. Resin composite was bonded incrementally to the prepared surfaces, using the adhesives Adper Scotchbond 1 [Adper Single Bond] and Adper Scotchbond 1 XT [Adper Single Bond 2] (3M-ESPE) according to the manufacturer's instructions. The bonded specimens were stored in distilled water at 37°C for 24 hours, sectioned into sticks and then subjected to a microtensile bond test. Data was analyzed with two-way ANOVA and Tukey's test. Additional specimens were immersed in a silver tracer solution for TEM examination of nanoleakage.

Results: No significant differences were found between both adhesive systems ($p>0.05$). However significant differences were observed among substrates ($p<0.01$), with enamel specimens showing lower bond strengths (29.1±0.4 MPa) than dentin specimens (43.5±1.8 MPa). For both systems, TEM micrographs of the resin-dentin interface demonstrated variable extent of nanoleakage.

Clinical Significance: The incorporation of nanofillers does not enhance the performance of the two-step total-etch adhesive system investigated.

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Introduction

Adhesive systems have been extensively used for esthetic restorations[1, 2]. They are classified into two main groups according to the etching procedure, which utilizes either phosphoric acid or acidic monomers. The former results in the complete removal of smear layer and demineralization of the underlying tooth structures. These etch-and-rinse adhesives may be applied either in three steps or two steps[3]. Two-step etch-and-rinse adhesives are preferred by clinicians due to their simpler and faster application in comparison with the three-step systems. However, to achieve satisfactory bonding, the adhesive should be liberally applied in order to saturate the exposed collagen fibril network and establish a sufficiently thick resin layer on the top of the hybrid layer. The presence of a thick resin layer acts as both a shock-absorber and prevents over-thinning of the adhesive that compromises its polymerization via oxygen inhibition [4-6].

The creation of a shock-absorbing resin layer is important not only to protect against the stress produced by the shrinkage of the composite resin, but also to absorb masticatory forces, tooth flexure effects and thermal cycling effects [6]. Several clinical trials [7-9] support this elastic bonding concept, with excellent results reported. A shock-absorbing layer may be produced either by applying several coats of an unfilled adhesive consecutively[5], or by incorporating nanofillers in the adhesive to improve its viscosity, so that the filled adhesive may be applied as a single layer[4, 10-12]. The latter method has been perceived by the marketing divisions of certain manufacturers to be beneficial in the development of adhesives which have simpler and faster applications.

Following this marketing strategy, a new filled version of the previously unfilled Adper Scotchbond 1 (also known as Adper Single Bond in USA) (3M ESPE (St. Paul, MN, USA) has recently been launched. This new filled one-bottle adhesive is known as Adper Scothbond 1 XT (also known as Adper Single Bond 2 in USA) (3 M ESPE), and is claimed by the manufacturer to contain characteristics conducive to simple and effective bonding to enamel and dentin. As with Adper Scotchbond 1, this product also contains VitreBond polyalkenoic acid copolymer. As a further improvement, 5 nm-diameter silica particles were incorporated in the amount of 10% weight, with the claim that the incorporation of nanofillers would further strengthen the bond and enhance the performance of the adhesive system. However, apart from the information supplied by the manufacturer, the benefits of the filled versus the unfilled version of this adhesive system have not been fully substantiated in literature.

The objective of this study was thus to analyze, through bond strength and ultrastructural evaluation, the adhesive potential on enamel and dentin of the filled version of a simplified adhesive in comparison with its predecessor. The null hypothesis tested was that the incorporation of nanofillers in a simplified etch-and-rinse adhesive has no effect on in vitro bonding performance.

Materials and Methods

Preparation of specimens for microtensile bond strength evaluation

Eighteen caries- and defect-free human third molars were stored in 0.5% chloramine T at 4°C and used within one month after retrieval. They were cleaned to remove all debris and calculus and then randomly divided into two equally-sized groups, E and D, for enamel and dentin bond strength testing and ultrastructural examination. Prior to the testing procedures, they were taken out of the disinfectant solution and stored in distilled water for 48 hours. In Group E, the most superficial portion of enamel on the buccal aspect of each tooth was removed using 180-grit silicon carbide paper under running water, in order to eliminate the surface aprismatic enamel layer and to create a flat, abraded enamel bonding surface. In Group D, the occlusal enamel of each tooth was removed using a slow-speed saw equipped with a diamond-impregnated blade (Digital Isomet, Buehler, Lake Bluff, IL, USA) under water irrigation, to expose a mid-coronal dentin surface. The dentin surface was polished with a 180 grit silicon carbide paper under running water for 20 seconds, to create a clinically-relevant smear layer. The prepared surfaces were examined under a stereoscopical microscope (Nikon type 102, Tokyo, Japan) at 30X magnification to ensure that no dentin was exposed in the teeth designated as Group E, and that the bonding surfaces of teeth designated as Group D were free of enamel.

Adper Scotchbond 1, the unfilled version, and Adper Scotchbond 1 XT, the new filled version of the simplified etch-and-rinse adhesive were bonded to the prepared surfaces (n=3) according to the manufacturer's instructions and light-cured using a halogen light-curing unit (VIP, Bisco Inc., Schamburg, IL, USA), with the curing intensity set at 600 mW/cm^2. A resin composite (Tetric Ceram, Ivoclar-Vivadent, Schaan, Liechtenstein) was incrementally applied to each tooth to form a core 6 mm in height. Each increment was individually light-cured for 40 seconds.

The restored teeth were then stored in distilled water at 37°C for 24 hours. After this period, they were sectioned perpendicularly to the bonded interface with the Isomet saw into a series of slabs. Each slab was further sectioned into 0.9 x 0.9 mm sticks, yielding a cross-sectional area of approximately 0.8 mm^2. The use of three teeth for each subgroup resulted in a total of 27-34 sticks in enamel and 63-76 sticks in dentin to test the microtensile bond strength. Sticks with premature failure were discarded and excluded from the compilation of the bond strength means.

The width and thickness of each stick was measured with a digital caliper, and glued with cyanoacrylate (Zapit, DVA, Corona, CA, USA) to the Geraldeli's device [13]. This jig consists of two parts joined together by a pair of post-hole joints. The Geraldeli's device was placed in a Bencor Multi-T testing assembly (Danville Engineering, San Ramon, CA, USA), which was mounted on an universal testing machine (Triax digital 50, Controls, Milano, Italy) at a crosshead speed of 0.5mm/min. Each specimen was stressed under tension to failure point. The load at failure point was recorded in Newtons and bond strength was then calculated in MPa.

Bond strength data from the four groups were statistically analyzed, with the number of sticks as the statistical unit. The data distribution was first analyzed for their normal distribution using the Kolmogorov-Smirnov test, and their equal variance using the Levene median test. A two-way ANOVA was subsequently applied to examine the effects of adhesive systems and substrates, and the interaction of these two factors on microtensile bond strength. All the analyses were processed by the SPSS 11.0 software (SPSS Inc., Chicago, IL, USA), with the level of significance set at 95% probability level.

Transmission electron microscopy

Three additional teeth with bonding surfaces prepared in dentin were used for each adhesive version. Both the filled and unfilled versions of the simplified adhesive were applied as previously described and coupled with a 2 mm thick layer of resin composite. After storage in distilled water for 24 hours, a 2 mm thick longitudinal slab was prepared from each tooth using the slow speed saw under water cooling. These slabs were coated with nail polish applied as close to the bonded interface as possible. The coated slabs were subsequently immersed in a 50 wt% ammoniacal silver nitrate tracer solution [14] in the dark for 24 hours.

The silver-impregnated slabs were rinsed thoroughly in running water for an hour. They were then immersed in a photodeveloping solution for 8 hours under a fluorescent light to reduce the diamine silver ion complexes into metallic silver grains within potential voids along the resin-dentin interfaces. The slabs were then processed for transmission electron microscopy (TEM). Undemineralized, epoxy resin-embedded, 90-100 nm thick sections were prepared according to the TEM protocol of Tay *et al.*[14]. Without further staining, the undemineralized slabs were examined using a TEM (Philips EM208S, Eindhoven, The Netherlands) operated at 80 kV.

Results

The mean and standard deviation of the microtensile bond strength values measured for all of the tested subgroups and the percentages of premature failures are reported in Table 1. The two-way ANOVA indicated that there was no significant difference between the adhesive systems tested ($p>0.05$). However, the dental substrate had a significant effect on bond strength ($p<0.01$), with enamel specimens showing lower bond strengths (29.09 $\pm$ 0.38 MPa) than dentin specimens (43.46 $\pm$ 1.8 MPa). The interaction between adhesive material and substrate was not significant ($p>0.05$). Pre-test failures occurred only on sectioning enamel specimens.

Examination of unstained, undemineralized TEM micrographs of the resin-dentin interface, after immersion in ammoniacal silver nitrate, revealed that nanoleakage was abundant along the base of the hybrid layer when both Adper Scotchbond 1 and Adper Scotchbond 1 XT were used in dentin (Figure 1 and 2). However in Adper Scotchbond 1 interfaces, silver deposition occurred predominantly in the form of isolated silver grains and water tree channels which were unevenly distributed in the hybrid layer (Figure 1). Conversely, with Adper Scotchbond 1 XT silver nitrate infiltration presented in the form of reticular patterns within the hybrid layer (Figure 2).

Higher magnification of these interfaces showed a phase separation of the polyalkenoic acid copolymer component, which could be seen in both the first and the second adhesive layer (Figure 3 and 4). However, with Adper Scotchbond 1 XT, in relation to the lower water content, phase separation was not as evident in the first layer, being more readily recognized in the second adhesive layer (Figure 4). These spherical polyalkenoic acid copolymer phases were found to consist of both larger and very fine silver grains that were confined into nanofiller clusters (Figure 5). The filled resin matrix was predominantly devoid of these silver grains.

Discussion

In the current study a newly introduced two-step total-etch adhesive and its predecessor were evaluated in their bonding ability to enamel and dentin. The non-trimming variable of the microtensile test was chosen for its reputation as a reliable adhesion testing technique that is capable of assessing the interfacial strength between an adhesive and the bonding substrate, thus avoiding the occurrence of cohesive failures during bonding testing due to a better stress distribution during loading [15]. Another advantage of this technique is that multiple specimens can be obtained from a single tooth by means of series of cuts made on a single tooth [16]. However it has been speculated that these cuts may induce the formation of internal defects into the specimens. Some authors [17-19] have already demonstrated that structural defects are commonly observed in SEM analysis, being more frequent on enamel than on dentin specimens. It has been hypothesized that the inherent fragility of enamel in the small cross-sections of microtensile specimens may be responsible for their higher frequency of defects, as well as their failure under relatively low loading levels in comparison to dentin. In fact, in the present trial, regardless of the adhesive system, enamel specimens constantly showed lower bond strength values than dentin specimens. This finding is in disagreement with the literature data provided by conventional tensile and shear bond strength tests [10, 20, 21], and years of clinical experience which confirm that enamel offers bonding conditions more favorable and consistently reliable than dentin. Another issue that should be considered is that pre-test failures occurred only on sectioning enamel specimens, emphasizing its fragility. It is possible that, as it is a a very brittle substrate, the values found for enamel are unrealistic measurements and the defects created into the interface or within the substrate can quickly increase [22], weakening the bond interface, consequently decreasing bond strength values recorded [19]. In order to

Table 1. Means, standard deviations and percentage of premature failures of the tested groups.

		Mean	Standard deviation	% of premature failure
Scotchbond 1	Dentin	42.18	6.82	0
	Enamel	28.82	1.05	31.70
Scotchbond 1 X T	Dentin	44.73	4.66	0
	Enamel	29.36	2.90	27.66

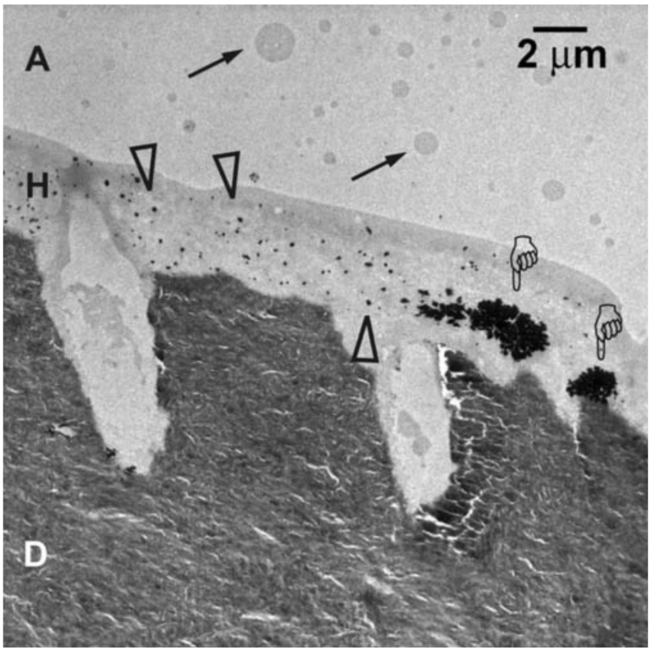

Figure 1. Unstained, undemineralized TEM micrograph of the resin-dentin interface using Adper Scotchbond 1. The specimen was examined after immersion in ammoniacal silver nitrate, demonstrating the two modes of silver impregnation: isolated silver grains (open arrowhead) scattered along the hybrid layer (H) and reticular patterns of nanoleakage silver deposits (pointer). Phase separation of the polyalkenoic acid copolymer component could be seen (arrow) into the adhesive layer (A). D: mineralized dentin.

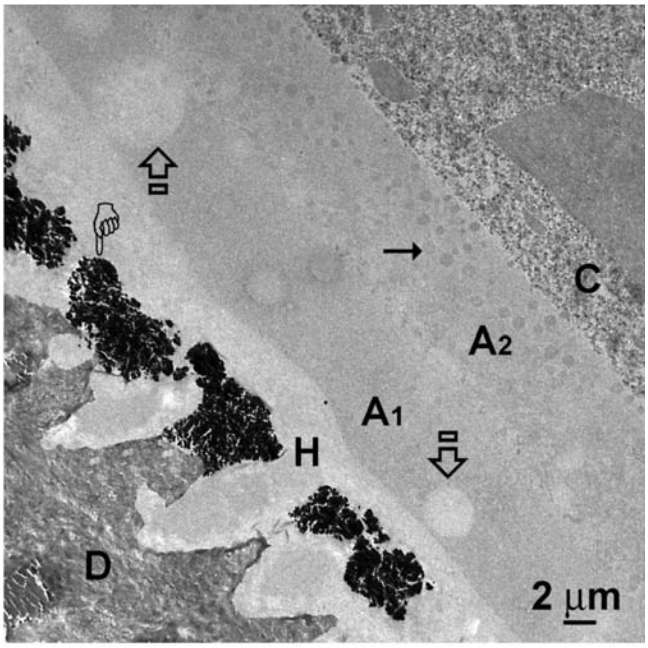

Figure 2. Unstained, undemineralized TEM micrograph of the resin-dentin interface using the experimental version of Adper Scotchbond 1 that contains nanofillers. The specimen was examined after immersion in ammoniacal silver nitrate. Two layers of the adhesive (A1 and A2) were applied as in the original Adper Scotchbond 1. Nanoleakage (pointer) is abundant along the base of the hybrid layer (H). Phase separation of the polyalkenoic acid copolymer component could be seen from both the first (open arrows) and the second (arrow) adhesive layer (A1 and A2 respectively). C: resin composite.

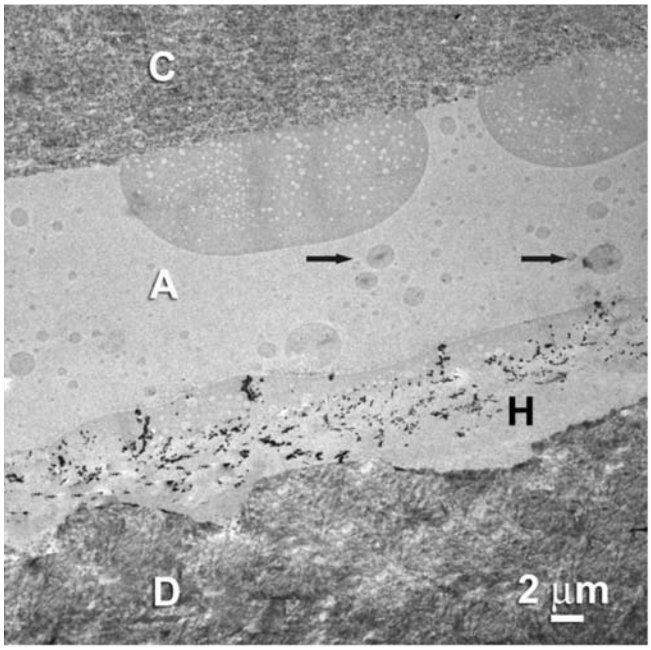

Figure 3. A higher magnification view of the hybrid layer (H) of Figure 1, showing isolated silver grains scattered along the hybrid layer and silver-filled water channels (water-trees). Phase separation of the polyalkenoic acid copolymer component (arrow) could also be seen all along the adhesive layer (A). D: mineralized dentin. C: resin composite.

measure the "true" bond strength in enamel, alternative cutting equipment, which places the least possible stress on the substrate, should be used in further research to evaluate bond efficacy of adhesive materials in enamel.

With regard to the effect of the improvement in the adhesive system, no statistical differences were found in the bond strength values between Adper Scotchbond 1 and Adper Scotchbond 1 XT. The TEM analysis revealed abundant nanoleakage along the base of the hybrid layer in dentin in both adhesive systems . Therefore, within the limitations of this study, it can be concluded that the incorporation of nanofillers in this adhesive neither strengthened the bond nor enhanced its sealing performance.

Since no difference was found between either system and the greatest number of factors affecting the quality of the bond in dentin than on enamel due to it's intrinsically heterogeneity, only TEM micrographs of the resin-dentin interface were done in this study. Isolated silver grains, water trees and reticular patterns of nanoleakage silver deposits were detected along the hybrid layer. It has been demonstrated that water trees represent regions in which minute quantities of water are retained within the adhesive-dentin interface [23]. According to these authors, the formation of water trees is a common finding, especially with ethanol-based total-etch adhesives, possibly in relation to more difficult water displacement. Conversely, the reticular patterns of nanoleakage silver deposits were probably caused by a

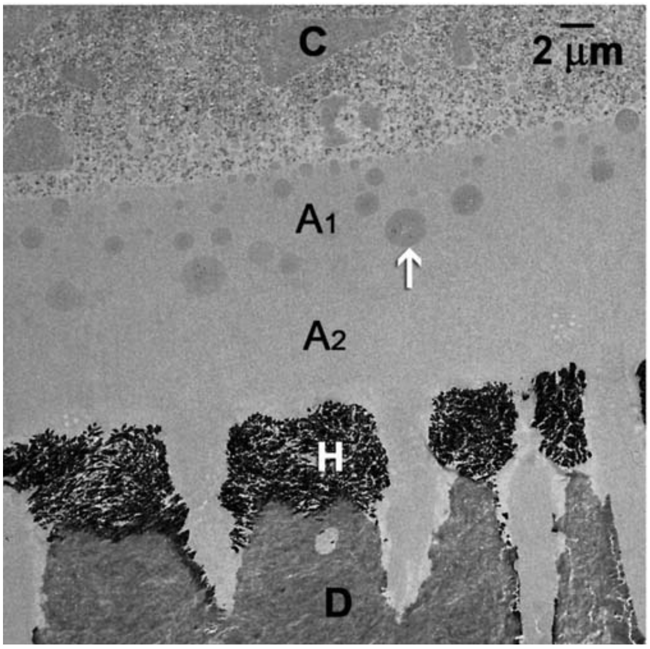

Figure 4. TEM of Adper Scotchbond 1 XT bonded to acid-etched dentin that was air-dried for 1 s. The demineralized collagen matrix collapsed and there was no resin infiltration. The collapsed matrix re-expanded when the specimen was immersed in silver nitrate, so that the entire matrix is now filled up with silver. Because there is also less water, phase separation was not so apparent in the first layer (A2), and phases of polyalkenoic acid copolymer (arrow) were more readily recognized in the second adhesive layer (A1).

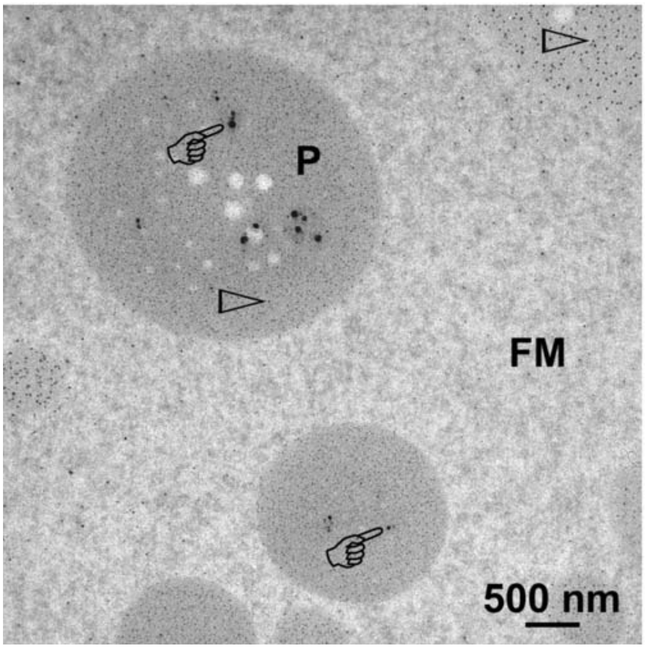

Figure 5. At higher magnification, the spherical polyalkkenoic acid copolymer phases (P) were found to consist of both larger (pointer) and very fine (open arrowheads) silver grains. The filled resin matrix (FM) was predominantly devoid of these silver grains.

collapsed demineralized collagen matrix that was not infiltrated by resin. This phenomenon was more clearly observed with Adper Scotchbond 1 XT (Figure 2), probably due to its lower water content when compared to Adper Scotchbond 1. The collapsed matrix re-expanded when the specimen was immersed in silver nitrate and the matrix remains filled up with silver.

The phase separation of the polyalkenoic acid copolymer component, also observed in this trial, had already been reported [23, 24]. According to Agee et al. (2003) the high degree of nanoleakage found on the hybrid layer of Adper Scotchbond 1 was probably due to the polyalkenoic acid copolymer, which may block adequate penetration of resin monomers inside the collagen fibril network. Since Adper Scotchbond 1 XT also contains this copolymer, the anticipated elevated degrees of nanoleakage on this product were evident.

The findings of the current study demonstrated that this new filled bonding system did not improve bond strength and sealing ability in enamel nor in dentin. However, further research is needed to evaluate the whole performance of this new adhesive system. More accurate conclusions on its performance in the oral environment should be derived from long clinical trials.

Acknowledgements

The authors are grateful to Programme Al,an, European Union Programme of high level scholarships for Latin America (identification number E03D08091BR).

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