Self-etch vs etch-and-rinse adhesives: effect of thermo-mechanical fatigue loading on marginal quality of bonded resin composite restorations

Roland Frankenbergera,*, Franklin R. Tayb

aDental Clinic I Operative Dentistry and Periodontology, University of Erlangen-Nuremberg, Glückstraße 11, D-91054 Erlangen, Germany
bPaediatric Dentistry and Orthodontics, Faculty of Dentistry, University of Hong Kong, 34 Hospital Road, Hong Kong SAR, China

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Adhesives;
Resin composites;
Fatigue

Summary
Objective. This study evaluated the marginal integrity of dentine adhesives bonded to enamel and dentine, before and after thermo-mechanical loading (TML).

Methods. MO cavities with proximal boxes beneath the CEJ were prepared in extracted human third molars. Direct resin composite restorations (Tetric Ceram) were bonded with 3-step etch-and-rinse (Syntac Classic, Solobond Plus, OptiBond FL), 2-step etch-and-rinse (Admira Bond, Single Bond), 2-step self-etch (AdheSE, Clearfil SE Bond), and 1-step self-etch (all-in-one) adhesives (Adper Prompt, Xeno III, iBond). Marginal gaps were analyzed using SEM of epoxy resin replicas. Bonded interfaces before TML were examined with TEM to identify pre-existing attributes for subsequent marginal disintegration.

Results. In enamel, high percentages of gap-free margins were initially identified for all adhesives. After TML, etch-and-rinse adhesives exhibited significantly higher percentages of gap-free margins (~90%) compared with two-step self-etch (~75%) and all-in-one (~55%) adhesives (p<0.05). iBond did not completely etch through the enamel smear layer. In dentine, 89-100% gap-free margins were initially observed. After TML, there were no statistical differences among etch-and-rinse (62-70%) and two-step self-etch (62-63%) adhesives (p>0.05). The all-in-one adhesives exhibited significantly less gap-free margins (<40%) in dentine (p<0.05), with iBond showing the worst marginal integrity (15%). The presence of pre-existing water channels within the adhesives probably expedited water sorption when restorations were under functional stresses.

Conclusion. Enamel bonding was more effective with phosphoric acid-etching. Etch-and-rinse and 2-step self-etch adhesives showed promising marginal adaptation
to dentine and may have a better clinical prognosis than the all-in-one bonding approach.

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Introduction

Durable adhesion of dental materials to tooth substrates is indispensable for clinical success with tooth-coloured restorative materials that shrink on polymerisation [1-3]. When direct resin composites are bonded to tooth structures using dentine adhesives, the initial and residual polymerisation stresses that are present along the cavity walls may result in gap formation, leakage, recurrent caries and pulpal irritation [4-6]. The detrimental effect of marginal gap formation cannot be offset even with the use of fluoride-releasing adhesives or restorative materials that prevent demineralization along cavity margins [7]. Thus, only hermetic sealing of restorations guarantees clinical success [8,9].

Phosphoric acid etching of enamel produces an increase in bonding surface area for micromechanical retention that has been shown to be clinically reliable [1,8]. High retention rates and excellent marginal seal have been reported in clinical techniques that involve bonding to phosphoric acid-etched enamel, as exemplified with the use of pit-and-fissure sealants, resin composite fillings, or ceramic inlays [1,2]. New approaches to bonding to enamel and dentine without phosphoric acid pretreatment have been introduced by the manufacturers of self-etch adhesives [9-12]. Two-step self-etch adhesives were characterised by separate chemical formulations for priming and bonding, utilising a self-etching, hydrophilic primer that is followed by the application of a comparatively more hydrophobic bonding agent [13-15].

The recently introduced 1-step self-etch (all-in-one) adhesives contain two liquids which are applied to tooth substrates after mixing [16-18]. The rationale for using two separate bottles for these adhesives is to isolate the potentially hydrolytically unstable acidic resin monomers from the water that is present for ionization of these monomers. The latest approach in simplifying bonding to enamel and dentine are the adhesives iBond (Heraeus Kulzer, Dormagen, Germany) and Brush & Bond (Sun Medical Inc., Shiga, Japan), in which all the adhesive components for etching, priming and bonding are supplied in a single bottle. The manufacturers claim that reliable results can be consistently achieved without the concern for the hydrolytic degradation of the acidic resin monomer 4-methacryloxyethyltrimellitic acid anhydride (4-META) in these adhesives. It has previously been shown that although mild self-etching adhesive systems are effective for bonding to bur-cut enamel, only minimal etching effects are achieved on uncut enamel and their use on the latter is not recommended [11,12,19,20].

Compared with adhesion to enamel, dentine is a completely different bonding substrate. Due to its tubular structure, the problem of dentinal fluid transudation, and the presence of the smear layer, bonding to dentine was introduced considerably later [21,22]. The main advantage arising from the use of dentine adhesives is their ability to reduce post-operative sensitivity and to support enamel margins of the restoration [1,2]. Dentine bonding is technique sensitive [23,24]. Therefore, one major goal for manufacturers in the development of new adhesives is to simplify their application procedures. The first step was to reduce conventional 3-step, etch-and-rinse adhesives to 2-step adhesives that combine the primers and bonding agents into a single solution [25]. Faster bonding and easier handling made these single-bottle adhesives very popular among dental practitioners all over the world [1].

Reports of post-operative sensitivity with the etch-and-rinse technique [26], irrespective of the 3-step or 2-step approach, have popularized the use of self-etch adhesive systems that are non-rinsing in nature and preserve the integrity of smear plugs within the dentinal tubules. Whereas 2-step self-etch systems have been reported to give reliable results, the all-in-one adhesives exhibited potential shortcomings in their incompatibility with autocuring resin composites and permeability to water movement after polymerisation [3,9,17,18].

The objective of the present study was to compare different approaches for bonding to enamel and dentine by use of a Class II fatigue loading design. Evaluation of marginal adaptation to enamel and dentine was performed with examination of epoxy resin replicas of the restorative margins under scanning electron microscopy (SEM) [25]. The null hypothesis tested was that there are no differences in the marginal integrity of either enamel or dentine margins in Class II cavities that were bonded with the different classes of dentine adhesives.
Materials and methods

Specimen selection and involved materials

One hundred and thirty intact, non-carious, unrestored human third molars, extracted for therapeutic reasons, were stored in an aqueous solution of 0.5% chloramine T at 4°C for up to 30 days. The teeth were debrided of residual plaque and calculus, and examined to ensure that they were free of defects under a light microscope at x20 magnification.

Standardised class II cavity preparations (OD, 4 mm in width bucco-lingually, 2 mm in depth at the bottom of the proximal box) with proximal margins located 1–2 mm below the cementoenamel junction were performed. The cavities were cut using coarse diamond burs under profuse water cooling (80 μm diamond, Two-Striper® Prep-Set, Premier, St Paul, USA), and finished with a 25 μm finishing diamond (one pair of diamonds per four cavities). Inner angles of the cavities were rounded and the margins were not bevelled to deliver comparable results to previous experiments [25]. The cavities were restored by use of different adhesives (Table 1).

Three teeth were used in each adhesive group for examination of the etching effect and bonding to cut enamel. After cavity preparation, one tooth was sectioned mesio-distally into two halves using a slow-speed saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water-cooling. Each half was conditioned with either phosphoric acid or the all-in-adhesive, but without further restoration with the resin composite. The adhesive was rinsed off with acetone in order to compare the etching effect of phosphoric acid vs the self-etch adhesives on cut enamel. The specimens were air-dried, mounted on aluminum stubs, sputter-coated with gold, and examined with a SEM (Cambridge Stereoscan 360, Cambridge, United Kingdom) operating at 20 kV. The other two teeth from each group were bonded with the respective adhesive and then restored with a microfilled composite with pre-polymerized fillers (EPIC-TMPT, Parkell Inc., Farmingdale, NY, USA) to facilitate ultramicrotomy for subsequent examination using transmission electron microscopy (TEM). The restored teeth were also in distilled water at 37°C for 21 days. A 1-mm thick, mesio-distal slab was sectioned from each Class II restoration using the slow speed saw under water cooling. These slabs were dehydrated in an ascending ethanol series and embedded in epoxy resin. Undemineralized 90–110 nm thick sections containing the resin-enamel interfaces were prepared according to the TEM preparation protocol described by Lai et al. [27]. The sections were collected on single-slot, carbon- and formvar-coated copper grids and were first examined unstained, using a transmission electron microscope (Philips EM208S, Eindhoven, The Netherlands) operating at 80 kV. After the initial TEM examination, the sections were further demineralized as reported by Hannig et al. [20]. The demineralized sections were rinsed with distilled water, double-stained with uranyl acetate and lead citrate for TEM re-examination.

An additional 1-mm thick slab was prepared from each Class II restoration for examination of the extent of nanoleakage along the resin-dentine interfaces. The slabs were immersed in 50 wt% ammoniacal silver nitrate for 24 h, according to the TEM silver tracer technique reported by Tay and Pashley [3,28]. Following the reduction of the diamine silver ions into metallic silver grains, the slabs were dehydrated in an ascending ethanol series and embedded in epoxy resin in preparation for ultramicrotomy. 90–100 nm thick sections containing the resin-dentine interfaces were prepared as described previously for enamel and examined with the TEM without further staining.

Marginal quality investigation

The prepared cavities (n=8) were treated with different classes of dentine adhesives according to the manufacturers’ instructions (Table 1). Eight teeth were randomly selected for each adhesive. The dentine adhesives and resin composite were polymerised with a Translux CL light-curing unit (Heraeus Kulzer, Dormagen, Germany). The intensity of the light was checked periodically with a radiometer (Demetron Research Corp, Danbury, CT, USA) to ensure that 400 mW/cm² was always delivered during the experiments. The adhesive was polymerized for 40s prior to application of the resin composite in all cases. The resin composite Tetric Ceram (Ivoclar Vivadent, Schaan, Liechtenstein; shade A2; batch no. E00194) was used for all experimental restorations.

Each cavity preparation was surrounded with a metal matrix band, bonded with the respective...
Table 1 Chemical compositions, batch numbers, dentine pre-treatment, bonding procedures, and manufacturers of the dentine adhesives tested.

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Components</th>
<th>Batch #</th>
<th>Composition</th>
<th>Application protocol</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syntac Classic (3-step etch-and-rinse)</td>
<td>Etchant</td>
<td>F51814</td>
<td>36% phosphoric acid</td>
<td>Etch enamel and dentine for 15 s, rinse, dry.</td>
<td>Ivoclar Vivadent</td>
</tr>
<tr>
<td></td>
<td>Primer</td>
<td>E52572</td>
<td>Maleic acid, TEGDMA, water, acetone</td>
<td>Apply Primer, leave undisurbed for 15 s, air-dry.</td>
<td>Schaan</td>
</tr>
<tr>
<td></td>
<td>Adhesive</td>
<td>E08386</td>
<td>PEGDMA, glutaraldehyde, water</td>
<td>Apply Adhesive, leave undisurbed for 10 s, air-dry.</td>
<td>Principality of Liechtenstein</td>
</tr>
<tr>
<td>Heliobond</td>
<td></td>
<td>E10061</td>
<td>BisGMA, TEGDMA, UDMA</td>
<td>Apply Bond, air-thin, light-cure.</td>
<td></td>
</tr>
<tr>
<td>Solobond Plus (3-step etch-and-rinse)</td>
<td>Etchant</td>
<td>26608</td>
<td>36% phosphoric acid</td>
<td>Etch for 15 s, rinse, dry gently.</td>
<td>Voco, Cuxhaven, Germany</td>
</tr>
<tr>
<td></td>
<td>Primer</td>
<td>29503</td>
<td>Water, acetone, maleic acid, acid-functionalized methacrylates, fluorides</td>
<td>Apply and air-thin.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adhesive</td>
<td>29503</td>
<td>Acetone, dimethacrylate, hydroxymethacrylate</td>
<td>Apply, air-thin and light-cure.</td>
<td></td>
</tr>
<tr>
<td>OptiBond FL (3-step etch-and-rinse)</td>
<td>Etchant</td>
<td>707583</td>
<td>37.5% phosphoric acid</td>
<td>Etch for 15 s, rinse, dry gently.</td>
<td>Kerr, Orange, CA, USA</td>
</tr>
<tr>
<td></td>
<td>Primer</td>
<td>709322</td>
<td>HEMA, GPDM, MMEP, ethanol, water, initiators</td>
<td>Scrub for 30 s, dry.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adhesive</td>
<td>709126</td>
<td>Bis-GMA, HEMA, GPDM, barium-aluminum borosilicate glass, disodium hexafluorosilicate, silica</td>
<td>Apply, air-thin and light-cure.</td>
<td></td>
</tr>
<tr>
<td>Admira Bond (2-step etch-and-rinse)</td>
<td>Etchant</td>
<td>26608</td>
<td>36% phosphoric acid</td>
<td>Etch for 15 s, rinse, dry gently.</td>
<td>Voco</td>
</tr>
<tr>
<td></td>
<td>Primer + Bond</td>
<td>25509</td>
<td>Acetone, bonding ormocer, dimethacrylates, functionalizing methacrylates, Initiators (camphorquinone, amine), stabilizer (BHT)</td>
<td>Scrub for 30 s, air-thin, light-cure.</td>
<td></td>
</tr>
<tr>
<td>Single Bond (2-step etch-and-rinse)</td>
<td>Etchant</td>
<td>7EJ</td>
<td>35% phosphoric acid</td>
<td>Etch for 15 s, rinse, dry gently.</td>
<td>3M ESPE, Seefeld, Germany</td>
</tr>
<tr>
<td></td>
<td>Primer + Bond</td>
<td>1FW</td>
<td>BisGMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiator, 3-8% water, ethanol</td>
<td>Scrub for 30 s, air-thin, light-cure.</td>
<td></td>
</tr>
<tr>
<td>AdheSE (2-step self-etch)</td>
<td>Primer</td>
<td>E35881</td>
<td>Dimethacrylate, phosphonic acid acrylate, water, stabilisers</td>
<td>Apply Primer, leave undisurbed for 30 s, air-dry.</td>
<td>Ivoclar Vivadent</td>
</tr>
</tbody>
</table>

(continued on next page)
adhesives, and restored incrementally with the resin composite in layers up to 2 mm thick. The increments were separately light-cured for 40 s each with the light source in contact with the coronal edge of the matrix band. After removal of the matrix band, the restorations were light-cured from their buccal and lingual aspects for an additional 20 s on each side. Prior to the finishing process, visible overhangs were removed using a posterior scaler (A8 5204S, Hu-Friedy, Leimen, Germany). Proximal margins were finished with flexible disks (SofLex Pop-on, 3M ESPE, St Paul, USA).

After storage in distilled water at 37 °C for 21 days, impressions (Provil Novo, Heraeus Kulzer, Hanau, Germany) of the teeth were taken and a first set of epoxy resin replicas (Alpha Die, Schuetz Dental, Rosbach, Germany) was made for SEM evaluation.

**Functional loading of class II cavities**

Thermo-mechanical loading of specimens was then performed in an artificial oral environment (‘Quasimodo’ chewing simulator, University of Erlangen, Germany). Two specimens were arranged in one simulator chamber in proximal contact, similar to the oral situation with the two restored marginal ridges in a normal intercuspation (Fig. 1). The two adjacent lateral ridges were occluded against a steatite (a multi-component semi-porous crystalline ceramic material)
antagonist (6 mm in diameter) for 100,000 cycles at 50 N at a frequency of 0.5 Hz. The specimens were simultaneously subjected to 2500 thermal cycles between +5 °C and +55 °C by filling the chambers with water in each temperature for 30 s. The mechanical action and the water temperature within the chewing chambers were checked periodically to ensure a reliable thermo-mechanical loading (TML) effect.

Figure 1 A photograph of the chewing simulator employed in the study. The insert at the right illustrates schematically the alignment of two specimens in one chamber of the chewing simulator.

Figure 2 SEM micrographs (1:200) of epoxy resin replicas reproduced from impressions of Class II cavities bonded with different dentine adhesives. RC: resin composite; D: dentine. A. Replica of Solobond Plus with ‘gap-free margin’ after 21 days of water storage, prior to thermo-mechanical loading. B. Gap formation (pointer) between adhesive and dentine, observed after thermo-mechanical loading (iBond). C. ‘Marginal irregularity’ between resin composite and dentine, observed after thermo-mechanical loading. The replica shows fluid transudation through dentine and the adhesive layer, as visible by blisters (Single Bond).
Analysis of marginal quality

After the completion of the 100,000 mechanical loading and the 2500 thermal cycles, impressions of the teeth were made again and another set of replica was made for each restoration. The replicas were mounted on aluminum stubs, sputter-coated with gold and examined under a SEM (Leitz ISI 50, Akashi, Tokyo, Japan) as before at x200 magnification.

SEM examination was performed by one operator having experience with quantitative margin analysis who was blinded to the restorative procedures. The marginal integrity between resin composite and dentine was expressed as a percentage of the entire margin length in enamel and dentine. Marginal qualities were classified according to the criteria 'continuous margin' (Fig. 2A), 'gap/irregularity' (Fig. 2B and C) and 'not judgeable/artifact'. Afterwards the percentage 'continuous margin' in relation to the individual judgeable margin was calculated as marginal integrity.

Statistical appraisal

Statistical analysis was performed using SPSS/PC+, Version 10 (SPSS Inc., Chicago, IL, USA) for Windows. As the majority of groups in each of the two investigations (i.e. enamel or dentine marginal integrity) did not exhibit normal data distribution (Kolmogorov-Smirnov test), non-parametric tests were used (Wilcoxon matched-pairs signed-ranks test, Mann-Whitney-U test) for pairwise comparisons at the 95% significance level.

Results

Morphological investigation

Bonding to cut enamel
SEM and TEM micrographs of the bonding of representative adhesive to cut enamel are shown in Figs. 3–6. iBond, a mild all-in-one adhesive, did not completely dissolve the enamel smear layer (Fig. 3A). The enamel hybrid layer comprised mostly of the smear layer, with incomplete and minimal etching of the underlying prismatic enamel (Fig. 3B). The enamel smear layer was completely dissolved in Xeno III, producing a mild surface etching effect of the enamel crystallites but with no differential etching of the enamel prisms (Fig. 4A). This all-in-one adhesive created a 1.5–3 μm thick hybrid layer in the underlying prismatic enamel (Fig. 4B), that consisted of predominantly intercrystallite infiltration (Fig. 4C) and with no resin tag formation.

Mild differential etching of the enamel prisms could be observed with the use of Adper Prompt (Fig. 5A), creating 5–8 μm thick enamel hybrid layers that consisted predominantly of intercrystallite infiltration.
infiltration, as the amount of differential etching was insufficient to produce frank resin tags (Fig. 5B). However, the adhesive was weak (Fig. 5B) and frequently separated from the surface of the hybrid layer, generating gaps between the adhesive layer and the latter (Fig. 5C). On the contrary, differential etching of enamel prisms could be clearly identified with phosphoric acid-etching (Fig. 6A), creating 8-10 μm thick hybrid layers in 3-step etch-and-rinse adhesives such as Syntac Classic that consisted of both intercrystallite infiltration (Fig. 6B) and resin tag formation (Fig. 6C).

**Bonding to dentine**
Phosphoric acid etching of dentine created 5-6 μm thick hybrid layers in 2-step etch-and-rinse adhesives such as Single Bond (Fig. 7A) and Admira Bond (Fig. 7C). Regions of incomplete resin infiltration could be identified in these adhesives, as indicated by the reticular patterns of nanoleakage within the hybrid layers. This feature was also observed in all the etch-and-rinse adhesives examined (not shown). Two additional modes of nanoleakage [35] could also be identified within the adhesive layer. For

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**Figure 4**  A. SEM micrograph depicting the etching effect of Xeno III on cut enamel. The smear layer was completely dissolved, exposing the etched prismatic enamel. There was very little differential etching of the enamel prisms and the prism boundaries could vaguely be recognised (black arrowheads). B. Unstained, undemineralised TEM micrograph of cut enamel bonded with Xeno III. The enamel smear layer was completely dissolved and a 1-3 μm thick enamel hybrid layer (H) could be observed. The interprismatic boundary (open arrowheads) of an enamel prism could be seen. Nanofiller clusters (pointer) were present within the adhesive (A). S: empty space. C. The corresponding stained, demineralised TEM micrograph showing the entrapment of enamel proteins within the hybrid layer (H). A: adhesive; S: empty space.
example, in Single Bond, silver-filled water channels (water trees [3]) were seen from the surface of the hybrid layer into the polyalkenoic acid copolymer component of the adhesive (Fig. 7B). Isolated silver grains could be observed throughout the hybrid layer and adhesive layer of Admira Bond (Fig. 7D).

An example of the bonding of a 2-step self-etch adhesive to dentine is illustrated with AdheSE in Fig. 8A and B. AdheSE completely dissolved the dentine smear layer and created a 2 μm thick hybrid layer within the intertubular dentine, with some nanoleakage observed within the hybrid layer. The adhesive layer was completely devoid of water trees. Similar features were observed in Clearfil SE Bond (not shown). By contrast, both water trees and isolated silver grains were extensively observed within the adhesive layer of the all-in-one adhesive iBond (Fig. 8C and D). These two modes of nanoleakage were also observed in the other two all-in-one adhesives, Xeno III and Adper Prompt (not shown).

Marginal quality investigation

Marginal quality in enamel
Results for marginal quality in enamel are presented in Table 2. Prior to TML, high percentages of gap-free margins were found. iBond with 89% gap-free margins exhibited significantly more gaps/
irregularities than Syntac Classic, Solobond Plus, OptiBond FL, Admira Bond, Single Bond, and Clearfil SE Bond (p < 0.05; Mann–Whitney U-test).

Comparing the results before and after TML, all adhesive systems showed a significant loss of gap-free margins (p < 0.05; Wilcoxon matched-pairs signed-ranks test). After TML, etch-and-rinse adhesives performed significantly better than self-etch adhesives (p < 0.05; Mann-Whitney U-test). Among the self-etch systems, the 2-step adhesives with separate bonding agents (AdheSE, Clearfil SE Bond) exhibited better marginal quality than the all-in-one adhesives; however, the differences were not statistically significant for clearfil SE Bond (p > 0.05; Mann-Whitney U-test). Only iBond showed a significantly smaller percentage of gap-free margins than the other adhesives (p < 0.05; Mann-Whitney U-test).

Marginal quality in dentine
Results for marginal adaptation in dentine are displayed in Table 3. Similar to enamel, high percentages of gap-free margins were found in dentine before TML. iBond with 88% gap-free margins, exhibited significantly more gaps/irregularities than Syntac Classic, Solobond Plus, OptiBond FL, AdheSE, and Clearfil SE Bond (p < 0.05; Mann-Whitney U-test).
Comparing the results before and after TML, all adhesive systems showed a significant decline in the percentages of gap-free margins ($p < 0.05$; Wilcoxon matched-pairs signed-ranks test). After TML, etch-and-rinse adhesives and 2-step self-etch adhesives performed significantly better than the all-in-one systems ($p < 0.05$; Mann-Whitney $U$-test). Among the all-in-one adhesives, iBond showed significantly less gap-free margins ($p < 0.05$; Mann-Whitney $U$-test).

**Discussion**

In this study, we investigated the marginal quality of four classes of dentine adhesives under simulated clinical conditions with the use of a chewing simulator. Understandably, clinical trials remain the gold standard in evaluating the performance of dental materials. However, one has also to take into consideration that the products under investigation may become obsolete by the time useful clinical
data are collected. This is further complicated by the time lag between obtaining the clinical results and having them published in peer-reviewed journals. Thus, preclinical screening via laboratory tests is still an important tool for the evaluation of dentine adhesives [29]. Bond strength tests are commonly carried out with quasistatic load until fracture. However, failure of clinical restorations due to high loads is exceptional [5,29]. More often, the materials or interfaces fail after repeated subcatastrophic loading, with stresses that are too small to provoke spontaneous failures during their initial applications [29]. Thus, the most frequent observation is gap formation between the resin composite and tooth substrates. These gaps may result from either insufficient compensation for the initial high polymerization shrinkage stresses that occur prior to occlusal loading, or from the lower, repeated stresses which are below the maximum stress the adhesive restoration could resist [25]. Therefore, fatigue tests provide a better understanding of the in vivo behaviour of dentine adhesives [25,29].

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**Figure 8** TEM micrographs of dentine bonded with representative self-etch adhesives prior to thermo-mechanical loading. C: composite; A: adhesive; H and between open arrows: hybrid layer; D: dentine. A. A low magnification view of the resin-dentine interface in the two-step self-etch adhesive AdheSE. B. A high magnification view of AdheSE, showing the presence of comparatively minimal nanoleakage within the hybrid layer. Some isolated silver grains were associated with the nanofiller clusters. C. A low magnification view of the resin-dentine interface in iBond, showing the presence of many silver-filled water channels (water trees—pointer) and isolated silver grains (open arrowhead) throughout the adhesive layer. D. A high magnification view of iBond, showing the presence of extensive nanoleakage within the hybrid layer. Primary water trees (open arrowheads) could be seen extending vertically from the surface of the hybrid layer, giving rise to silver-filled water blisters (asterisk) with the adhesive layer. Secondary water trees (pointer) extended circumferentially from these water blisters.
The results of this study clearly indicated that conventional phosphoric acid-etching remains the most reliable mode of pre-treatment in obtaining a durable and more fatigue-resistant enamel bond [20,30,31]. Although most of the self-etch adhesives bonded well to cut enamel prior to functional and thermal stresses, they were significantly less effective after fatigue testing. It is known from earlier reports that the micromorphological interaction of etch-and-rinse adhesives extends deeper into enamel. On the other hand, it is also known that self-etching systems provide a network of intercrystallite retention leading to a large surface for bonding [1,12,20]. This kind of enamel bond produced by these self-etching systems proved to be able of initially compensating for polymerisation shrinkage stresses. However, marginal quality of these interfaces seems to be lower when compared to the etch-and-rinse adhesives. Although aggressive self-etch adhesives like Adper Prompt creates hybrid layers that approach the thickness of those created by adhesive systems that utilise phosphoric acid-etching, it may be the lack of frank resin tags in the self-etch systems that is responsible for their compromised marginal quality. Although a flat hybrid layer that relies solely on intercrystallite retention is mechanically retentive, in-plane crack propagation may occur more easily in the presence of stress raisers such as minute air voids that are trapped along the resin-enamel interface. On the contrary, the incorporation of resin tags provides a three-dimensional grasp of the etched enamel. This may deter crack propagation via crack branching or deflection that consume fracture energy, thereby improving the fracture toughness of the interface and increasing its resistance to fatigue stresses. A similar retardation in crack propagation at the bone-cement interface has been observed with the penetration of polymethyl methacrylate bone cement spikes or ‘posts’ into cancellous bone [32,33]. For iBond, the inability to etch completely through the enamel smear layer produced thin, incomplete hybridisation of the subsurface prismatic enamel. This may additionally account for the decline in marginal integrity after TML in this mild self-etch adhesive, with the weak link possibly occurring between the hybridised enamel smear layer and the underlying unetched prismatic enamel.

There were also significant differences between 2-step self-etch systems and the all-in-one adhesives in their ability to withstand stresses generated via fatigue testing. The difference between 2-step and all-in-one self-etching systems should not be related to different ways of interacting with enamel. It is probably attributed to the fact that the all-in-one adhesives are more susceptible to water sorption. In the absence of a coupling hydrophobic bonding agent, they behave as permeable membranes after polymerization [17,18]. This may expedite water sorption between the partially demineralized enamel and the restorative material, plasticizing and eventually weakening the bonded enamel interfaces.

The present study also demonstrated pronounced differences among the adhesives in their bonding performance on dentine, with the general trend that conventional systems with separate primers and bonding agents perform better than simplified systems that combine the functions of priming and bonding, irrespective of the etch-and-rinse or the self-etch approach. The results of

<p>| Table 2 Results of the SEM analysis of enamel margins before and after thermo-mechanical loading (TML). |
|-------------------------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Gap-free margins in enamel [%] (SA)</th>
<th>Prior to TML</th>
<th>After TML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syntac classic</td>
<td>100A</td>
<td>92.9 (7.4)A</td>
<td></td>
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<tr>
<td>Solobond Plus</td>
<td>100A</td>
<td>94.3 (6.7)A</td>
<td></td>
</tr>
<tr>
<td>OptiBond FL</td>
<td>100A</td>
<td>94.7 (5.3)A</td>
<td></td>
</tr>
<tr>
<td>Admira Bond</td>
<td>99.3 (2.1)A</td>
<td>90.6 (9.3)A</td>
<td></td>
</tr>
<tr>
<td>Single Bond</td>
<td>98.9 (2.2)A</td>
<td>94.5 (7.2)A</td>
<td></td>
</tr>
<tr>
<td>AdheSE</td>
<td>92.1 (6.1)AB</td>
<td>80.0 (10.0)B</td>
<td></td>
</tr>
<tr>
<td>Clearfil SE Bond</td>
<td>96.3 (5.9)A</td>
<td>71.3 (14.1)BC</td>
<td></td>
</tr>
<tr>
<td>Adper Prompt</td>
<td>91.2 (9.9)AB</td>
<td>56.2 (14.8)C</td>
<td></td>
</tr>
<tr>
<td>Xeno III</td>
<td>94.7 (7.3)AB</td>
<td>54.6 (18.1)C</td>
<td></td>
</tr>
<tr>
<td>iBond</td>
<td>89.0 (9.7)B</td>
<td>54.0 (14.3)C</td>
<td></td>
</tr>
</tbody>
</table>

Same letters within one column indicate no statistically significant difference (p > 0.05, Mann-Whitney-U test).

<p>| Table 3 Results of the SEM analysis of dentine margins before and after thermo-mechanical loading (TML). |
|-------------------------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Gap-free margins in dentine [%] (SA)</th>
<th>Prior to TML</th>
<th>After TML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syntac Classic</td>
<td>100A</td>
<td>69.8 (14.0)A</td>
<td></td>
</tr>
<tr>
<td>Solobond Plus</td>
<td>97.9 (3.3)A</td>
<td>70.3 (19.7)A</td>
<td></td>
</tr>
<tr>
<td>OptiBond FL</td>
<td>100A</td>
<td>65.6 (14.0)A</td>
<td></td>
</tr>
<tr>
<td>Admira Bond</td>
<td>98.9 (2.0)A</td>
<td>64.2 (22.5)A</td>
<td></td>
</tr>
<tr>
<td>Single Bond</td>
<td>96.5 (5.5)AB</td>
<td>62.5 (16.1)A</td>
<td></td>
</tr>
<tr>
<td>AdheSE</td>
<td>98.9 (3.2)A</td>
<td>62.8 (21.0)A</td>
<td></td>
</tr>
<tr>
<td>Clearfil SE Bond</td>
<td>100A</td>
<td>62.9 (16.8)A</td>
<td></td>
</tr>
<tr>
<td>Adper Prompt</td>
<td>94.7 (7.8)AB</td>
<td>34.0 (15.7)B</td>
<td></td>
</tr>
<tr>
<td>Xeno III</td>
<td>95.2 (6.9)AB</td>
<td>38.1 (13.5)B</td>
<td></td>
</tr>
<tr>
<td>iBond</td>
<td>87.7 (10.4)B</td>
<td>15.0 (11.2)C</td>
<td></td>
</tr>
</tbody>
</table>

Same letters within one column indicate no statistically significant difference (p > 0.05, Mann-Whitney-U test).
the functional cavity test clearly showed that all adhesives performed very well initially in their capacity to compensate for shrinkage stresses generated during the polymerisation of the resin composite. This is reflected by the high percentages of gap-free margins after setting of the resin composite. Incorrect handling or chemical incompatibility between adhesive and resin composite of different manufacturers can be ruled out, as shown by the successful initial results. However, it is disenchanted to observe that after 100,000 loading cycles, the fatigue phenomenon has a profound influence on the bonded dentine margins, resulting in up to 85% gap formation over time.

The most important property of dentine adhesives relating to marginal quality seems to be the presence of a hydrophobic bonding agent [25]. All the adhesive systems that utilize separate bonding resins exhibited promising results that are independent of a phosphoric acid-etching step. Exceptions were the 2-step etch-and-rinse adhesives Admira Bond and Single Bond. In particular, replicas of the Single Bond specimens exhibited water transudation from the dentine aspects of the specimens, as demonstrated by the occurrence of blisters within the adhesive and swelling of the adhesive layer itself. This may be perceived as the first sign of water sorption that is precipitated by the permeability of this adhesive, with the release of the water during impression taking.

Although nanoleakage was universally observed in dentine hybrid layers of all the etch-and-rinse and self-etch adhesives, the adhesive layer in Single Bond is characterised by the presence of water trees [3] in the polyalkenoic acid copolymer component (Fig. 7A and B). Although these water trees were absent in Admira Bond, isolated silver grains could be detected throughout the entire adhesive layer (Fig. 7C and D). These features were not observed in the 2-step self-etch adhesive AdheSE (Fig. 8A and B), but were present in abundance in the all-in-one adhesives such as iBond (Fig. 8C and D). It has been suggested recently that the water trees represent channels containing free, unbound water that are trapped within the adhesive, while the isolated silver grains represent bound water that are attached via hydrogen bonding to the ionic or hydrophilic domains within the adhesive [34]. Thus the water trees and isolated silver grains are the morphologic manifestation of sites within the adhesive wherein water sorption and movement is likely to occur. Whereas the water trees provide the venues for rapid capillary fluid flow through the adhesive [35], the isolated water grains represent sites within the adhesive in which ions and small molecules can jump from nanopore to nanopore via the process of ion hopping [36,37], being the molecular mechanism for diffusion of water through the adhesive [38].

In the absence of a more hydrophobic coating in the simplified adhesive systems, rapid water sorption via the water trees and isolated silver grains can occur via the hydrophilic and permeable adhesive layer. Compared with the 2-step etch-and-rinse adhesives, water sorption is likely to be more prominent in all-in-one adhesives due to the incorporation of high concentration of ionic (acidic) resin monomers. This may account for the severe compromise in marginal integrity when dentine bonded with the all-in-one adhesives were subjected to TML. Although it is known that compression stresses result in a decrease, while tensile stresses result in an increase in the rate of water sorption through polymerized resins [39,40], it is possible that the release of compression stresses during each loading cycle creates a partial vacuum that actively promotes water transport via capillary fluid flow through the water trees that pre-existed prior to TML. It is intriguing that water sorption is rapid enough to cause a severe degradation of the marginal integrity during the period in which TML was performed. By measuring the electric impedance of hydrophobic vs hydrophilic resin films, it was shown that 20 μm thick hydrophobic resin films gave an initial, high impedance value of 1.5×10^9 ohms to electric current flow and did not change over time. Conversely, 20 μm thick hydrophilic resin films gave an initial impedance of 7×10^6 ohms (almost 1000-fold less impedance), that rapidly fell to 1×10^5 ohms after four days (i.e. another 1000-fold decrease in electric impedance in four days) [David Pashley—personal communication]. These data illustrated the extremely rapid rate in which water sorption can occur in the very hydrophilic all-in-one adhesives. As all dentine adhesives contain hydrophilic resin components to a variable extent, it is not surprising therefore to see a decline in marginal integrity for all adhesives, although such a phenomenon was exacerbated in the all-in-one adhesives. A recent long-term water storage study also showed that simplified 2-step etch-and-rinse adhesives performed worse compared to 3-step etch-and-rinse systems [30]. From a morphologic perspective, it has also been shown that the propensity for the occurrence of isolated silver grains and water trees increased when exposed bonded dentine specimens were aged in water for 12 months [41]. Further studies should be performed to correlate the morphologic expression of these two modes of silver deposition and the changes in electric impedance of bonded dentine with and
without TML, in order to confirm the hypothesis that water sorption is expedited during TML.

We have to reject the null hypothesis that are no differences in the marginal integrity of either enamel or dentine margins in Class II cavities that were bonded with the different classes of dentine adhesives. The enamel and dentine bonding fatigue performances reported in this study are in agreement with the conclusion of a recently published TML study [42], in that simplified adhesive systems cannot be recommended for unrestricted clinical use in Class II restorations. With respect to the use of self-etch adhesives, 2-step self-etch adhesives exhibited potentially more promising results than the all-in-one bonding approach.

Conclusions

All adhesives under investigation exhibited a certain amount of deterioration relating to marginal quality in enamel and dentine.

Regarding bonding efficacy to enamel and dentine, conventional 3-step etch-and-rinse adhesives are still not surpassed by the newer simplified adhesive systems.

For self-etch adhesives, the all-in-one bonding approach was less effective than 2-step self-etch systems that encompass the use of separate hydrophobic bonding resins, especially in bonding to dentine.

References


