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Bond strength of a mild self-etch adhesive with and without prior acid-etching

K.L. Van Landuyt, P. Kanumilli, J. De Munck, M. Peumans, P. Lambrechts, B. Van Meerbeek*

Leuven BIOMAT Research Cluster, Department of Conservative Dentistry, School of Dentistry, Oral Pathology and Maxillo-Facial Surgery, Catholic University of Leuven, Kapucijnenvoer 7, B-3000 Leuven, Belgium

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Clearfil SE;
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Dentin

Summary: The self-etch approach provides dentists with a generation of user-friendly and less technique-sensitive adhesives. Nevertheless, some concern has been raised regarding their bonding effectiveness to enamel, in particular when so-called 'mild' self-etch adhesives are employed.
Objectives: The purpose of this study was to test the hypothesis that the two-step self-etch adhesive Clearfil SE Bond (C-SE; Kuraray, Osaka, Japan) bonds equally effective to enamel/dentin either with or without prior etching with phosphoric acid.
Methods: Bur-cut enamel/dentin surfaces prepared from human molars were partially split in two halves by cutting a shallow groove. One half was first etched with 40% phosphoric acid (K-etchant), while protecting the other half by holding a razor blade in the groove. Next, C-SE was applied strictly following the manufacturer's instructions, after which the surface was built up using Z100 (3M Espe). After 24-h water storage, micro-specimens were prepared with the interface circularly constricted using a Micro-Specimen Former, prior to micro-tensile bond strength (MPa) measurement. In addition, interfaces of C-SE with enamel/dentin prepared with and without beforehand acid etching were examined by Feg-SEM and TEM.
Results: Beforehand etching significantly increased the bonding effectiveness of C-SE to enamel. A clearly more micro-retentive surface was revealed by TEM and Feg-SEM when enamel was etched. Phosphoric-acid etching prior to C-SE application on dentin significantly decreased the μTBS to dentin. TEM provided indications of a low-quality hybrid layer after beforehand phosphoric-acid etching.
Conclusion: Using C-SE, additional etching with phosphoric acid to improve bonding effectiveness should be limited to enamel.

* Corresponding author. Tel.: +32 16 33 75 87; fax: +32 16 33 27 52.
E-mail address: bart.vanmeerbeek@med.kuleuven.ac.be (B. Van Meerbeek).

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Introduction

Regarding user friendliness and technique sensitivity, the self-etch approach appears most promising. As self-etch adhesives make use of acidic monomers that can etch and prime simultaneously, the separate etching and water-rinsing phase can be omitted. It has been suggested that this reduces the technique sensitivity and improves the efficiency in clinical procedures by reducing the chair-side time.[1] As the critical and difficult-to-standardize etching, rinsing and drying steps are omitted, technique sensitivity associated with bonding to dehydrated demineralized dentin is eliminated[2] and, in spite of recent findings, incomplete resin infiltration of demineralized dentin should theoretically be prevented by virtue of the simultaneous and concomitant demineralization and infiltration.[3,4]

Nevertheless, some concern remains regarding both short- and long-term bonding effectiveness of self-etch adhesives to enamel, in particular when so-called 'mild' (pH around 2 or above) self-etch adhesives are employed.[5,6] Some manufacturers even recommend the adjunctive use of phosphoric acid when bonding to enamel and especially in case of non-instrumented enamel. Since enamel bonding is primarily based on micromechanical interlocking of a low-viscosity resin into micro-porosities,[7] the extent and the depth of the etching pattern should logically influence the bonding performance of an adhesive. It was repeatedly demonstrated that this etching pattern largely depends on the acidity of the conditioner.[6,8,9] In literature, however, no consensus exists upon the use of mild self-etch adhesives on enamel. Several authors have reported lower bonding effectiveness for mild self-etch adhesives,[6,10] whereas other authors found similar results as for etch-and-rinse adhesives.[8,11]

It can be hypothesized that converting a two-step self-etch adhesive into a three-step etch-and-rinse adhesive by prior acid-etching may raise the bonding effectiveness, especially to enamel. Therefore, we determined the effect of a preceding phosphoric-acid conditioning step on the bonding effectiveness of a two-step self-etch adhesive (Clearfil SE Bond (C-SE), Kuraray, Osaka, Japan) to enamel and dentin, using a micro-tensile bond strength (μTBS) protocol. Additionally, the interface was characterized by transmission and scanning electron microscopy (TEM and SEM).

Materials and methods

One experimental and one control group was tested. In the experimental group (C-SE etch), Clearfil SE Bond was applied on beforehand phosphoric-acid-etched enamel and dentin, and in the control group (C-SE non-etch), it was applied according to the manufacturer's instructions on non-etched enamel and dentin.

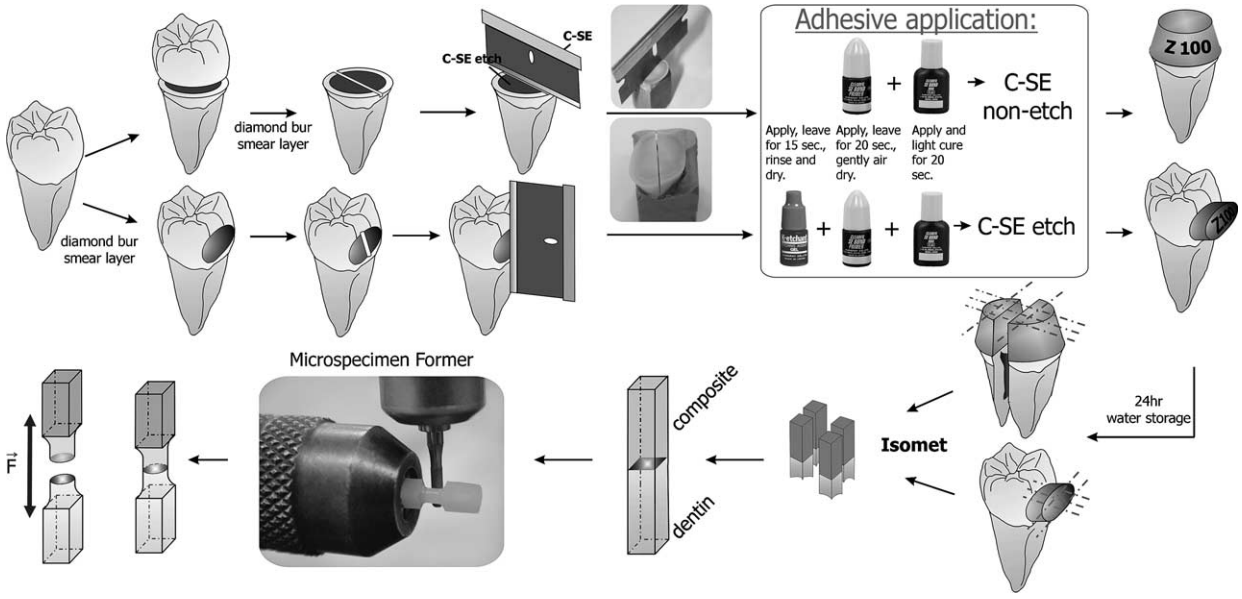

Figure 1 Study set-up.

μTBS-testing

The set-up of the study is schematically presented in Fig. 1. Non-carious human third molars (gathered following informed consent approved by the Commission for Medical Ethics of KULeuven) were stored in 0.5% chloramine/water at 4 °C and were used within 1 month after extraction. Teeth with signs of fluorosis were excluded. To prepare dentin samples, the occlusal crown third was removed with a diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA), thereby exposing a flat mid-coronal dentin surface. A standardized bur-cut smear layer was produced by removing a thin layer of the surface using a Micro-Specimen Former (University of Iowa, Iowa City, IA, USA), equipped with a high-speed regular-grit (100 μm) diamond bur (842, Komet, Lemgo, Germany). For enamel, a flat surface was ground using the same bur at the buccal and lingual surface of a tooth. Both the dentin and enamel surfaces were divided in two equal parts by a groove of 0.5 mm depth in which an extra-thin razor blade (0.15 mm) was positioned. Only one half of each surface was etched with phosphoric acid (K-etchant, Kuraray) for 15 s, while the other surface was protected from the acid by the razor blade. After rinsing with running water for 10 s, dentin was air-dried while preventing extensive dehydration. Subsequently, Clearfil SE Bond was applied according to the manufacturer's instructions to the whole surface and composite build-ups were made with Z100 (3M ESPE, Seefeld, Germany) in three or four layers to a height of 5–6 mm.

After storage overnight in distilled water (37 °C), rectangular sticks (2×2 mm wide; 8–9 mm long) were sectioned perpendicular to the adhesive-tooth interface using the Isomet saw. Only the four central dentin sticks were used to eliminate substrate regional variability.[12,13] The sticks were trimmed at the interface into an hourglass shape (diameter of ±1.1 mm) using the MicroSpecimen Former, equipped with a fine-grit (30 μm) diamond (5835KREF, Komet) in a high-speed handpiece under air/water coolant. The specimens were fixed to Ciucchi's jig with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Ohtawara, Japan) and stressed in tension at a crosshead speed of 1 mm/min using a universal testing device (LRX, Lloyd, Hampshire, UK). The μTBS was derived by dividing the imposed force at time of fracture by the bond area (mm^2). Statistical differences were examined using two-way ANOVA ($\alpha=0.05$), with acid-etching and the variable 'tooth' as predicting factors. The mode of failure was determined with a stereomicroscope at 50× magnification.

Representative dentin and composite μTBS-fracture planes, exhibiting the most frequently observed failure mode and a μTBS close to the mean, were processed for field-emission gun scanning electron microscopy (Feg-SEM; Philips XL30, Eindhoven, The Netherlands), using common specimen processing described previously.[14]

TEM interface characterization

C-SE was applied both to phosphoric acid-etched and non-etched bur-cut enamel and dentin surfaces. The

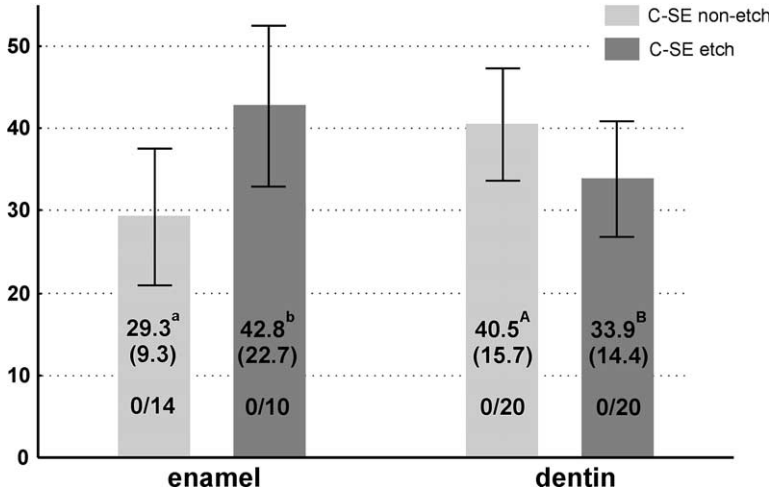

Figure 2 μTBS of the experimental and control group for enamel and dentin. Bars denote mean μTBS and whiskers define 95% confidence intervals. Inside the bars, the mean μTBS value, the standard deviation in brackets and the number of pre-testing failures/total number of tested specimens is indicated. Means with the same superscript are not significantly different within their group.

Table 1 Failure analysis performed by light microscope and confirmed by Feg-SEM analysis.

		C-SE non-etch	C-SE etch
Enamel	Adhesive failure	0	0
	Mixed failure	5	3
	Cohesive failure in enamel	1	1
	Cohesive failure in composite	8	6
Dentin	Adhesive failure	0	5
	Mixed failure	9	7
	Cohesive failure in enamel	5	2
	Cohesive failure in composite	6	6

specimens were processed for TEM according to the procedure described in detail by Van Meerbeek et al.[15] As to enamel, only non-demineralized specimens were processed. For dentin, non-demineralized and lab-demineralized (10% formaldehyde-formic acid for 36 h) ultrathin sections were cut (Ultracut UCT, Leica, Vienna, Austria), and examined unstained and positively stained (5% uranyl acetate for 20 min/saturated lead citrate for 3 min) using TEM (Philips CM10, Eindhoven, The Netherlands). Additional adhesive-enamel/dentin interfaces, stained with 50 wt% ammoniacal silver nitrate solution, were prepared according to a nano-leakage detection protocol described before by Tay et al.[16]

Results

The μTBSs are summarized in Fig. 2. The respective modes of failure are listed in Table 1. Figs. 3–6 show the interaction of C-SE non-etch and C-SE etch on enamel and dentin.

When bonded to enamel, the μTBS significantly increased by beforehand acid-etching. Nevertheless, failure analysis revealed no substantial difference in failure pattern between both groups. As for dentin, however, prior phosphoric-acid etching significantly decreased the bond strength. Along with this decrease in bond strength, an increased number of adhesive failures were observed (Table 1 and Fig. 6) for C-SE etch.

TEM observations of enamel revealed very little interaction of C-SE with non-etched enamel (Fig. 3(a)). After etching, the typical ragged pattern of eroded enamel and distinct macro- and micro-resin tags could be observed at the interface (Fig. 3(b)). Silver-stained enamel specimens did not show any sign of nanoleakage for either C-SE non-etch or C-SE etch. As to dentin, a 1 μm thick hybrid layer and hybridized smear plugs were formed in the C-SE non-etch group (Fig. 4). Dentin was only partially demineralized, leaving hydroxyapatite crystals dispersed in the hybrid layer. Uranyl acetate and lead citrate stained the hybrid layer heavily and silver-staining led to distinct deposition of silver at the top of the hybrid layer. In the C-SE etch group, phosphoric-acid etching yielded a hybrid layer with a thickness varying between 3 and 5 μm and distinct resin tags (Fig. 5). A transition zone, which was partially demineralized, could be seen at the bottom of the hybrid layer. After staining with uranyl acetate and lead citrate, a number of samples showed a slightly decreasing gradient in electrondensity with the hybrid layer depth. When non-demineralized dentin samples were stained with silver nitrate, a striking reticular pattern of nanoleakage became obvious throughout the hybrid layer, although most nanoleakage was seen at the bottom of the hybrid layer.

Discussion

The development of mild self-etch adhesives has brought some promising opportunities. Unlike with

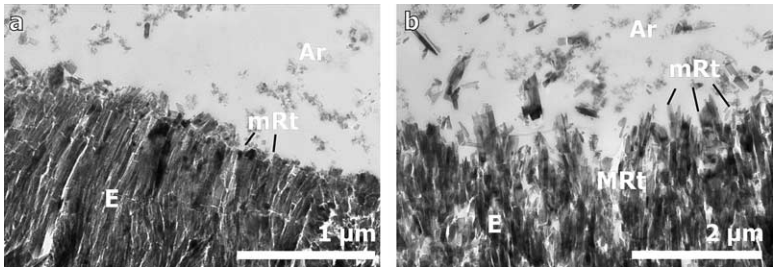

Figure 3 Transmission electron microscopy photomicrographs of C-SE non-etch (a) and C-SE etch (b) on enamel. The interface of C-SE bonded to non-etched enamel revealed little interaction, whereas after phosphoric acid etching both micro- and macro-resin tags can be observed. No evidence of nanoleakage could be observed in either group (Ar, Adhesive resin; E, Enamel; mRt, Micro-resin tag, MRt: Macro-resin tag).

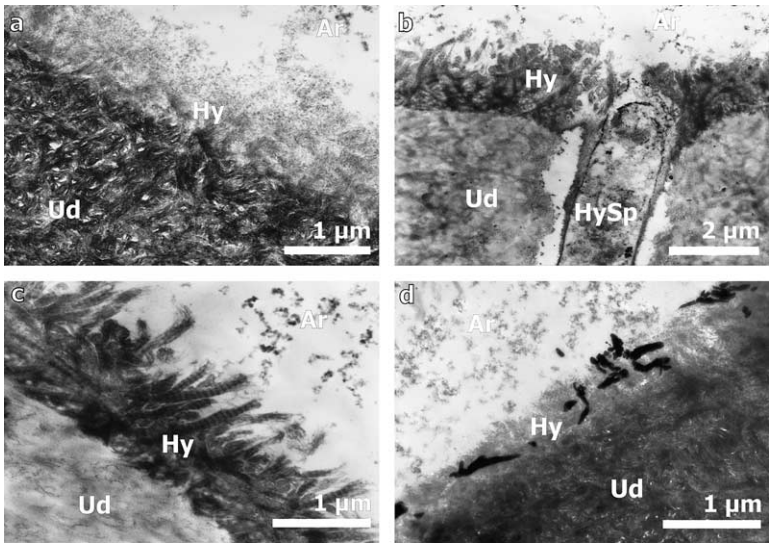

Figure 4 Transmission electron microscopy photomicrographs of C-SE non-etch to smear-layer covered dentin. (a) Non-demineralized, non-stained TEM giving a typical view of the hybrid layer produced by C-SE on smear-layer covered dentin. Dentin is only partially demineralized, as hydroxyapatite crystals scattered throughout the 1 μm-thick hybrid layer can be observed, (b) and (c) Demineralized, stained (uranyl-acetate and lead citrate) TEM. Note the hybridized smear plug, and the shag-carpet appearance at the transition to the adhesive resin. (d) Non-demineralized, ammonical silver-nitrate stained TEM. Throughout the hybrid layer and most often at the top of hybrid layer, dens silver deposits can be observed (Ar, Adhesive resin; Hy, Hybrid layer; HySp, Hybridized smear plug; Ud, Undemineralized dentin).

etch and rinse adhesives, not all hydroxyapatite is removed from the hybrid layer in dentin as the demineralization by mild self-etch adhesives (pH around 2) is restricted both in depth and in extent. Whereas research increasingly indicates that etch and rinse adhesives suffer from poor adaptation of the bonding resin to the denuded collagen fibrils,[17,18] a chemical interaction between residual

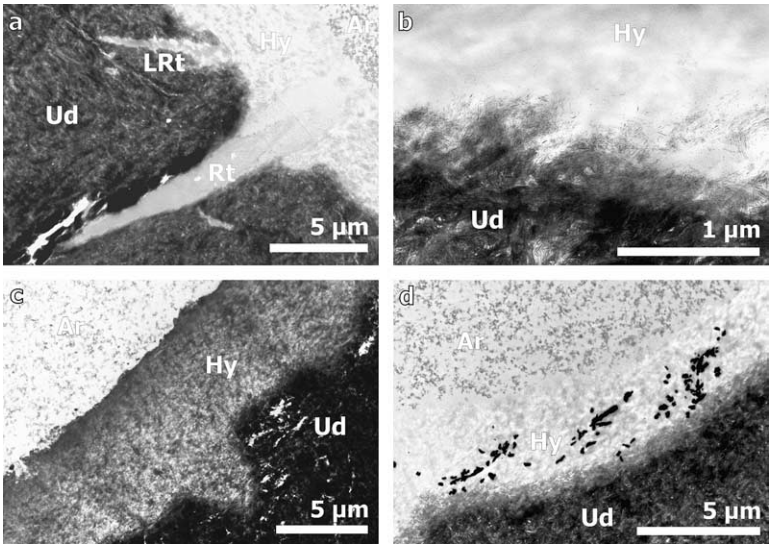

Figure 5 Transmission electron microscopy photomicrographs of C-SE etch to smear-layer covered dentin. (a) Unstained, non-demineralized TEM, revealing the formation of hybrid layer of approximately 5 μm with distinct resin tags. (b) Detail of the hybrid layer in (a), showing the transition between demineralized dentin and intact dentin. Note that there is a partially demineralized transition zone, due to the additional etching effect of the primer of C-SE. (c) Non-demineralized, stained (uranyl-acetate and lead citrate) TEM. Note the slightly decreasing gradient of electrondensity in the hybrid layer, which may be an indication for suboptimal infiltration. (d) Non-demineralized silver-nitrate stained TEM revealing dense deposits of silver nitrate throughout the hybrid layer. Most often they were detected at the bottom half of the hybrid layer, (Ar, Adhesive resin; Hy, Hybrid layer; LRt, Lateral resin tag; Rt, Resin tag; Ud, Undemineralized dentin).

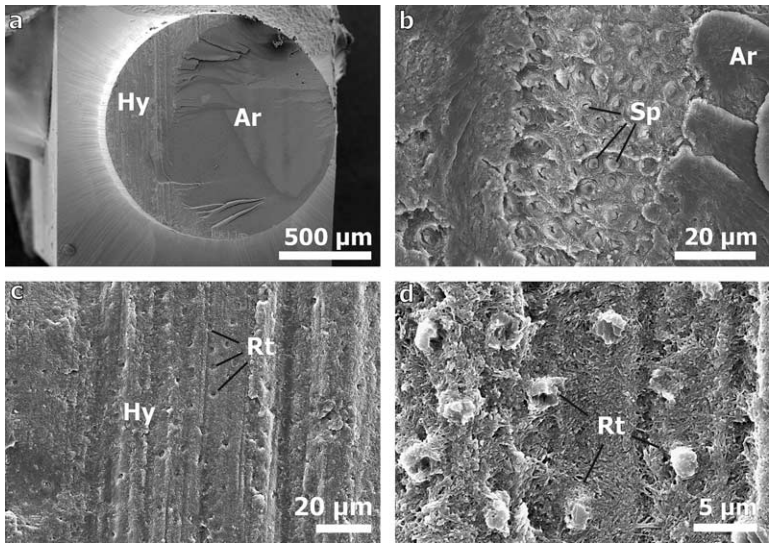

Figure 6 Field-emission gun scanning electron microscopy photomicrographs of C-SE non-etch and C-SE etch after micro-tensile bond strength testing. (a) and (b) Feg-SEM of C-SE non-etch on dentin after µTBS. (a) Dentin side after µTBS showing a typical mixed failure mode. (b) Detail of (a), note the smear plugs left in the dentin tubules. (c) and (d) Feg-SEM of C-SE etch on dentin after micro-tensile bond strength testing, showing adhesive failure. (c) Dentin side, dentin tubules are occluded by resin tags. (d) Composite side showing fracture at the bottom of the hybrid layer. Note the poorly resin-enveloped collagen fibrils (Ar, Adhesive resin; Hy, Hybrid layer; Rt, Resin tag; Sp, Smear plug).

hydroxyapatite and the functional monomers in self-etch adhesives is expected to improve bonding. Research has pointed out that the functional monomers in self-etch adhesives can chemically interact with hydroxyapatite within a clinically manageable time, and this chemical interaction has been hypothesized with better resistance towards degradation by prevention of micro- and nanoleakage.[17–20]

Nevertheless, on enamel, the use of mild self-etch adhesives has raised some concern.[10,21] The shallower etching pattern on enamel and subsequent reduced micro-mechanical retention might jeopardize bond strength and durability.[22] So far, literature does not provide a straightforward answer whether mild self-etch adhesives bonded to enamel can resist the mechanical and chemical challenges of the oral cavity as well as etch-and-rinse adhesives do. Moreover, the results of bond strength studies on this subject are conflicting.

In this study, we examined whether an additional preceding etching step with phosphoric acid provides any supplementary effect on the bond strength of the commercial adhesive Clearfil SE Bond by a standard micro-tensile bond strength methodology. By virtue of the 'split-tooth design', in which each enamel/dentin surface received both experimental treatments, a pair-wise test was carried out. This more powerful statistical test is able to rule out the variability introduced by using different teeth and application methods.

The outcome of the µTBS test clearly indicated that phosphoric-acid etching indeed increased the bond strength to enamel, but had an undeniably adverse effect on the bond strength to dentin. Our findings are corrobated by studies of several other authors, who also report increased bond strength to enamel, but impaired bond strength to dentin.[23–26]

Feg-SEM and TEM of the enamel interface in the non-etch group revealed hardly any microscopically detectable resin-tag formation, whereas distinct interaction between C-SE and enamel was observed when priorly acid-etched (Fig. 3). Our findings are in agreement with the observations of several other authors.[23,27] Therefore, the most plausible explanation for the increased µTBS is the increase in enamel porosity, resulting in an increased resin-interlocking and micro-mechanical retention. In spite of the weak correlation between enamel-etching depth/pattern and bond strength found in literature,[6,8–11] the aggressiveness of the enamel treatment may play an important role. In this study, enamel was covered with a clinically relevant bur-produced thick smear layer, in contrast to many other studies. Koibuchi et al. showed how the use of 600-grit paper could lead to overestimated bond strengths when using self-etch adhesives.[28] Moreover, in our study the same adhesive was converted into a 3-step etch and rinse adhesive, which

justifies a clear judgment of the effect of phosphoric-acid etching, as it becomes increasingly clear that the adhesive's specific ingredient composition also strongly influences bonding effectiveness.

The lack of silver staining in both the C-SE non-etch and C-SE etch group indicates the formation of a void-free resin entanglement in enamel (Fig. 3). This may explain the good results of Clearfil SE Bond in clinical studies, even when enamel was not acid-etched.[29,30] Finally, if the functional monomers chemically interact with hydroxyapatite, this should evidently also contribute to the bonding effectiveness to enamel, despite the low etching aggressiveness of the primer. The significant increase in bond strength to enamel may thus be the result of a twofold mechanism: increased micromechanical retention in addition to the chemical interaction.

When bonded to acid-etched dentin, the bond strength of C-SE was significantly reduced. Several authors have suggested incomplete infiltration of the demineralized collagen network by the bonding resin[23,24] as the underlying cause for this decrease in bond strength. Since the C-SE etch group was applied according to a 'dry bonding' procedure, collapse and shrinkage of the collagen network should have occurred.[31] Any collapse, even partial, may hinder efficient resin infiltration, leading to porous zones, in particular at the bottom of the hybrid layer.[32,33] Nevertheless, the water-containing primer of C-SE must be able to re-expand this network at least in part.[34] The partially demineralized transition zone clearly visible at the bottom of the hybrid layer is suggestive for good penetration of the primer solution (Fig. 5(b)). Phosphoric-acid etching generally results in a rather abrupt transition of exposed collagen to unaffected dentin so that the presently observed partially demineralized zone at the bottom of the hybrid layer must be ascribed to additional demineralization induced by the self-etching functional monomers.[35] While the primer may have penetrated adequately, the filled bonding resin of C-SE still may have been hampered to penetrate the exposed collagen network completely. Our TEM images that were stained with uranyl acetate and lead citrate intermittently showed a hybrid layer with a slightly decreasing gradient in electrondensity from top to bottom. This indeed suggests suboptimal infiltration of the bottom part of the hybrid layer (Fig. 5). When silver-stained however, dense deposits of silver could be observed throughout the whole hybrid layer, but they were more prevalent in the bottom half of the hybrid layer (Fig. 5(d)). This consistent pattern of silver-staining resembles the reticular mode of silver-staining observed by Tay et al.[16,36] On the other hand, the C-SE non-etch group also exhibited some distinct nanoleakage, though mainly at the top of the hybrid layer. Besides incomplete resin infiltration, such silver stained regions may represent according to Tay sites of retained water and subsequent incomplete resin infiltration, and also permeable regions that result from the interaction of the basic diamine ions with acidic/hydrophilic resin components.[16,36] Even though one can only speculate as to the actual cause(s) of these silver deposits, the consistency of these silver deposits in the thick hybrid layer after etching are a strong indication for the existence of submicron gaps, and must subsequently be regarded as inherent weaknesses in the hybrid layer. The inferior quality of the hybrid layer after acid-etching is also reflected in the increased number of interfacial failures (Table 1). Besides the inferior quality of the hybrid layer, the lack of chemical interaction between the functional monomer 10-MDP in C-SE and hydroxyapatite may also account for a lower bonding effectiveness, as hydroxyapatite is no longer available throughout the hybrid layer after phosphoric-acid etching. C-SE has consistently been reported with high bond strengths that approach those of 3-step etch-and-rinse adhesives.[2,37–40] Apart from the superior etching qualities of 10-MDP, the good performance of this adhesive in spite of the small hybrid layer and the absence of resin tags may be ascribed to the affinity of 10-MDP for hydroxyapatite.[20] Moreover, the bond strength after acid-etching to dentin may also have been jeopardized by increased 'transdentinal' permeability of the dentin. Pashley et al. showed that removing the smear layer led to a drastic increase in wetness of the dentin due to the water flux through the dentinal tubules, which can influence bond strength adversely.[41,42]

In spite of the improved results for enamel, this study is not conclusive as to the preferability and need of selective acid etching in clinical situations. Miguez et al. suggested supplementary etching for restorations that rely mainly on enamel bonding.[43] However, only long-term durability studies can indicate whether it is worth adding an additional time-taking etching step. In an in-house randomized clinical study, which is actually a superior durability study, the effect of additional enamel etching was evaluated as well. Peumans et al. found that acid-etching enamel had no effect on the restoration retention which was 100% after 3 years. Nevertheless, etched enamel tended to have

sligthly fewer marginal defects than not-etched enamel.[30]

From this study can be concluded that the bonding effectiveness of Clearfil SE Bond can be improved by selectively etching the enamel margins of a cavity with phosphoric acid. However, etching should be limited to enamel only, as etching dentin led to the formation of a low-quality hybrid layer prone to nanoleakage, and to impaired bond strengths to dentin.

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