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Effect of cavity configuration and aging on the bonding effectiveness of six adhesives to dentin

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KEYWORDS
Adhesion; Etch-and-rinse; Self-etch; Micro-tensile bond strength; Polymerization shrinkage; Durability; Aging

Summary *Objectives*: The purpose of this study was to determine the effect polymerization contraction stress may have on bond durability.
Methods: Bonding effectiveness was assessed by micro-tensile bond strength testing (μTBS) and electron microscopy. The μTBS to flat dentin surfaces and in standardized cavities was determined (this after 1 day as well as 1 year water storage). Six adhesives representing all current classes were applied: two etch-and-rinse (OptiBond FL, Kerr; Scotchbond 1, 3M ESPE), two self-etch (Clearfil SE Bond, Kuraray; Adper Prompt, 3M ESPE) and two glass-ionomer (Fuji Bond LC, GC; Reactmer, Shofu) adhesives.
Results: The conventional 3-step etch-and-rinse adhesive OptiBond FL bonded most effectively to dentin, and appeared insensitive to polymerization shrinkage stress and water degradation. The 2-step self-etch adhesive Clearfil SE Bond most closely approached this superior bonding effectiveness and only slightly lost bond strength after 1-year water exposure. The 2-step etch-and-rinse adhesive Scotchbond 1 and the 'strong' 1-step self-etch adhesive Adper Prompt appeared very sensitive to cavity configuration and water-aging effects. The 2-step resin-modified glass-ionomer adhesive Fuji Bond LC only suffered from shrinkage stress, but not from 1-year water-exposure. Remarkable also is the apparent repairability of the 'mild' 1-step glass-ionomer adhesive Reactmer when stored for 1 year in water, in spite of the very low 1-day μTBS.
Significance: Simplified bonding procedures do not necessarily imply improved bonding performance, especially in the long term.

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Introduction

Restoring teeth with minimal sacrifice of sound tooth structure forms the basis of today's restorative practice.[1] Essential in achievement of this concept are adhesives that provide strong and durable bonding to the remaining sound enamel and dentin. Many laboratory reports have proven that modern adhesives do effectively bond to tooth tissue, at least in the short term.[2,3] Clinically, marginal deterioration of composite restorations remains however problematic in the long term and still forms the major reason to replace adhesive restorations.[4,5]

Consequently, the long-term stability of bonding, in particular to dentin, remains questionable.[6,7] A factor known to promote bond degradation is long-term water exposure.[8–11] Bond deterioration by water storage might be caused by degradation of interface components, such as denaturation of collagen and/or elution of degraded or insufficiently cured resin.[6,12] Otherwise, bonding to enamel is known to be more stable over time, especially using etch-and-rinse adhesives.[13] Bonding to etched enamel has recently even been shown to seal the more vulnerable resin–dentin bond and so to protect it against water degradation.[7,13]

Most studies currently investigating the durability of adhesion do, however, not take into account polymerization contraction stresses. These stresses put resin–tooth interfaces under severe tension, in particular when restoring cavities with high C-factor, and thus yield less chance for relaxation of shrinkage stress.[14] Such pre-stressed interfaces may be more susceptible to degradation. They may for instance explain the relatively fast in vivo degradation noted for high C-factor Class-I restorations,[15] even when adhesives are used that in vitro predicted durable bonding.[16]

The main objective of this study was therefore to determine the effect polymerization contraction stress may have on bond durability. A micro-tensile bond strength (μTBS) protocol was used to determine the bonding effectiveness of six adhesives representing the three classes of today's adhesive approaches ('etch-and-rinse', 'self-etch' or 'glass-ionomer' approach following the classification by Van Meerbeek et al.[17,18]). The hypothesis tested was that adhesives bonded equally well to dentin at the bottom of Class-I cavities as to flat 'laboratory' dentin surfaces. In addition, the degradation resistance of resin–dentin bonds formed at the bottom of Class-I cavities was tested by μTBS determination after the restored teeth were aged in water for 1 year.

Materials and methods

μTBS-testing

Sixty-five non-carious human third molars (gathered following informed consent approved by the Commission for Medical Ethics of the Catholic University of Leuven) were stored in 0.5% chloramine solution at 4 °C and used within 1 month after extraction. First, all teeth were mounted in gypsum blocks in order to ease manipulation. A standard box-type Class-I cavity ($4.5 \times 4.5\ mm^2$) was then prepared at the occlusal crown center with the pulpal floor ending at mid-coronal dentin, using a high-speed hand piece with a cylindrical medium-grit (100 μm) diamond bur (842; Komet, Lemgo, Germany) mounted in a MicroSpecimen Former (University of Iowa, Iowa City, IA, USA). Next, all specimens were randomly divided into six groups, and subjected to a bonding treatment strictly according to the manufacturer's instructions using one of the six adhesives listed in Table 1. The cavity was filled in three horizontal layers with Z100 (3M ESPE, St Paul, MN, USA). Light-curing was done using an Optilux 500 (Demetron/Kerr, Danbury, CT, USA) device with a light output not less than $550\ mW/cm^2$. The teeth were stored either for 24 h or for 1 year at 37 °C in 0.5% chloramine in water, to prevent bacterial growth.[9] After storage, the teeth were sectioned perpendicular to the adhesive–tooth interface using an Isomet diamond saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA) to obtain rectangular sticks ($1.8 \times 1.8\ mm^2$ wide; 8–9 mm long). Out of each tooth, four sticks were sectioned from the central cavity floor. They were mounted in the pin-chuck of the MicroSpecimen Former and trimmed at the biomaterial–tooth interface to

Table 1 List of adhesives investigated.

Product name	Manufacturer	Class of adhesive[a]
OptiBond FL	Kerr, Orange, CA, USA	3-Step etch-and-rinse adhesive
Scotchbond 1[b]	3M ESPE, Seefeld, Germany	2-Step etch-and-rinse adhesive
Clearfil SE Bond	Kuraray, Osaka, Japan	2-Step self-etch adhesive
Adper Prompt	3M ESPE	1-Step self-etch adhesive
FujiBond LC	GC, Tokyo, Japan	2-Step glass-ionomer adhesive
Reactmer	Shofu, Kyoto, Japan	1-Step glass-ionomer adhesive

[a] According to Van Meerbeek et al.[18]
[b] Marketed in US as 'Adper Single Bond'.

a cylindrical hour-glass shape with a bonding surface of about 1 mm^2 using a fine cylindrical diamond bur (835KREF, Komet, Lemgo, Germany) in a high-speed handpiece under air/water spray coolant. Specimens were then fixed to Ciucchi's jig with cyanoacrylate glue (Model Repair II Blue, Sankin Kogyo, Tochigi, Japan) and stressed at a crosshead speed of 1 mm/min until failure in a LRX testing device (LRX, Lloyd, Hampshire, UK) using a load cell of 100 N. The μTBS was expressed in MPa, as derived from dividing the imposed force (N) at the time of fracture by the bond area (mm^2). When specimens failed before actual testing, a bond strength of 0 MPa was included in the calculation of the mean μTBS. The actual number of pre-testing failures (ptf) was explicitly noted as well. The mode of failure was determined light-microscopically at a magnification of 50× using a stereomicroscope, and recorded as either 'cohesive failure in dentin', 'adhesive failure' or 'mixed adhesive and/or cohesive resin failure'.

Following an identical specimen preparation protocol, the μTBS of the six adhesives to flat 'laboratory' mid-coronal dentin surfaces was determined (control). Again, four μTBS sticks were sectioned from the mid/mid-coronal dentin area, trimmed at the biomaterial–tooth interface to a cylindrical hour-glass shape, and eventually pulled apart in the LRX material tester. A detailed description of specimen processing has previously been described by De Munck et al.[3]

Statistical analysis

Kruskal–Wallis analysis and Dwass–Steel–Chritchlow–Fligner multiple comparisons were used to determine statistical differences in μTBS between adhesives applied to either flat 'laboratory' dentin or to Class-I cavity bottom dentin, respectively, after 1-day as well as after 1-year water storage, at a significance level of 0.05. Similar statistics were carried out to assess the effect of cavity configuration and aging on the bonding effectiveness of each adhesive separately.

Failure analysis using Fe-SEM and TEM

From each group, representative μTBS-specimens were processed for field-emission scanning electron microscopy (Fe-SEM, Philips XL30, Eindhoven, The Netherlands) using common specimen processing including fixation, dehydration, chemical drying, and gold-sputter coating.[19] Some selected Fe-SEM specimens with particular ultra-structural features were further processed for transmission electron microscopy (TEM). They were immersed for 12 h in epoxy resin prior to embedding in molds.[20] Non-demineralized 70–90 nm sections through the fracture plane were cut using a diamond knife (Diatome, Bienne, Switzerland) in an ultramicrotome (Ultracut UCT, Leica, Vienna, Austria). For evaluation of collagen, TEM sections were positively stained with 5% uranyl acetate (UA) for 20 min and saturated lead citrate (LC) for 3 min prior to TEM examination (Philips CM10, Eindhoven, The Netherlands).

Results

The mean μTBS, standard deviations and the ratio's of the number of pre-testing failures over the total number of specimens (*n*) are summarized per adhesive and experimental condition in Table 2, and graphically presented in box-whisker plots in Fig. 1. The results from light-microscopy failure analysis are presented in Table 3. The results from multiple comparisons statistical analysis (*p*-values) are mentioned in Table 4 for bonding to flat 'laboratory' dentin (control), and in Table 5 for

Table 2 μTBS to flat 'laboratory' dentin, and to Class-I cavity bottom dentin after 1-day and 1-year water storage.

	1 Day, flat 'laboratory' dentin		1 Day, Class-1 cavity bottom dentin		1 Year, Class-1 cavity bottom dentin	
	μTBS	ptf/*n*	μTBS	ptf/*n*	μTBS	ptf/*n*
OptiBond FL	47.3 (13.1)^{a}	0/12	51.5 (10.3)^{a}	0/12	40.7 (11.9)^{a}	0/9
Scotchbond 1	42.0 (11.4)^{a,b}	0/12	11.9 (6.0)^{c}	15/20	0^{c}	11/11
Clearfil SE Bond	48.1 (11.5)^{a}	0/12	41.3 (8.4)^{a}	0/16	26.8 (10.6)^{a,b}	0/8
Adper Prompt	14.8 (8.4)^{d}	2/12	7.2 (9.8)^{c}	12/23	3.2 (6.2)^{c}	9/12
Fuji Bond LC	31.3 (8.0)^{b,c}	0/18	19.9 (6.2)^{b}	0/10	19.4 (5.8)^{b}	0/10
Reactmer	28.4 (9.9)^{c}	0/18	4.0 (7.6)^{c}	17/24	27.5 (6.1)^{b}	0/12

ptf, pre-testing failure; *n*, total number of specimens; (SD). Means with the same superscript are not significantly different within their group (column).

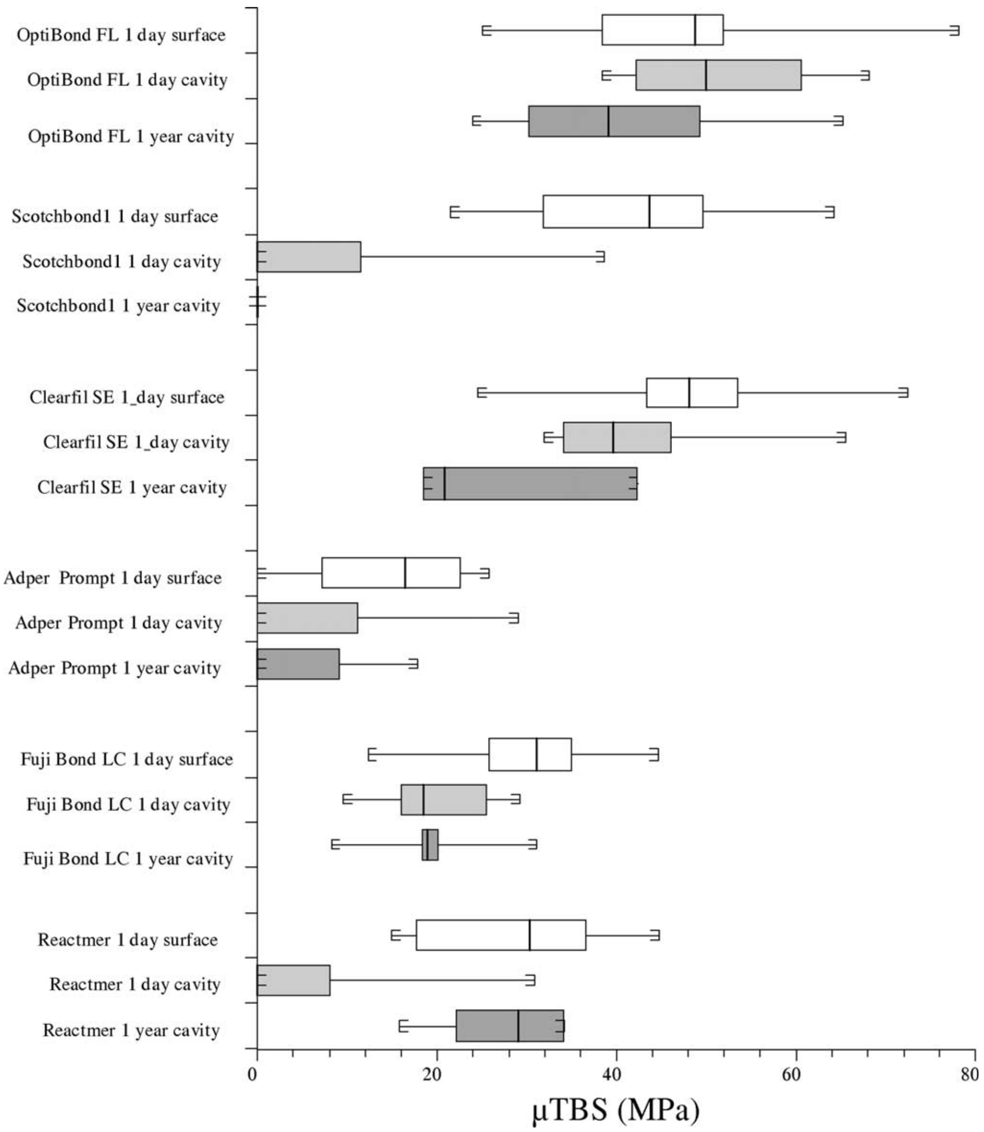

Figure 1 μTBS to dentin. The boxes represent non-parametric statistics (lower quartile, median, upper quartile) with the whiskers extending to minimum and maximum value. The central vertical line represents the median value. The color of the box refers to the experimental group: white, μTBS to flat dentin after 1-day water storage; gray, μTBS to cavity bottom dentin after 1-day water storage; dark gray, μTBS to cavity bottom dentin after 1-year water storage.

bonding to Class-I cavity bottom dentin after 1-day and 1-year water storage. Finally, the statistical results of the effect of cavity configuration and aging on bonding effectiveness are mentioned per adhesive in Table 6.

When bonded to flat laboratory 'dentin' (1-day water storage; Fig. 1, Tables 2 and 4), no significant difference in bonding effectiveness was recorded between, respectively, the 3- and 2-step etch-and-rinse adhesive, Optibond FL and Scotchbond 1, and the 2-step self-etch adhesive, Clearfil SE Bond. Their bonding effectiveness was significantly higher than that of the 1-step self-etch adhesive, Adper Prompt, and that of the 2- and 1-step glass-ionomer adhesives, Fuji Bond LC and Reactmer. The only exception is the μTBS of Scotchbond 1 that is not significantly different from that of Fuji Bond LC and only nearly significantly different from that of Reactmer. There is no significant difference in bonding performance between both glass-ionomer adhesives, Fuji Bond LC and Reactmer. Significantly the lowest μTBS was recorded for the 1-step self-etch adhesive Adper Prompt.

When bonded to Class-I cavity bottom dentin (1-day water storage; Fig. 1, Tables 2 and 5 top right), the μTBS of the 3-step etch-and-rinse adhesive OptiBond FL and of the 2-step self-etch adhesive Clearfil SE Bond were significantly higher than that of all other adhesives. The glass-ionomer adhesive Fuji Bond LC bonded relatively well to

Table 3 Failure mode analysis.

	Cohesive failure in dentin	Adhesive failure (+pre-testing failure)	Mixed adhesive and/or cohesive resin failure	Total specimen number
1 Day, flat 'laboratory' dentin				
OptiBond FL	5	0 + 0	7	12
Scotchbond 1	3	0 + 0	9	12
Clearfil SE Bond	6	0 + 0	6	12
Adper Prompt	0	0 + 2	10	12
Fuji Bond LC	0	0 + 0	18	18
Reactmer	0	8 + 0	10	18
1 day, Class-1 cavity bottom dentin				
OptiBond FL	6	0 + 0	6	12
Scotchbond 1	0	4 + 15	1	20
Clearfil SE Bond	4	4 + 0	8	16
Adper Prompt	0	4 + 11	8	23
Fuji Bond LC	0	2 + 0	8	10
Reactmer	1	6 + 17	0	24
1 year, Class-1 cavity bottom dentin				
OptiBond FL	3	2 + 0	4	9
Scotchbond 1	0	0 + 11	0	11
Clearfil SE Bond	2	0 + 0	6	8
Adper prompt	0	0 + 0	3 + 9	12
Fuji Bond LC	0	1 + 0	9	10
Reactmer	0	0 + 0	12	12

Class-I cavity bottom dentin and significantly better than Scotchbond 1, Adper Prompt, and Reactmer. Basically due to the high number of pre-testing failures, recorded as 0 MPa, the bonding effectiveness of the 2-step etch-and-rinse adhesive Scotchbond 1 was as low as that of Adper Prompt and Reactmer.

When bonded to Class-I cavity bottom dentin and after 1-year water storage (Fig. 1, Tables 2 and 5 bottom left), again the highest μTBS was recorded for the 3-step etch-and-rinse adhesive OptiBond FL, which also again was only not significantly different from the μTBS recorded for the 2-step self-etch adhesive Clearfil SE Bond. In contrast with the 1-day results, no significant difference could be found in bonding effectiveness between Clearfil SE Bond and both glass-ionomer adhesives, Fuji Bond LC and Reactmer. Again the significantly lowest μTBS to Class-I cavity bottom dentin after 1-year of water storage was recorded for the 2-step etch-and-rinse adhesive Scotchbond 1 and the 1-step self-etch adhesive Adper Prompt.

Comparing the bonding effectiveness to Class-I cavity bottom dentin with that to flat 'laboratory' dentin (1-day water storage; Table 6 first column), the factor cavity configuration did not affect the bonding effectiveness of the 3-step etch-and-rinse adhesive OptiBond FL and of the 2-step self-etch

Table 4 *p*-Values of all pairwise Kruskal–Wallis comparisons (Dwass–Steel–Chritchlow–Fligne) for μTBS to flat 'laboratory' dentin (1-day water storage).

1 Day surface	OptiBond FL	Scotchbond1	Clearfil SE Bond	Adper Prompt	Fuji Bond LC	Reactmer
OptiBond FL	***	0.7696	>0.9999	*0.0006*	*0.0092*	*0.0043*
Scotchbond 1		***	0.8014	*0.001*	0.1015	**0.0519**
Clearfil SE Bond			***	*0.0006*	*0.0037*	*0.0012*
Adper Prompt				***	*0.0008*	*0.0317*
Fuji Bond LC					***	0.9886
Reactmer						***

p-Values in italics are smaller than 0.05 and thus indicate significant difference; *p*-value in bold indicates nearly significant difference.

Table 5 *p*-Values of all pairwise Kruskal–Wallis comparisons (Dwass–Steel–Chritchlow–Fligne) for μTBS to Class-1 cavity bottom dentin after 1-day water storage (top right) and after 1-year water storage (bottom left).

1 Year	1 Day					
	OptiBond FL	Scotchbond1	Clearfil SE Bond	Adper Prompt	Fuji Bond LC	Reactmer
OptiBond FL	***	*< 0.0001*	0.0972	*< 0.0001*	*0.0011*	*< 0.0001*
Scotchbond 1	*0.0005*	***	*< 0.0001*	0.8837	*0.0093*	>0.9999
Clearfil SE Bond	0.2763	*0.0007*	***	*< 0.0001*	*0.0004*	*< 0.0001*
Adper prompt	*0.0009*	0.5089	*0.0014*	***	*0.0166*	0.8291
Fuji Bond LC	*0.008*	*0.0004*	0.5973	*0.0018*	***	*0.0003*
Reactmer	*0.0415*	*0.0002*	0.9825	*0.0004*	**0.0623**	***

p-Values in italics are smaller than 0.05 and thus indicate significant difference; *p*-value in bold indicates nearly significant difference.

adhesive Clearfil SE Bond. A significant bond-reducing effect was recorded for the 2-step etch-and-rinse adhesive Scotchbond 1 and the 2- and 1-step glass-ionomer adhesives, Fuji Bond LC and Reactmer. A nearly significantly lower (borderline) bonding performance was recorded for the 1-step self-etch adhesive Adper Prompt.

Evaluating the effect of aging (Class-I cavity dentin after 1-year water storage versus after 1-day water storage; Table 6 second column), a significant bond-reducing effect was only recorded for the 2-step self-etch adhesive Clearfil SE Bond, while a remarkable significant bond-enhancing effect was recorded for the 1-step glass-ionomer adhesive Reactmer. The aging factor was not significant for all other adhesives (OptiBond FL, Scotchbond 1, Adper Prompt, and Fuji Bond LC).

With regard to failure patterns, typical failure patterns are shown in Figs. 2–6 for the six adhesives tested following the three experimental conditions. Most failures were recorded as 'mixed adhesive and/or cohesive resin failure', irrespective of adhesive and experimental condition (Table 3). Only in case a rather high number of pre-testing failures were recorded (Scotchbond 1 bonded to Class-1 cavity bottom dentin after 1-day and 1-year water storage, and Reactmer bonded to Class-1 cavity bottom dentin after 1-day water storage), the most frequent failure pattern was recorded as 'adhesive', and was clearly associated with a relatively low bonding effectiveness. An exception to this observation are the failure patterns recorded for Adper Prompt, which failed predominantly mixed adhesively and cohesively in resin (Table 3, Fig. 4). When a relatively high μTBS was measured (in particular for Optibond FL and Clearfil SE Bond), the specimens tended to fail more cohesively in dentin. Noteworthy is also that after 1-day as well as after 1-year water storage, a large part of the Fuji Bond LC specimens failed (at least partially) cohesively within the glass-ionomer adhesive (Fig. 5). This indicates that the actual bonding effectiveness of Fuji Bond LC was not assessed because the cohesive strength of the glass-ionomer material itself was lower than, or at least as low as, the interfacial bond strength. The latter was partially corroborated by Fe-SEM analysis of the fractured surfaces, which revealed that actually some failures were located at the glass-ionomer/composite interface (Fig. 5).

Adhesive failure patterns were typically recognized by exposure of similarly curved scratches (circles) with a diameter corresponding to the diameter (1.4 mm) of the diamond bur used for cavity preparation (Figs. 2–6). The 3-step etch-and-rinse adhesive OptiBond FL hardly ever failed 'adhesively'. When this occurred after 1-year water storage, the adhesively failed parts never exhibited poorly resin-impregnated collagen fibrils (image not shown), confirming the high hybridization efficacy of this 3-step etch-and-rinse approach. On the contrary, the rather low bonding effectiveness recorded for the 2-step etch-and-rinse adhesive Scotchbond 1 was associated with a high number of adhesive failures (Table 3), often exhibiting poorly resin-impregnated collagen (Fig. 2).

Table 6 *p*-Values for significancy of effect of cavity configuration and aging (Kruskal–Wallis, Dwass–Steel–Chritchlow–Fligner comparisons).

	Cavity configuration	Aging
OptiBond FL	0.6618	0.0706
Scotchbond 1	*< 0.0001*	0.1789
Clearfil SE Bond	0.0921	*0.0272*
Adper prompt	**0.0667**	0.4204
Fuji Bond LC	*0.0044*	>0.9999
Reactmer	*< 0.0001*	*< 0.0001*

p-Values in italics are smaller than 0.05 and thus indicate significant difference; *p*-value in bold indicates nearly significant difference.

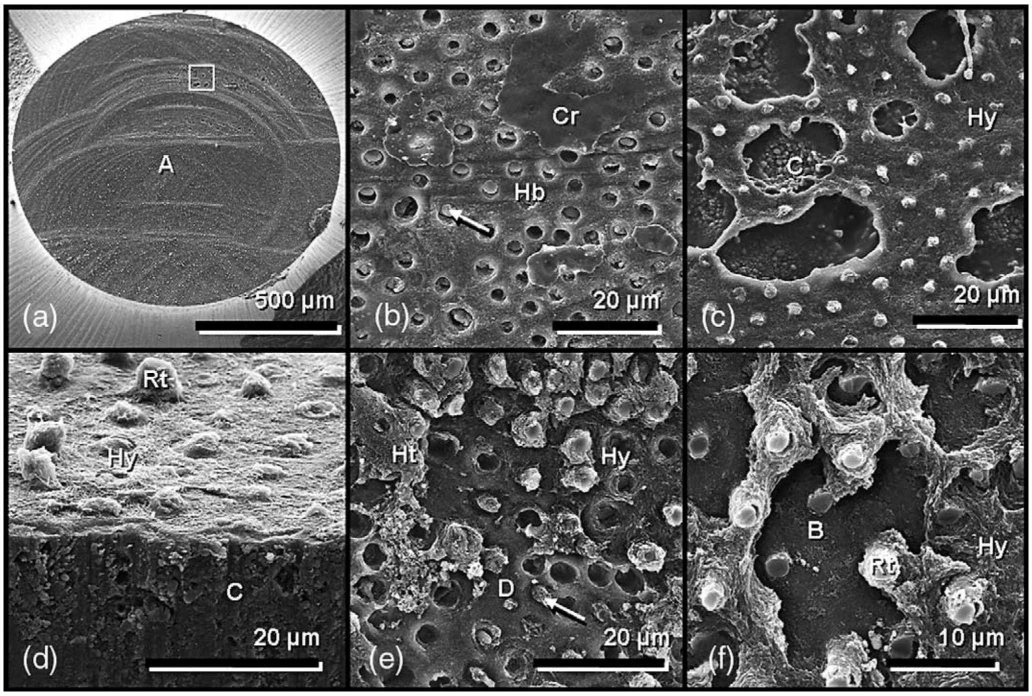

Figure 2 Electron microscopic evaluation of Scotchbond 1. (a) Fe-SEM photomicrograph of the fractured surface of Scotchbond 1 applied in a cavity after 1-day water storage (dentin side). In contrast to when Scotchbond 1 was applied to a flat dentin surface, the failure occurred completely adhesively (A). (b) Magnification of a (box). On some sites, the dentin surface remained covered with a thin layer of resin (Cr); other areas failed at the bottom of the hybrid layer (Hb). Tubules are occluded by resin tags (arrow). (c) Composite counterpart at a similar area as in b. At some sites, the hybrid layer (Hy) remained attached to the resin composite (C). (d) Magnification of the edge of the composite side of the μTBS-specimen, viewed at an angle of 60°. The hybrid layer (Hy) remained attached to the composite (C). Loose collagen fibrils incompletely enveloped by resin were disclosed. Consequently, the main failure site occurred within or at the bottom of the hybrid layer. Rt, resin tag. (e) Fe-SEM photomicrograph of the fractured surface of Scotchbond 1 applied in a cavity after 1-year water storage (dentin side). Again, the specimen failed mainly adhesively at the bottom of the hybrid layer, exposing unaffected dentin (D), as well as it failed within the hybrid layer (Hy), exposing non-resin-enveloped collagen fibrils. Some areas failed near the top of the hybrid layer (Ht). Arrow, resin tag. (f) Composite counterpart of e. The same failure sites can be detected; they occurred at the top of hybrid layer (Hy), uncovering the bonding layer (B) and within or at the bottom of the hybrid layer, exposing non-resin-enveloped collagen fibrils.

Discussion

Numerous studies reported on bonding performance of adhesives as measured following a μTBS-protocol. However, most were performed on flat 'laboratory' dentin surfaces that have a low C-factor of 1/5.[16,21,22] In a tooth cavity, shrinkage stress is, however, generated during polymerization of the composite, pulling the adhesive from the cavity wall.[14,23] This phenomenon is especially pronounced in a Class-I cavity (used in this study) with five bonded walls and only one free surface, revealing a C-factor of 5/1. High shrinkage stresses may induce gaps between the restoration and the cavity wall/floor that must result in micro-leakage, post-operative pain and other related clinical problems.[5,24]

Six adhesives representing all different classes of adhesives were tested (Table 1).[17,18] To insure minimal variation in polymerization shrinkage stress, standardized cavities were made using a template and the MicroSpecimen Former. In addition, the same resin composite (Z100, 3M ESPE), known for its relatively high E-modulus,[25] was used for all adhesives. Because of the high C-factor of the experimental cavities (5/1) and the high E-modulus of the resin composite used, we assumed that this in vitro generated shrinkage stress approaches the highest stress generated clinically. Qualitative Fe-SEM and TEM examination of the fracture planes combined with fractographic analysis was used to substantiate the bond strength data.

Previous studies have shown that with increasing C-factor the μTBS decreases.[11,26] In this study, the factor 'cavity configuration' did not weaken the bond produced by the 3-step etch-and-rinse adhesive OptiBond FL and the 2-step self-etch adhesive Clearfil SE Bond. Among the different

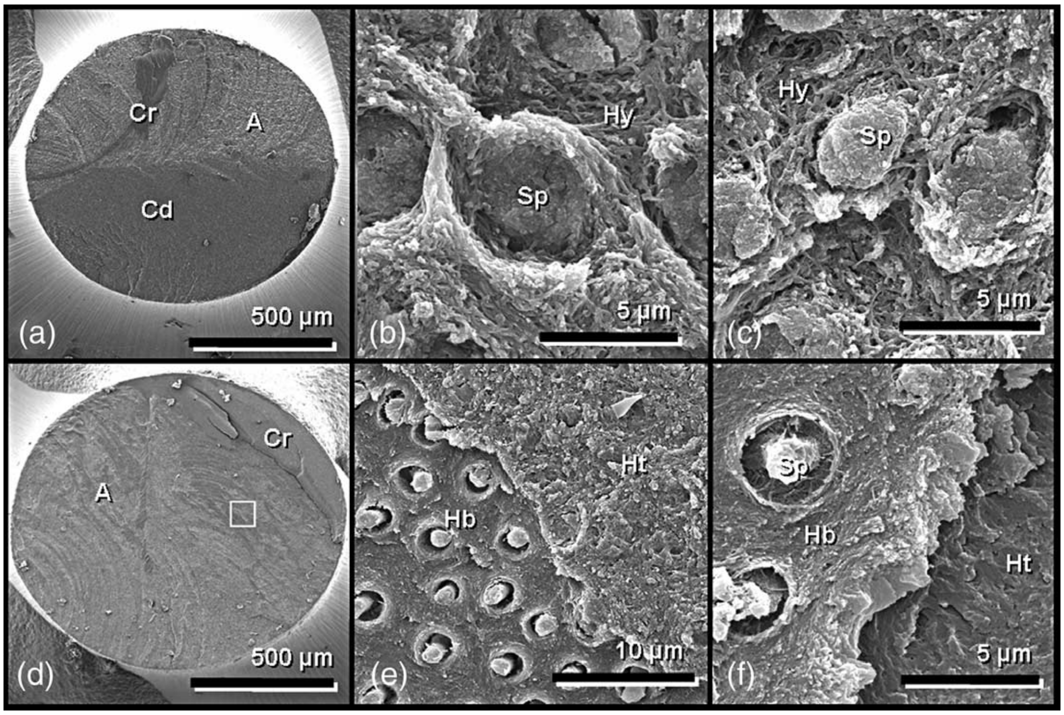

Figure 3 Electron microscopic evaluation of Clearfil SE Bond. (a) Fe-SEM photomicrograph of the fractured surface of Clearfil SE Bond applied in a cavity after 1-day water storage (dentin side). The failure was recorded as 'mixed' including adhesive failure (A) and cohesive failure in resin (Cr) and dentin (Cd). (b) Higher magnification of an adhesively failed area of a. Hybridized smear plugs (Sp) and a resin-infiltrated collagen matrix can be detected, suggesting that the specimen failed within the hybrid layer (Hy). (c) Composite counterpart of b. Hy, hybrid layer that remained attached to the composite part; Sp, hybridized smear plug. (d) Fe-SEM photomicrograph of the fractured surface of Clearfil SE Bond applied in a cavity after 1-year water storage (dentin side). The failure was recorded as 'mixed' including adhesive failure (A) and cohesive failure in resin (Cr). (e) Magnification of an adhesively failed area of d (box). The failure seems to be located either at the top (Ht) or the bottom (Hb) of the thin (± 1 μm) hybrid layer. (f) Composite counterpart of a similar site as e. Hb, failure at the bottom of the hybrid layer; Ht, failure at the top of the hybrid layer; Sp, hybridized smear plug.

classes of adhesives,[18] the 3-step etch-and-rinse adhesive OptiBond FL often presented with the highest bond strength values.[2,3,18,27] This superior bonding effectiveness must probably, to a large extent, be attributed to optimal dentin hybridization, as was demonstrated in several ultra-morphologic interface analyses.[17,18,28–31] The conventional 3-step application procedure that guarantees a low technique-sensitive application procedure, the specific monomer/solvent cocktail of the primer solution (containing glycerophosphoric acid dimethacrylate or GPDM, 2-hydroxyethyl methacrylate or HEMA, and phthalic acid mono ethyl methacrylate or PAMM in an ethanol–water solution), the viscous solvent-free and glass-filled adhesive, and other ingredients such as an adequate polymerization initiator, may all have contributed to this in vitro and in vivo very successfully and reliably performing adhesive.[2,7,17,30,32] Also in this study, this 3-step approach resulted in the highest μTBS in Class-I cavities, after 1-day as well as after 1-year water storage. Because of this high bond strength, the specimens nearly never failed solely 'adhesively', as some of the less performing adhesives do (Table 3, Fig. 2).

Among the self-etch adhesives, the 2-step adhesive Clearfil SE Bond has also consistently been associated with favorable laboratory results. In particular when bonded to dentin, Clearfil SE Bond did not underscore OptiBond FL, despite its being applied following a self-etch approach with one application step less.[16,17,27] Recently, a randomized controlled clinical trial did not reveal any difference in clinical behavior, when Clearfil SE Bond was applied following manufacturer's instructions or when enamel was selectively acid-etched with 40% phosphoric acid prior to the application of Clearfil SE Bond.[18,33] The specific molecular composition with 10-methacryloxydecyl dihydrogen phosphate (10-MDP) as functional monomer has recently been proven to be capable of interacting intimately with residual hydroxyapatite that remained within the shallow 1-μm hybrid layer[18,34] From the three

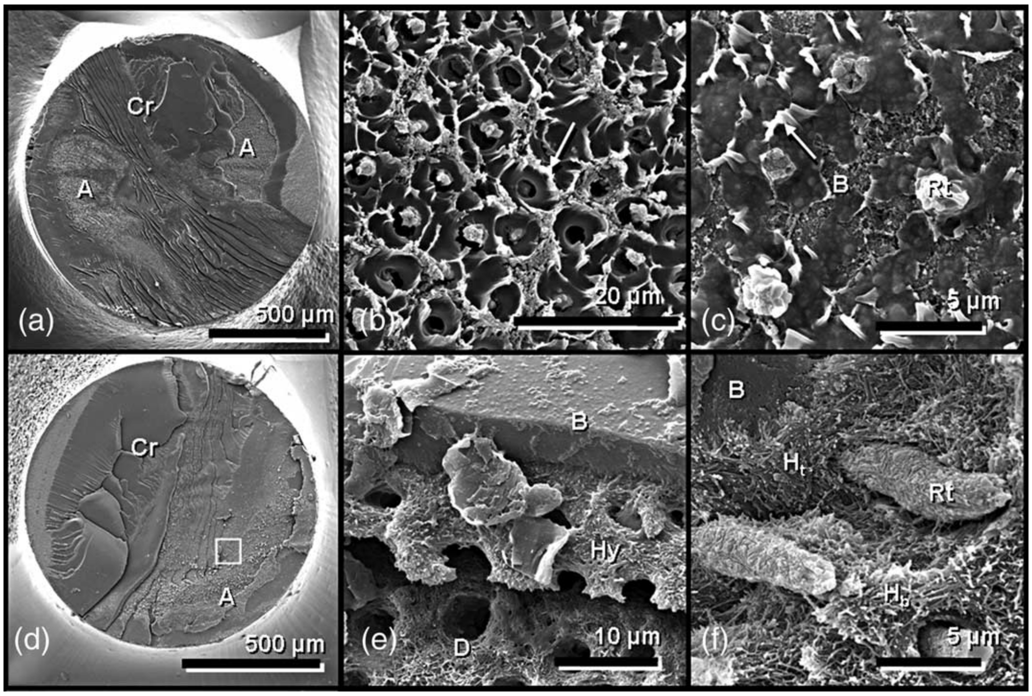

Figure 4 Electron microscopic evaluation of Adper Prompt. (a) Fe-SEM photomicrograph of the fractured surface of Adper Prompt applied in a cavity after 1-day water storage (dentin side). The failure was recorded as 'mixed' including adhesive failure (A) and cohesive in resin (Cr). (b) Magnification of an adhesively failed area. Tubules are widely opened by the strong self-etch adhesive and filled with resin tags. A reticular pattern is formed by a resin rim that surrounds the tubuli (arrow). (c) Fe-SEM photomicrograph of the corresponding composite counterpart of b. The heads of the resin tags are still attached to the adhesive resin layer. Consequently, the specimen failed mainly at the top of the hybrid layer. (d) Fe-SEM photomicrograph of the fractured surface of Adper Prompt applied in a cavity after 1-year water storage (dentin side). The failure was recorded as 'mixed' including adhesive failure (A) and cohesive in resin (Cr). (e) Magnification of the transition zone between adhesive and cohesive failure, viewed at an angle of 45° (box in d). The three components that constitute the resin–dentin interaction zone can easily be distinguished: bonding layer (B), hybrid layer (Hy) and unaffected dentin (D). (f) Composite side of a similar site as e. B, bonding; Hb, bottom of the hybrid layer; Ht, top of the hybrid layer; Rt, resin tag.

monomers investigated (besides 10-MDP, also 4-methacryloxyethyl trimellitic acid or 4-MET, and 2-methacryloxyethyl phenyl hydrogen phosphate or phenyl-P), the chemical bonding potential of 10-MDP with hydroxyapatite was significantly the highest and the most hydrolytically stable.[34]

Another reason contributing to the superior bonding effectiveness of OptiBond FL and Clearfil SE Bond, might be the particle-filled adhesive resin that is typically applied in a relatively thick layer. It has been hypothesized before that a relatively thick adhesive layer may act as an intermediary stress reliever to compensate for the shrinkage stress imposed during polymerization of the composite to the resin-cavity wall/bottom bond.[18,35–40] Finite element analysis also revealed that with increasing thickness or decreasing elastic modulus of the adhesive resin, the shrinkage stresses can be considerably decreased.[41] Consequently, this elastic bonding concept may, to a large extent, explain the good resistance OptiBond FL and Clearfil SE Bond have against polymerization shrinkage, when applied in a high C-factor cavity (Table 6).

The cavity configuration significantly affected the bonding performance of the 2-step etch-and-rinse adhesive Scotchbond 1. When bonded to flat 'laboratory' dentin, Scotchbond 1 presented with bond strengths as high as that measured for the conventional 3-step system OptiBond FL. Identical results were obtained before.[2,7] When Scotchbond 1 was applied in a cavity, however, different factors may have compromised its bonding capacity. The risk on improper drying of etched dentin increased because of the narrow dimensions of the Class-I cavities prepared in this study. Likewise, it must also have been more difficult to adequately remove residual solvents (water and ethanol) from the combined primer/adhesive resin. Pooling of the adhesive was especially inevitable in the cavity corners, having lead to thicker adhesive layers, but also more difficult solvent removal in such regions. In this regard, a study by Zheng et al.[22] reported

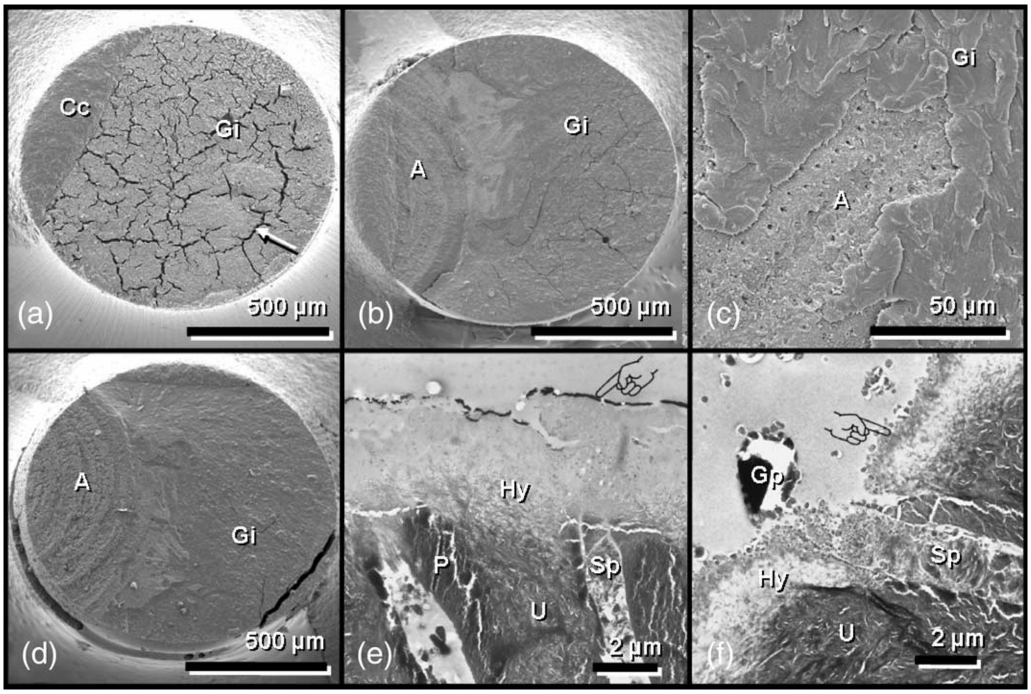

Figure 5 Electron microscopic evaluation of Fuji Bond LC. (a) Fe-SEM photomicrograph of the fractured surface of Fuji Bond LC. The whole surface remained covered with glass-ionomer (Gi), which is cracked (arrow) because of the dehydration procedure and high vacuum in the SEM. Cc, contamination of surface with conductive carbon cement. (b) Fe-SEM photomicrograph of the fractured surface of Fuji Bond LC applied in a cavity after 1-year water storage (dentin side). The failure was recorded as 'mixed' including adhesive (A) failure and cohesive in glass-ionomer (Gi). (c) Higher magnification of b. A, adhesive failure; Gi, cohesive failure in glass-ionomer. (d) Fe-SEM photomicrograph of the corresponding composite side of b. A, adhesive failure; Gi, cohesive failure in glass-ionomer. (e) Unstained, non-demineralized TEM section of b. The thin black line (hand pointer) originates from the gold-coating procedure, applied for SEM evaluation, and delimitates the actual failure site. The specimen failed at the transition of gel phase and matrix. Hy, hybrid layer; P, peri-tubular dentin; Sp, smear plug; U, unaffected dentin. (f) Stained, non-demineralized TEM section of b. The specimen failed cohesively in the glass-ionomer. The specimen only merely reacted with the heavy metals, resulting in a selective staining of the gel phase (hand pointer). Gp, glass particle; Hy, hybrid layer; Sp, smear plug; U, unaffected dentin.

that the μTBS of Scotchbond 1 decreased with increasing thickness of the adhesive; it even dropped to 0 MPa at a thickness of 400 μm. In contrast to 2-step etch-and-rinse adhesives, such application errors are less critical using 3-step adhesives, since resin is applied in two separate steps, of which the application of the adhesive (final step) may partially compensate for less-optimal resin infiltration achieved with the primer. Interfacial TEM characterization of Scotchbond 1 bonded to dentin also revealed varying hybridization efficiency.[30,42] In particular, the polyalkenoic acid co-polymer within the combined primer/adhesive has been thought to affect hybridization efficiency. Due to its relatively large molecular weight (MW = 14,000–20,000) and its high affinity for calcium, it forms a film that only partially infiltrates the exposed collagen fibril network and thus may block effective infiltration of other monomers.[28,43] All these compromising factors may for instance also explain the rather varying results Scotchbond 1 obtained in Class-V clinical studies.[44,45] The above-mentioned factors, even pronounced due to the small cavity size, along with the high elastic modulus of the resin-composite used (E-modulus of Z100 = 21 GPa) probably resulted in a rather poor bond at the cavity bottom that could not withstand the high polymerization shrinkage stress inherent to the high C-factor of the Class-I cavity. Consequently, gaps must have been formed at the interface, and been responsible for specimen failure during specimen preparation (pre-testing failures; Table 2). Failure analysis revealed that Scotchbond 1 failed after 1-day (as well as after 1-year) water storage nearly solely 'adhesively', whereas in a previous study when bonded to flat dentin (even after 4-year water storage), Scotchbond 1 only failed 'adhesively' in on average 35% of the bond surface.[7] Before, we have shown that using a μTBS-protocol low bond strengths correlated with high percentages of 'adhesive' failures.[7] After 1-day water storage,

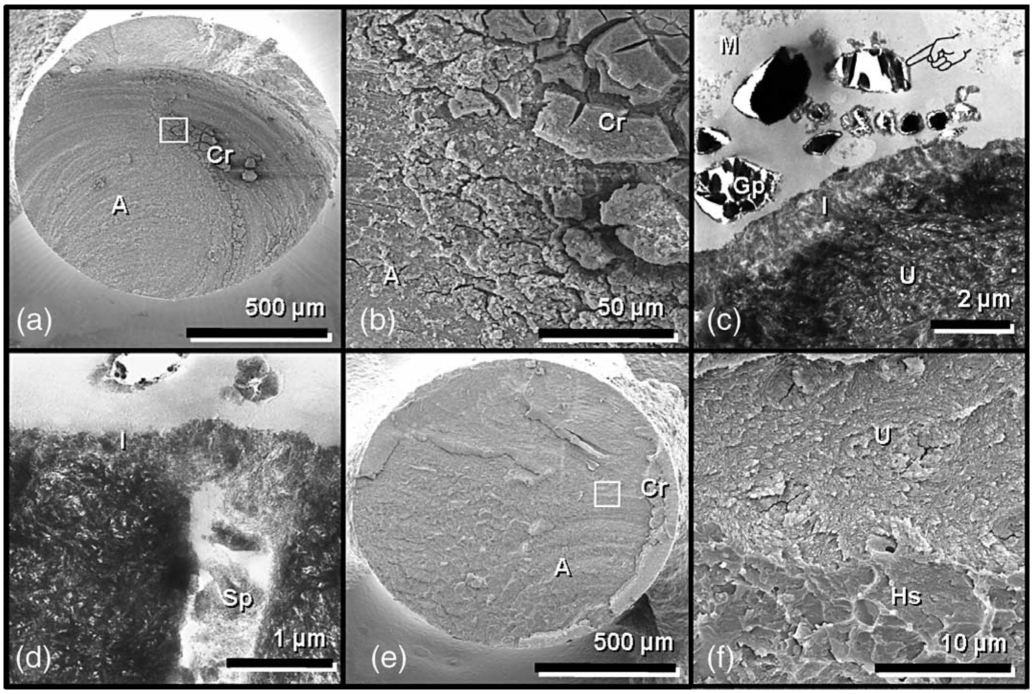

Figure 6 Reactmer after 1-day water storage. (a) Fe-SEM photomicrograph of the fractured surface of Reactmer applied in a cavity after 1-day water storage (dentin side). A, adhesive failure; Cr, cohesive failure in resin. (b) Magnification of a (box). No dentinal tubuli can be detected in the adhesively failed area. Cr, cohesive failure in resin. (c) Unstained, non-demineralized TEM photomicrograph of an intact Reactmer-dentin interface after 24-h water storage. Gp, glass particle (note the surrounding rim, hand pointer); I, interaction zone; M, matrix; U, unaffected dentin. (d) Unstained, non-demineralized TEM photomicrograph of an intact Reactmer-dentin interface. At this side, nearly no interaction (I) can be noticed and the tubulus remained occluded by a smear plug (Sp). (e) Fe-SEM photomicrograph of the fractured surface of Reactmer applied in a cavity after 1-year water storage (dentin side). The failure was recorded as 'mixed' including adhesive (A) failure and cohesive failure in resin (Cr). (f) Magnification of an adhesive failed (A) area of a (box). On some sites, the hybridized smear layer (Hs) had detached from the unaffected dentin (U).

most of the failures were located at the base of the hybrid layer, where SEM revealed many loose collagen fibrils that did not appear enveloped by resin (Fig. 2). This confirms the poorer hybridization efficiency mentioned above. A weak layer of exposed collagen that was insufficiently impregnated by resin may have been present in between an incompletely formed hybrid layer and the underlying unaffected dentin. Similar rather disappointing results, related to technique-sensitivity, were reported in a study, in which the long-term bonding effectiveness of Scotchbond 1 was assessed following a μTBS-protocol after cyclic thermal and mechanical loading.[46]

Currently, glass-ionomers are the only self-adhesive restorative materials,[17,47,48] though also their bonding effectiveness gains from beforehand conditioning with a low-concentrated polyalkenoic acid (10–20%) conditioner.[31] Their bonding mechanism is twofold. The polyalkenoic acid conditioner superficially and partially demineralizes dentin, leaving HAp around exposed collagen fibrils. As a result, a submicron hybrid layer is formed that provides micro-mechanical retention. In addition, the residual HAp within the hybrid layer serves as a receptor for chemical interaction with the carboxyl groups of the polyalkenoic acid.[48] By adding methacrylate monomers, resin-modified glass-ionomers can be used to bond resin composites to tooth substrates. Even though this bonding strategy in vitro commonly underscores that of conventional etch-and-rinse adhesives,[17,18,49] the resin-modified glass-ionomer Fuji Bond LC has been highly successful in vivo in Class-V studies for periods up to five years.[17,18,33,50] Nevertheless, in this study Fuji Bond LC appeared sensitive to the cavity configuration, since its μTBS slightly, but significantly decreased in Class-I cavities.

Reactmer should be regarded as a one-step resin-based adhesive. However, due to the incorporation of fillers that are produced from the reaction of ion-leachable glass with polyalkenoic acid, it was introduced as a one-step glass-ionomer or so-called 'giomer' on the dental market. After 24 h, a very low μTBS was recorded and nearly all failures were solely 'adhesive', with only few Reactmer remnants left on the dentin surface (Table 3, Fig. 6). This rather low bonding effectiveness must be attributed to the Class-I configuration, as this adhesive in conjunction with the same composite performed remarkably better to a flat surface after 24 h of water storage.[3] Additional TEM analysis revealed that Reactmer only superficially interacts with the dentin surface. Hence, the smear layer may not have been fully dissolved, but only infiltrated and consequently stabilized, as can be concluded from the following observations: (1) the interaction layer between dentin and Reactmer was very irregular. The thickness of the interaction zone varied from 1 μm to nearly no interaction (Fig. 6); (2) the dentinal tubules appeared still filled with smear and (3) the primer has a relatively high pH of 3.2.[3] From these data, one could speculatively expect that the 24-h micro-mechanical bonding effectiveness might have been relatively low and especially sensitive to shrinkage stress induced by polymerization of the composite in the high C-factor Class-I cavity, as shown by the low μTBS after 24 h (Table 2).

The effect of cavity configuration on the bonding effectiveness of the 'strong' one-step self-etch adhesive Adper Prompt was only nearly significant. However, this must be regarded as statistically significant, taking into account the significantly higher number of pre-testing failures when placed in a Class-I cavity. Like in this study when applied to flat or to Class-I cavity bottom dentin, it was shown before that this one-step self-etch adhesive and its pre-decessor Prompt L-pop produced μTBSs that were among the lowest ones recorded for adhesives from the diverse classes of contemporary adhesives.[2,18] This lower bonding performance is also reflected in the varying clinical results reported from Class-V studies. Relatively favorable short-time retention rates of 100% at 6 months and 96% at 1 year were recorded, respectively, by Mũnoz et al.[51] and by Boghosian.[52] However, relatively high loss rates of 21% at 2 years and even 35% at 1 year were reported by, respectively, van Dijken[45] and Brackett et al.[53] Several explanations such as inhibition of polymerization of the restorative composite on top due to its high acidity,[54] incomplete wetting and insufficiently thick adhesive layer,[55] and phase separation between hydrophilic and hydrophobic ingredients and resultant sensitivity to hydrolysis,[17,18,56] have been advanced to explain this lower bonding performance to dentin as compared to more conventional etch-and-rinse and self-etch adhesives.

Besides reduced bond strength in Class-I cavities, pre-stressed interfaces may also be more susceptible to degradation, for example by gaps and micro-voids that facilitate fluid exchange along the interface. In this study, the long-term degradation of resin–dentin bonds formed in Class-I cavities was studied by exposure to water for 1 year at 37 °C. When sensitive to aging, the μTBS of the adhesive should have been reduced after 1-year water storage. Nevertheless, as the restored teeth were stored intact, the occlusal seal produced by bonding of the adhesive to the outer enamel margin of the Class-I cavities may have protected the bond of the adhesive to the Class-I bottom dentin against degradation. This beneficial effect was demonstrated before when four etch-and-rinse adhesives were applied to dentin disks surrounded by an enamel rim.[7] Again, the bonding performance of the 3-step etch-and-rinse adhesive Optibond FL appeared stable despite the 1-year water storage, though the *p*-value nearly reached the level of significance (Table 6). This confirms our previous results when OptiBond FL was bonded to flat dentin and even after exposure to water for 4 years did not appear to have lost bond strength.[7]

The μTBS of the 'mild' 2-step self-etch adhesive Clearfil SE Bond decreased significantly after 1 year of water storage. Although the failure modes did not change noteworthy over time, SEM analysis pointed out that adhesive failures after 1 day occured pre-dominantly more within the hybrid layer, whereas after 1 year the failures were located more at the transition of the hybrid layer to unaffected dentin (Fig. 3).

Using the 2-step etch-and-rinse adhesive Scotchbond 1, all specimens failed prior to being tested so that no μTBS could be recorded when it was bonded to Class-I cavity bottom dentin and exposed to water for 1 year. Also in our previous study, Scotchbond 1 appeared sensitive to 4-year water aging, except when the Scotchbond-1-dentin bond was all-around sealed by a resin–enamel bond.[7] In that study, Scotchbond 1 was applied to flat dentin disks. Also in this study, some protection must have been provided by bonding of Scotchbond 1 to the occlusal enamel margins of the Class-I restorations. However, this appeared insufficient already after 1 year. This may indicate that the additional polymerization stress in Class-I cavities rendered the bonding performance even more vulnerable to water degradation.

When using the 2-step glass-ionomer adhesive Fuji Bond LC, no difference was found between 1-day and 1-year μTBS. This is in contrast with a similarly conducted study where Fuji Bond LC was bonded to flat dentin surfaces and stored for 4 years in water.[58] In that study, the μTBS dramatically decreased, mainly because of degradation of the glass-ionomer matrix itself. By storing the entirely restored tooth in water in this study, for only 1 year, it can be assumed that the water exposure of the glass-ionomer matrix at the pulpal floor of the cavity and the resultant damage to it must have been limited, thereby explaining the difference with the former results. This is also confirmed by a non-changing failure analysis over the 1-year period (Table 3). Also SEM analysis did not reveal any structural changes over time (Fig. 5). TEM analysis on the other hand showed that sites rated as 'adhesive' failure, actually failed at the transition of the gel phase to the glass-ionomer matrix, leaving the hybrid layer intact (Fig. 5). This effect should be further investigated in depth.

Remarkably, the 'mild' 1-step self-etch adhesive Reactmer was the only adhesive, of which the μTBS to Class-I cavity bottom dentin did not decrease after one year of water storage. It even increased considerably (and highly significantly). No pre-testing failures were recorded after 1-year water storage, whereas about 70% of the specimens failed prior to being tested for the 1-day water-stored Class-I specimens (Fig. 1 and Table 2). Also the main failure mode changed from exclusively 'adhesive' after 1 day to 'mixed' after 1 year (Table 3), and thus sustains the hypothesis of improved bonding effectiveness over time. The most plausible explanation for this remarkable effect is that the glass-ionomer phase within Reactmer may by a kind of ion-exchange mechanism have additionally chemically interacted with dentin. These water-dependent reactions may have taken a few weeks to establish[57], especially at the pulpal floor of the Class-I restoration, a site relatively remote from the water source. Alternatively, maturation of the glass-ionomer adhesive with time may have enforced its cohesive strength and subsequently also its μTBS. This ongoing reaction may then also have induced expansion of the adhesive, and so relieved polymerization stress and avoided gap formation[57]. This expansion effect in combination with chemical interaction with dentin may be indicative of a kind of 'repair' effect, when compared with the poor 1-day bond performance.

No further significant reduction in μTBS was recorded for the 1-step self-etch adhesive Adper Prompt after 1-year of water storage, though an already low bonding performance was achieved at 1 day.

In conclusion, the conventional 3-step etch-and-rinse adhesive still remained most effective in bonding to dentin, and appeared insensitive to effects of increased polymerization shrinkage stress and water degradation. Most closely approaching this superior bonding effectiveness of OptiBond FL, the 'mild' 2-step self-etch adhesive Clearfil SE Bond only slightly lost bond strength after 1-year exposure to water. The 2-step etch-and-rinse adhesive Scotchbond 1 and the 'strong' 1-step self-etch adhesive Adper Prompt appeared very sensitive to cavity configuration and water-aging effects, whereas the 2-step resin-modified glass-ionomer adhesive Fuji Bond LC only suffered from higher shrinkage stress, but not from the 1-year water-exposure. Remarkable is the apparent repairability of the 'mild' 1-step self-etch or glass-ionomer adhesive Reactmer when stored for 1 year in water. In general, simplified bonding procedures do not necessarily imply improved bonding performance, especially on the long term. The application of technique-sensitive adhesives such as the 2-step etch-and-rinse adhesive ScotchBond 1 and the 1-step self-etch adhesive Adper Prompt in more complex configurations leads to dramatic bond deterioration, on a short as well as long-term basis.

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