©Operative Dentistry, 2006, **30-1**, 456-465

Effect of Water Storage on the Bonding Effectiveness of 6 Adhesives to Class I Cavity Dentin

J De Munck • K Shirai • Y Yoshida • S Inoue
KL Van Landuyt • P Lambrechts • K Suzuki • H Shintani • B Van Meerbeek

Clinical Relevance

Recently developed "user-friendly" adhesives do not perform as well as traditional 3-step etch-and-rinse adhesives in the long term.

*Jan De Munck, DDS, PhD, postdoctoral research fellow, Leuven BIOMAT Research Cluster, Department of Conservative Dentistry, School of Dentistry, Oral Pathology and Maxillo-facial Surgery, Catholic University of Leuven, Belgium

Kenichi Shirai, DDS, PhD, Leuven BIOMAT Research Cluster, Department of Conservative Dentistry, School of Dentistry, Oral Pathology and Maxillo-Facial Surgery, Catholic University of Leuven, Belgium and Department of Operative Dentistry, Hiroshima University Graduate School of Dentistry, Japan

Yasuhiro Yoshida, DDS, PhD, associate professor, Department of Biomaterials, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan

Satoshi Inoue, DDS, PhD, lecturer, Division for General Dentistry, Hokkaido University Dental Hospital, Sapporo, Japan

Kirsten Van Landuyt, DDS, PhD-student, Aspirant Fund for Scientific Research of Flanders, Leuven BIOMAT Research Cluster, Department of Conservative Dentistry, School of Dentistry, Oral Pathology and Maxillo-facial Surgery, Catholic University of Leuven, Belgium

Paul Lambrechts, DDS, PhD, full professor, Leuven BIOMAT Research Cluster, Department of Conservative Dentistry, School of Dentistry, Oral Pathology and Maxillo-facial Surgery, Catholic University of Leuven, Belgium

Kazuomi Suzuki, DDS, PhD, full professor, Department of Biomaterials, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan

Hideaki Shintani, DDS, PhD, full professor, Hiroshima University Graduate School of Dentistry, Japan

Bart Van Meerbeek, DDS, PhD, professor, Leuven BIOMAT Research Cluster, Department of Conservative Dentistry, School of Dentistry, Oral Pathology and Maxillo-facial Surgery, Catholic University of Leuven, Belgium

*Reprint request: Kapucijnenvoer 7, B-3000 Leuven, Belgium; e-mail: jan.demunck@med.kuleuven.be

DOI: 10.2341/05-57

SUMMARY

Adhesive-dentin interfaces degrade with time. This study determined the effect water storage may have on the bonding effectiveness of adhesives to occlusal Class I cavity-bottom dentin. Six adhesives, all representing contemporary classes of adhesives, were applied: a 3-step (OptiBond FL, Kerr) and 2-step (Scotchbond 1*, 3M ESPE) etch-and-rinse adhesive, a 2-step (Clearfil SE, Kuraray) and 1-step (Adper prompt, 3M ESPE) self-etch adhesive and a 2-step (FujiBond LC, GC) and 1-step (Reactmer, Shofu) resin-modified glass-ionomer adhesive. Bonding effectiveness was assessed by microtensile bond strength testing (µTBS) and electron microscopy (Feg-SEM and TEM). The µTBS was determined after 1 day and 1

Scotchbond 1 is known as Single Bond outside of Europe.

year water storage of the entire restored cavity (indirect exposure of the adhesive-dentin interface to water) and prepared µTBS-beams (direct exposure of the adhesive-dentin interface to water). The hypotheses tested were: (1) resin-dentin bonds formed at the bottom of Class I cavities resist 1-year water storage and (2) an adjacent composite-enamel bond protects the composite-dentin bond against degradation. Non-parametric Kruskal-Wallis analysis statistically analyzed the µTBSs. The first hypothesis was rejected, as only the µTBS of OptiBond FL and Clearfil SE did not significantly decrease after 1-year direct and/or indirect water storage. The second hypothesis was corroborated, as the bonding effectiveness of most simplified adhesives (Scotchbond 1, Adper Prompt, FujiBond LC and Reactmer) approached 0 (because of the frequent pre-testing failures) after 1-year direct water exposure. The second hypothesis concluded that the 3-step etch-and-rinse adhesive must still be regarded the "gold standard." Though µTBS decreased significantly, Clearfil SE, as a 2-step self-etch adhesive, was the only simplified adhesive to perform reliably after 1-year direct water exposure.

INTRODUCTION

Restoring teeth with minimal sacrifice to sound tooth structure following the concept of "minimal invasive dentistry" forms the basis for today's restorative practice.[1] Essential in the achievement of this concept are the adhesives that provide strong, durable bonding to the remaining sound tooth tissue. Many laboratory reports have proven that modern adhesives effectively bond to tooth tissue, at least in the short-term, as well as to flat surfaces.[2-3]

Most *in vitro* bond strength studies use flat surfaces to test the bonding effectiveness of dental adhesives. Clinically, however, adhesives are applied in cavities, which result in higher polymerization contraction stress (and technique sensitivity). This stress puts the resin-tooth interfaces under severe tension during the critical setting of the adhesive, particularly when restoring cavities with a high C-factor.[4] Such pre-stressed interfaces are more similar to the clinical situation but may be more susceptible to degradation.[5]

A factor known to promote bond degradation is long-term water exposure. Bond deterioration by water storage might be caused by degradation of the interface components, such as the denaturation of collagen and/or the elution of degraded or insufficiently cured resin.[6] Therefore, this study determined the durability of adhesion by long-term exposure to water.

In this study, a microtensile bond strength (µTBS) protocol was used to determine the bonding effectiveness of 6 adhesives, representing the 3 classes of today's adhesive approaches ("etch-and-rinse," "self-etch" and "glass-ionomer") in a clinically relevant setting, using tooth cavities. The hypotheses tested were: (1) resin-dentin bonds formed at the bottom of Class I cavities resist 1-year water storage and (2) an adjacent composite-enamel bond can protect the composite-dentin bond against degradation.

METHODS AND MATERIALS

µTBS-testing

For this study, non-carious human third molars (gathered following informed consent, approved by the Commission for Medical Ethics of the Catholic University of Leuven), stored in 0.5% chloramine solution at 4°C were 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 x 4.5 mm) was then prepared at the occlusal crown center, with the pulpal floor ending at mid-coronal dentin, using a high-speed handpiece 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 6 groups and subjected to bonding treatment strictly according to the manufacturer's instructions, using 1 of the 6 adhesives that represent all types of contemporary adhesives[7]: a 3-step etch-and-rinse adhesive (OptiBond FL, Kerr, Orange, CA, USA), a 2-step etch-and-rinse adhesive (Scotchbond 1*, 3M ESPE, St Paul, MN, USA), a 2-step self-etch adhesive (Clearfil SE, Kuraray, Osaka, Japan), a 1-step self-etch adhesive (Adper Prompt, 3M ESPE; applied in a single layer), a 2-step resin-modified glass-ionomer adhesive (FujiBond LC, GC, Tokyo, Japan) and a 1-step resin-modified glass-ionomer adhesive (Reactmer, Shofu, Kyoto, Japan). The cavity was filled in 3 horizontal layers with Z100 (3M ESPE). 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.

Next, the teeth were randomly assigned to 1 of the 3 experimental groups (Figure 1). For each adhesive, 3 teeth were tested after 24 hour storage in water (24-hour cavity/indirect: indirect exposure of the adhesive-dentin interface to water for 24 hours, while still protected by a circumferential adhesive-enamel bond), 3 teeth were tested after 1 year storage in water (1 year-cavity/indirect: indirect exposure of the adhesive-dentin interface to water for 1 year, while still protected by a circumferential adhesive-enamel bond) and, from 3 teeth, µTBS specimens were prepared and stored for 1 year prior to being tested (1 year-cavity/direct: direct exposure of the adhesive-dentin interface to water for 1 year, while there was no circumferential adhesive-enamel bond present). The specimens were stored at 37°C in 0.5% chloramine in water to prevent bacterial growth.[8]

After storage, the restored cavities 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 x 1.8 mm wide; 8-9-mm long). Out of each tooth, 4 sticks were sectioned from the central cavity floor (Figure 1). The sticks were mounted in the pin-chuck of the MicroSpecimen Former and trimmed at the biomaterial-tooth interface to 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. The 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/minute until failure in a LRX testing device (LRX, Lloyd, Hampshire, UK) using a load 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 was also explicitly noted. The mode of failure was determined light-microscopically at a magnification of 50x using a stereomicroscope and recorded as either "cohesive failure in dentin," "interfacial failure" or "mixed interfacial and/or cohesive resin failure."

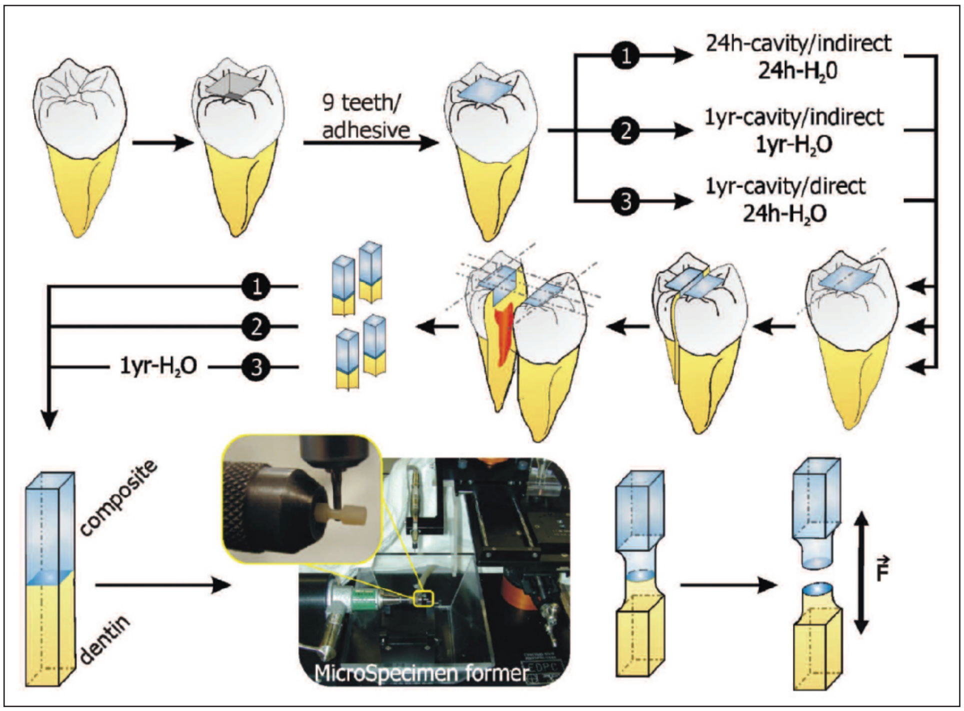

Figure 1. *Schematic study design.*

Statistical Analysis

Kruskal-Wallis analysis and Dwass-Steel-Chritchlow-Fligner multiple comparisons were used to determine statistical differences (at a significance level of 0.05) in µTBS among all adhesives to evaluate the effect of 1-year indirect and direct exposure to water.

Failure Analysis Using Feg-SEM and TEM

From each group, representative µTBS-specimens were processed

Table 1: *µTBS to Dentin*

µTBS (SD) ptf/n	24-hour Cavity/Indirect	1-year Cavity/Indirect	1-year Cavity/Indirect
OptiBond FL	51.5^{a} (10.3) 0/12	40.7^{a,c} (11.9) 0/9	43.6^{a,b} (12.9) 0/12
Scotchbond 1	11.9^{d,f,g} (6.0) 15/20	0^{g} 11/11	0.4^{g} (1.3) 11/12
Clearfil SE	41.3^{a} (8.4) 0/16	26.8^{a,c,d} (10.6) 0/8	18.4^{c} (9.4) 0/12^{f}
Adper Prompt	7.2^{d,f,g} (9.8) 12/23	3.2^{g} (6.2) 9/12	0^{g} (0) 12/12
FujiBond LC	19.9^{c,f} (6.2) 0/10	19.4^{c,f} (5.8) 0/10	0.6^{g} (2.1) 10/11
Reactmer	4.0^{g} (7.6) 17/24	27.5^{b,c,e} (6.1) 0/12	5.6^{d,e,f,g} (9.5) 5/8

µTBS = micro-tensile bond strength, value in MPa; ptf = pre-testing failure; n = total number of specimens; SD = standard deviation. Means with the same superscript are not significantly different.

for field-emission gun scanning electron microscopy (Feg-SEM, Philips XL30, Eindhoven, The Netherlands) using common specimen processing, including fixation, dehydration, chemical drying and gold-sputter coating.[9] Some selected Feg-SEM specimens with particular ultra-structural features were further processed for transmission electron microscopy (TEM). The specimens were immersed for 12 hours in epoxy resin, prior to embedding in molds.[10] 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 minutes and saturated with lead citrate (LC) for 3 minutes prior to TEM examination (Philips CM10, Eindhoven, The Netherlands).

RESULTS

The mean µTBS, standard deviation and ratio of the number of pre-testing failures (ptf) over the total number of specimens (n) are summarized per adhesive and experimental condition in Table 1, and they are graphically presented in box-whisker plots in Figure 2. The results from multiple comparison statistical analysis are summarized in homogeneous groups (Table 1) and specified for the effects of indirect and direct water exposure for each adhesive in Table 2. The results from light-microscopy failure analysis are presented in Table 3.

When bonded to Class I cavity bottom dentin (24-hour cavity/indirect; Figure 2, Table 1), the µTBS of the 3-step etch-and-rinse adhesive OptiBond FL and the 2-step self-etch adhesive Clearfil SE were significantly higher than all other adhesives. The glass-ionomer adhesive FujiBond LC bonded relatively well to Class I cavity bottom dentin and bonded 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 Adper Prompt and Reactmer.

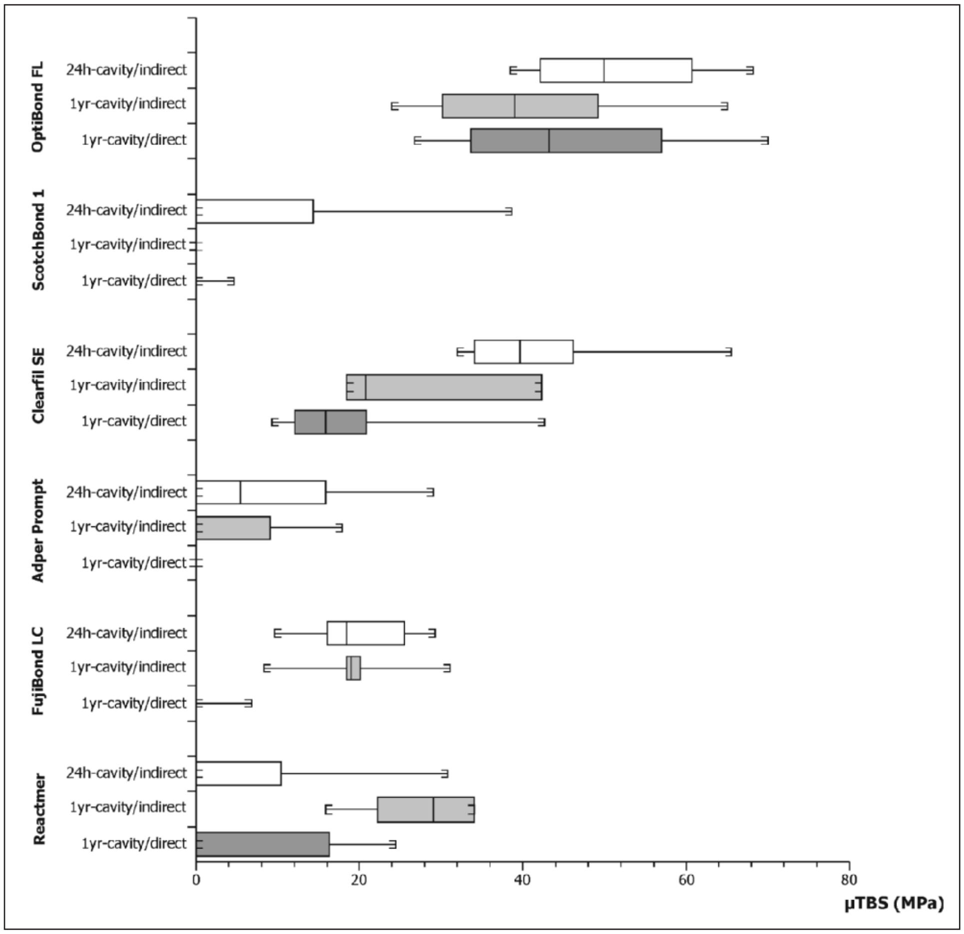

Figure 2. *Effect of water storage on the µTBS to dentin of adhesives applied in Class I cavities. The box represents the spreading of the data between the first and third quartile. The central vertical line represents the median. The whiskers denote the range of variance.*

Table 2: p-*values of the Kruskal-Wallis Pairwise Comparisons for the Effect Indirect and Direct Water Exposure*

Effect	1-year Indirect Water Exposure[1]	1-year Direct Water Exposure[2]	Indirect/ Direct[3]
OptiBond FL	0.75	0.991	>0.999
Scotchbond 1	0.898	0.991	>0.999
Clearfil SE	0.473	0.008	0.612
Adper Prompt	0.985	0.185	0.942
FujiBond LC	>0.999	0.005	0.005
Reactmer	0.001	>0.999	0.099

[1]24-hour cavity/indirect vs 1-year cavity/indirect; [2]24-hour cavity/indirect vs 1-year cavity/direct; [3]1-year cavity/indirect vs 1-year cavity/direct.

Table 3: *Failure Mode Analysis (light microscopy)*				
24-hour Cavity/Indirect	**Cohesive Failure**	**Interfacial Failure (+ptf)**	**Mixed Failure* (+ptf)**	**n**
OptiBond FL	6	0 + 0	6	12
Scotchbond 1	0	4 + 15	1	20
Clearfil SE	4	4 + 0	8	16
Adper Prompt	0	4 + 11	8	23
FujiBond LC	0	2 + 0	8	10
Reactmer	1	6 + 17	0	24
1-year Cavity/Indirect	**Cohesive Failure**	**Interfacial Failure (+ptf)**	**Mixed Failure* (+ptf)**	**n**
OptiBond FL	3	2 + 0	4	9
Scotchbond 1	0	0 + 11	0	11
Clearfil SE	2	0 + 0	6	8
Adper Prompt	0	0 + 0	3 + 9	12
FujiBond LC	0	1 + 0	9	10
Reactmer	0	0 + 0	12	12
1-year Cavity/Direct	**Cohesive Failure**	**Interfacial Failure (+ptf)**	**Mixed Failure* (+ptf)**	**n**
OptiBond FL	6	1 + 0	5	12
Scotchbond 1	0	1 + 11	0	12
Clearfil SE	2	10 + 0	0	12
Adper Prompt	0	0 + 12	0	12
FujiBond LC	0	1 + 10	0	11
Reactmer	1	1 + 5	1	8

**mixed failure = interfacial and/or cohesive resin failure; ptf = pre-testing failure.*

When bonded to Class I cavity bottom dentin and after 1-year indirect exposure to water (1-year cavity/indirect, Figure 2, Table 1), again the highest µTBS was recorded for the 3-step etch-and-rinse adhesive OptiBond FL, which, again, was significantly higher than all other approaches, except for the 2-step self-etch adhesive Clearfil SE (p=0.804). In contrast to the 24-hour results, no significant difference was found in bonding effectiveness between Clearfil SE and both glass-ionomer adhesives, FujiBond LC and Reactmer. Again, the significantly lowest µTBS to Class I cavity bottom dentin after 1-year indirect water storage was recorded for the 2-step etch-and-rinse adhesive Scotchbond 1 and the 1-step self-etch adhesive Adper Prompt.

When bonded to Class I cavity bottom dentin and stored in water for 1 year in the form of µTBS beams (1 year-cavity/direct, Figure 2, Table 1), the highest µTBS was again recorded for the 3-step etch-and-rinse adhesive OptiBond FL and was at least double any other µTBS recorded in this test. All other adhesives failed predominantly during specimen preparation, except Clearfil SE, which performed significantly lower but still reliably, as no pre-testing failures were observed after 1-year direct water exposure.

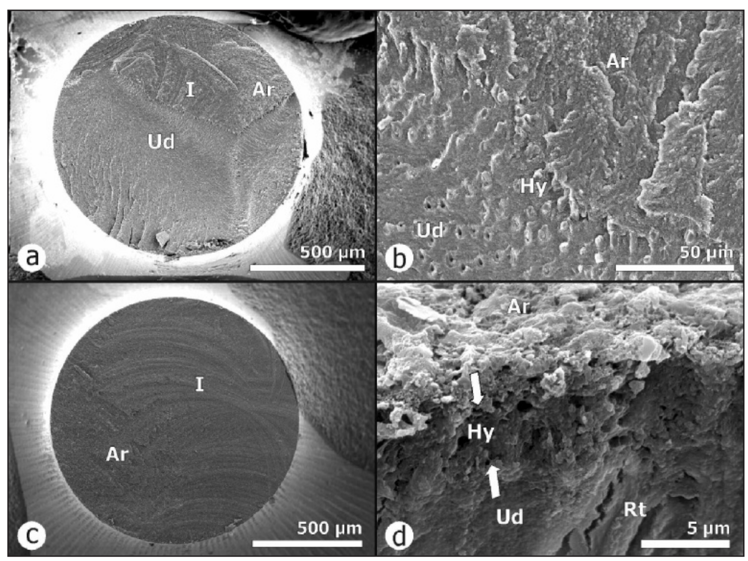

Figure 3. *Electron microscopic evaluation of OptiBond FL. a) Feg-SEM photomicrograph of a fracture surface from a 24-hour cavity/indirect specimen (dentin side). The specimen exhibited a mixed failure pattern involving the adhesive resin (Ar), as well as unaffected dentin (Ud). I = Interfacial failure. b) Higher magnification of (a) at the transition area between failure in resin and dentin. The fracture hardly progressed along the hybrid layer (Hy). c) Fracture surface of a 1-year cavity/direct specimen. Most of the failure occurred at the dentin-biomaterial interface (I), as witnessed by the typical circular scratch marks from cavity preparation. d) Fractured cross-section of (c) at an interfacially failed area. On top of the hybrid layer (Hy, in between arrows), a thin layer of adhesive resin can be noticed (Ar). Rt = Resin tag.*

Evaluating the effect of 1-year indirect exposure to water in a Class I cavity (24 hour-cavity/indirect vs 1 year-cavity/indirect, Table 2), the factor aging was not significant for most adhesives (OptiBond FL, Scotchbond 1, Clearfil SE, Adper Prompt and FujiBond LC), apart from a remarkably significant bond-enhancing effect for the 1-step glass-ionomer adhesive Reactmer.

Evaluating the effect of 1-year direct exposure to water in a Class I cavity (24 hour-cavity/indirect vs 1 year-cavity/direct, Table 2), again, the 3-step etch-and-rinse adhesive seemed most durable, as no significant decrease in µTBS was measured. A significant decrease in µTBS was, however, recorded for the 2-step self-etch and glass-ionomer adhesive. No significant decrease was observed for the other adhesives (Scotchbond 1, Adper Prompt and FujiBond LC), primarily because of the already low values recorded at 24 hours.

Despite the lowest µTBS for simplified adhesives being recorded after 1-year direct water exposure, only FujiBond LC and Reactmer recorded a statistically significant decrease when compared to 1-year indirect exposure (1 year-cavity/indirect vs 1 year-cavity/direct, Table 2).

With regard to failure patterns, typical patterns are shown in Figures 3 through 8 for the 6 adhesives tested following the 3 experimental conditions. In general, a high µTBS was correlated with a higher tendency to fail within dentin or composite (in particular, for Optibond FL and Clearfil SE). Most failures were recorded as "mixed interfacial and/or cohesive resin failure," irrespective of the adhesive and experimental condition (Table 3). Interfacial failure patterns were typically recognized by exposure of similarly curved scratches with a radius corresponding to the diamond bur used for cavity preparation (Figures 3 through 8). The 3-step etch-and-rinse adhesive hardly ever failed "interfacially" and, when this occurred, the fracture surface was still covered with a thin resinous layer. Moreover, none of the fracture surfaces exhibited poorly resin-impregnated collagen fibrils, confirming the high hybridization efficacy of this 3-step etch-and-rinse approach (Figure 3).

The rather low bonding effectiveness recorded for the 2-step etch-and-rinse adhesive (Scotchbond 1) was associated with a high number of interfacial failures (Table 3), often exhibiting poorly resin-impregnated collagen (Figure 4).

Light microscopic failure analysis of Clearfil SE 1-year cavity/indirect specimens revealed a predominantly "mixed" failure pattern (Table 3). The 1-year cavity/direct specimens, on the other hand, exhibited

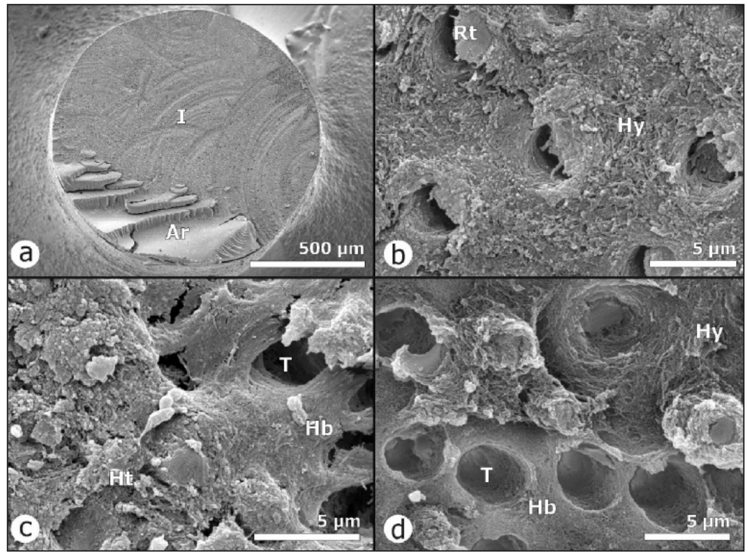

Figure 4. *Electron microscopic evaluation of Scotchbond 1. a) Feg-SEM photomicrograph of a mainly interfacially (I) failed 24-hour cavity/indirect specimen (dentin side). Typical circular scratch marks from cavity preparation can be observed. Ar = Adhesive resin. b) Higher magnification of (a). The specimen failed within the hybrid layer (Hy). Note the presence of seemingly unprotected collagen fibrils. Rt = Resin tag. c) Failure surface of a 1-year cavity/indirect specimen. At some sites, the specimen failed at the top of the hybrid layer (Ht), while at other areas, failure occurred at the bottom of the hybrid layer (Hb). T = Dentinal tubule. d) Fracture surface of a 1-year cavity/direct specimen that failed mainly at the bottom of the hybrid layer (Hb), exposing unaffected dentin. Other parts failed within the hybrid layer (Hy), exposing non-resin-enveloped collagen fibrils.*

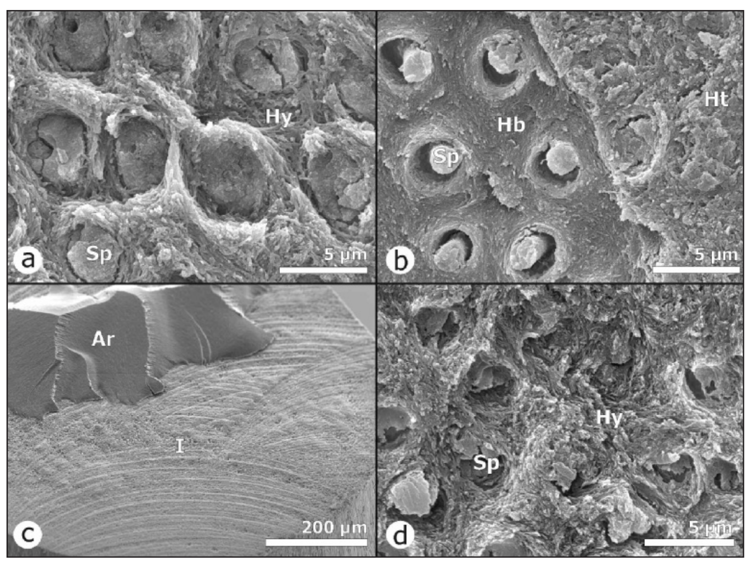

Figure 5. *Electron microscopic evaluation of Clearfil SE. a) Feg-SEM photomicrograph of the fractured surface of a 24-hour cavity/indirect specimen (dentin side). Magnification of an area that failed within the hybrid layer (Hy); Hybridized smear plugs (Sp) and a resin-infiltrated collagen matrix (Hy) can be observed. b) Feg-SEM photomicrograph of a 1-year cavity/indirect specimen at the dentin side. The failure was located either at the top (Ht) or the bottom (Hb) of the thin (±1 µm) hybrid layer. c) Feg-SEM photomicrograph of a 1-year cavity/direct specimen at the dentin side viewed at an angle of 60°. Ar = Adhesive resin, I = Interfacial failure. d) Magnification of an interfacially failed area of (c). The specimen failed mainly within the hybrid layer (Hy).*

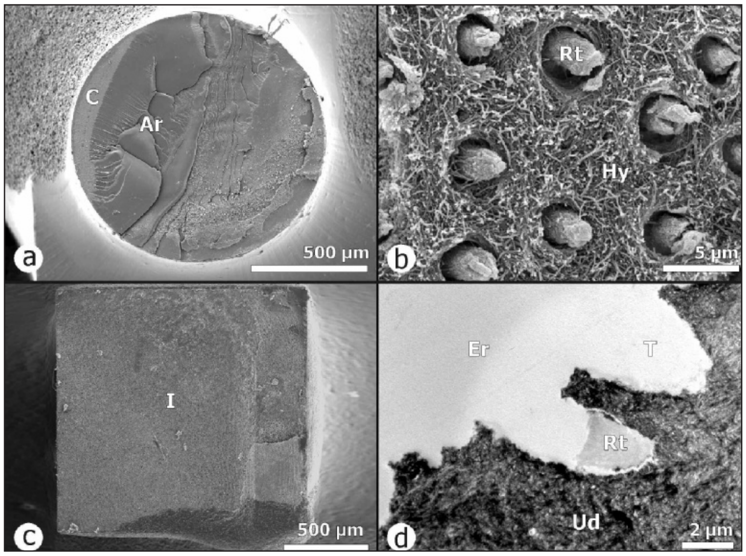

Figure 6. *Electron microscopic evaluation of Adper Prompt. a) Feg-SEM photomicrograph of the fractured surface of a 1-year cavity/indirect specimen (dentin side). The specimen failed mainly within the resinous part of the beam. Ar = Adhesive resin, C = Composite. b) Magnification of the composite side of (a). The specimen failed within the hybrid layer (Hy). Lots of loose collagen fibrils can be noticed, indicating that the resin must have disappeared (during water storage and/or specimen processing). Rt = Resin tag. c) Photomicrograph of the fractured surface of a 1-year cavity/direct specimen (pre-testing failure, dentin side). I = Interfacial failure. d) Non-demineralized, non-stained TEM photomicrograph of a similar specimen as (c). The hybrid layer completely detached from the unaffected dentin (Ud), leaving only small remnants of resin tags (Rt) in the tubules (T). Er =*

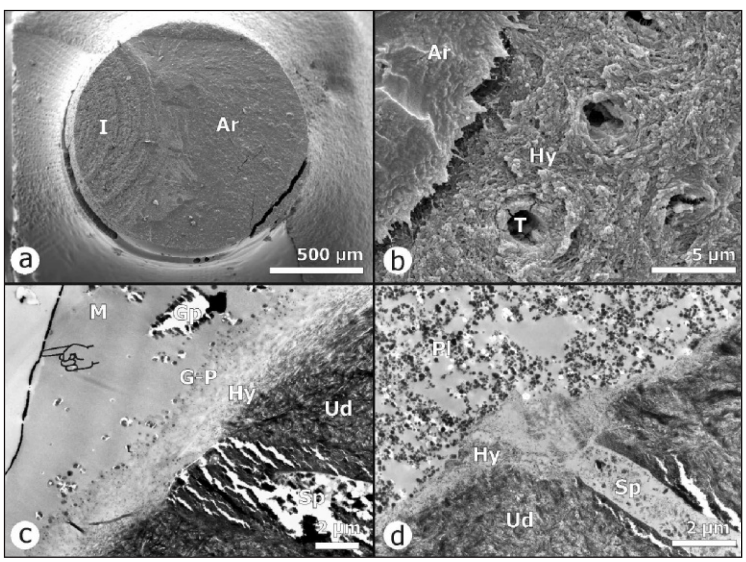

Figure 7. *Electron microscopic evaluation of FujiBond LC. a) Fracture surface of a 1-year cavity/indirect specimen at the dentin side that failed at the interface (I) and within the resin-modified glass-ionomer (Ar). b) Higher magnification of (a). Ar = Adhesive resin, Hy = hybrid layer, T= Dentinal tubule. c) Stained, non-demineralized TEM section of (b). The specimen failed cohesively in the glass-ionomer. The specimen only slightly reacted with the heavy metals, resulting in a selective staining of the gel phase (G-P). The tin black line, indicated by the hand pointer, originates from the gold sputtering procedure and deliminates the actual fracture location. M = matrix, Gp = Glass particle, Sp = hybridized smear plug, Ud = unaffected dentin. d) Unstained, non-demineralized TEM section of a 1-year cavity/direct specimen. The fracture surface was after µTBS testing protected by a thin layer of Clearfil Protect Liner F (Pl). The specimen failed at the top of the hybrid layer (Hy).*

not only a significantly decreased µTBS, but also more solely interfacial failures (Table 3 and Figure 5).

In case low µTBS values were recorded or the specimen failed prematurely, the most frequent failure pattern observed was "interfacial" failure. Interesting to note are the failure patterns recorded for Adper Prompt. The control 24-hour cavity/indirect group failed predominantly mixed interfacially or cohesively in resin (Table 3). After 1-year of indirect exposure to water, the main part of the failures occurred within the adhesive resin, with only small parts failing at the interface (Table 3). After 1 year of direct water exposure (1-year cavity/direct) most failures occurred interfacially. More in-depth Feg-SEM and TEM evaluation revealed that failures recorded initially as "interfacial" by light microscopy, actually occurred at the bottom of the hybrid layer (Figure 6).

It is also noteworthy that FujiBond LC 24-hour cavity/indirect specimens, as well as 1-year cavity/indirect specimens, failed mainly (at least partially) within the glass-ionomer adhesive itself (Figure 7). This indicates that the actual bonding effectiveness of FujiBond LC was probably not assessed, because the cohesive strength of the glass-ionomer material itself appeared lower than or at least as low as the interfacial bond strength. The latter was corroborated by Feg-SEM analysis of the fractured surfaces, which revealed that some failures were actually located at the glass-ionomer/composite interface (Figure 7).

Most fracture surfaces of Reactmer-dentin interfaces disclosed large areas of interfacial failure (Table 3). As Reactmer hardly demineralized the surface, the interaction zone is very thin and mainly represents the hybridized smear layer (Figure 8). On many fracture surfaces, this interaction zone detached from the underlying dentin, revealing unaffected dentin.

DISCUSSION

Previous studies have shown that, with increasing C-factor, the µTBS decreases.[11-13] A determining factor in that regard is the thickness, strength and stability of the adhesive layer. In a previous study, the µTBS of most adhesives did decrease when applied in a Class I cavity. Only adhesives with a separate hydrophobic bonding resin (2-step self-etch and 3-step etch-and-rinse approach) were able to withstand polymerization shrinkage stress in high C-factor cavities, even though a very stiff composite was used. The authors hypothesized that the resultant thick adhesive layer acted as an intermediary stress reliever to compensate for the shrinkage stress imposed during polymerization, following the "elastic bonding" concept.[14-18] Consequently, this elastic bonding concept may, to a large extent, explain the good resistance these multi-step adhesives show against polymerization shrinkage when applied in a high C-factor cavity.

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. In case the restored teeth were stored intact (1-year cavity/indirect), the occlusal seal produced by bonding the adhesive to the outer enamel margin of the occlusal Class I cavities may have protected the bond of the adhesive to the Class I bottom dentin against degradation. This beneficial effect was demonstrated previously, when 4 etch-and-rinse adhesives were applied to dentin disks surrounded by an enamel rim.[19] In that study, the µTBS of two 3-step etch-and-rinse adhesives bonded to a flat surface did not decrease significantly, irrespective of the storage conditions being either directly or indirectly exposed to the aqueous environment. The current results confirm this observation, as the bonding performance of the 3-step etch-and-rinse adhesive Optibond FL appeared stable during the 1-year indirect and direct water exposure.

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 a previous study by the authors, Scotchbond 1 appeared sensitive to 4-year water aging, except when the Scotchbond 1-dentin bond was sealed by a resin-enamel bond.[19] In the latter study, Scotchbond 1 was applied to flat dentin disks. Also, in this study, the bonding of Scotchbond 1 to the occlusal enamel margins of Class I restorations must have provided some protection against deterioration of the bond. However, this appeared insufficient after 1 year. This may indicate that the additional polymerization stress in Class I cavities rendered the bonding performance more vulnerable to water degradation. Inadequate solvent evaporation can result in inferior bonding effectiveness. When applied in thick layers, it is inevitable that the adhesive resin pools in cavity corners, by which, due to inadequate solvent removal, Scotchbond 1 is unable to produce an effective bond.[20] Moreover, this partially cured HEMA-hydrogel also jeopardizes the long-term bonding effectiveness by fluid migration through the adhesive resin.[21] In addition to these effects, the sealing ability of this adhesive may, in this study, also have been harmed by micro-cracks induced by high polymerization shrinkage stress. Altogether, inadequate solvent evaporation on its own may have resulted in a permeable interface, and this effect must have been intensified by micro-cracks induced in a weakened adhesive layer by high polymerization stress, as simulated in this laboratory study and, as it probably occurs in many clinical situations. The resultant permeable adhesive layer must have enabled the hydrolytic breakdown of various interface components and the subsequent leaching out of breakdown products (along with uncured components). This probably explains the significant reduction in bonding effectiveness over relatively short clinical periods of time.

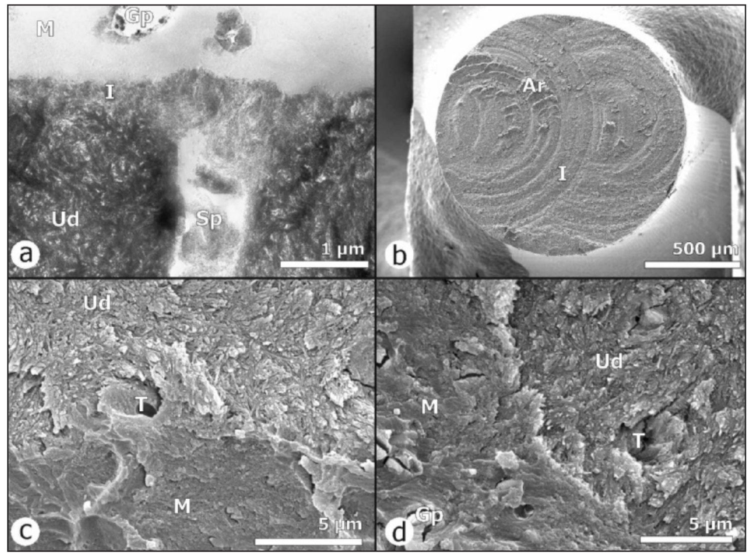

Figure 8. *Electron microscopic evaluation of Reactmer. a) Unstained, non-demineralized TEM photomicrograph of an intact Reactmer-dentin interface. Gp = glass particle, I = Interaction zone; M = Matrix; Sp = Smear plug; Ud = Unaffected dentin. The interaction zone is very thin on this section, but varied considerably, suggesting that this layer represents a hybridized smear layer, rather than a true hybrid layer. b) Feg-SEM photomicrograph of the fractured surface of a 1-year cavity/indirect specimen (dentin side). The specimen failed mainly at the Reactmer-dentin interface (I), apart from some small areas that failed within the adhesive resin itself (Ar). c) Higher magnification of (b) at the dentin side. On some sites, the hybridized smear layer detached, exposing unaffected dentin (Ud). M= Matrix of adhesive resin, T = dentinal tubule. d) Feg-SEM photomicrograph of a 1-year cavity/direct specimen at the dentin side. On most sites, the hybridized smear layer detached from the unaffected dentin.*

The µTBS of the "mild" 2-step self-etch adhesive Clearfil SE did not decrease significantly over 1-year indirect water exposure (Table 2). Although the failure modes did not change over time, Feg-SEM analysis revealed that interfacial failures after 1 day occurred predominantly more within the hybrid layer; whereas, after 1 year, the failures were located more at the transition of the hybrid layer to unaffected dentin (Figure 5). The µTBS of Clearfil SE to dentin was more affected by direct water exposure (1-year cavity/direct, Table 2). Also, the failure patterns shifted toward solely interfacial failures for the directly exposed specimens. These results are in agreement with the values obtained by Armstrong and others,[22] who observed a median µTBS value of 21.6 MPa after 15 months of direct water exposure, which was about half of the baseline µTBS.

No significant reduction in µTBS was recorded for the 1-step self-etch adhesive Adper Prompt after 1 year of indirect water exposure, mainly because of the already low bonding performance recorded at 1 day. After 1-year direct water exposure, bonding effectiveness was so low that none of the specimens could be tested. Along with decreasing bond strengths, an increasing number of interfacial failures were observed. After 1 year of direct water exposure, most specimens failed at the bottom of the hybrid layer (Figure 6), suggesting degeneration of collagen and resin parts over time.

For the 2-step glass-ionomer adhesive FujiBond LC, no difference in µTBS was found between the 24-hour-cavity/indirect and 1-year cavity/indirect specimens. This is in contrast with a similarly conducted study, where FujiBond LC was bonded to flat dentin surfaces and stored for 4 years in water.[23] In that study, the µTBS dramatically decreased, mainly because of degradation of the glass-ionomer matrix itself. Probably, the exposure time was too short (1 vs 4 year), and the diffusion path too long (bottom of Class I cavity) for the diffusion rate-dependent degradation processes to significantly affect the resin-modified glass ionomer/dentin bond. This is confirmed by a non-changing failure analysis over the 1-year period (Table 3). Also, Feg-SEM analysis did not reveal any structural changes over time for the indirectly exposed group (Figure 7). On the other hand, the bond strengths of the specimens directly exposed to water decreased to nearly zero. Also, the failure site shifted to solely interfacial failures (Table 3). TEM also revealed that the interface failed at the top of the hybrid layer (Figure 7d). Consequently, it can be assumed that the mechanical properties of the gel phase,[24] deposited on top of the hybrid layer, must have deteriorated. This process must be diffusion-dependent, as it did not occur in the indirect water exposure group.

Remarkably, the "mild" 1-step self-etch adhesive Reactmer was the only adhesive that showed no increase in µTBS to Class I cavity bottom dentin after 1 year of water storage. It even increased considerably (and highly significantly). No pre-testing failures were recorded in the 1-year cavity/indirect group; whereas, about 70% of the 24 hour-cavity/indirect specimens failed prior to being tested (Table 1). Also, the main failure mode changed from exclusively "interfacial" after 1 day to "mixed" after 1 year (Table 3) and, thus, confirms 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 also chemically interacted with dentin. These water-dependent reactions may have taken a few weeks to establish,[25] 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, its µTBS. These ongoing reactions then may also have induced expansion of the adhesive, and so relieved polymerization stress and avoided gap formation.[25] This expansion effect, in combination with the chemical interaction with dentin, may be indicative of a kind of "repair" effect, when compared with poor 24-hour bond performance. The clinical benefits of this repair process are, however, doubtful, as the resultant bond is very sensitive to direct water exposure. After 1-year direct water exposure, the µTBS of Reactmer to dentin approached zero (Table 1).

CONCLUSIONS

The conventional 3-step etch-and-rinse adhesive still remained most effective in bonding to dentin and appeared insensitive to the effects of increased polymerization shrinkage stress and water degradation. Most closely approaching this "gold standard" was the "mild" 2-step self-etch adhesive Clearfil SE that, despite a significant decrease in µTBS, was the only adhesive to perform reliably after 1 year of direct 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 water-aging effects. The 2-step resin-modified glass-ionomer adhesive FujiBond LC only suffered from higher shrinkage stress but not from 1-year indirect water exposure; direct water exposure was, however, also detrimental for this resin-modified glass-ionomer adhesive. Remarkable is the apparent repairability of the "mild" 1-step glass-ionomer adhesive Reactmer when the entirely restored cavities were stored for 1 year in water; this effect may be clinically less relevant, as the bonds formed did not withstand 1-year direct water exposure.

In general, simplified bonding procedures did not necessarily imply improved bonding performance, especially in 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, led to a dramatic bond deterioration in the long term.

(Received 18 April 2005)

References

1. Degrange M & Roulet JF (1997) *Minimally Invasive Restorations with Bonding* Quintessence Publishing Chicago.

2. Inoue S, Vargas MA, Abe Y, Yoshida Y, Lambrechts P, Vanherle G, Sano H & Van Meerbeek B (2001) Microtensile bond strength of eleven modern adhesives to dentin *Journal of Adhesive Dentistry* **3(3)** 237-245.

3. De Munck J, Van Meerbeek B, Satoshi I, Vargas M, Yoshida Y, Armstrong S, Lambrechts P & Vanherle G (2003) Microtensile bond strengths of one- and two-step self-etch adhesives to bur-cut enamel and dentin *American Journal of Dentistry* **16(6)** 414-420.

4. Feilzer AJ, de Gee AJ & Davidson CL (1987) Setting stress in composite resin in relation to configuration of the restoration *Journal of Dental Research* **66(11)** 1636-1639.

5. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H & Oguchi H (2000) *In vivo* degradation of resin-dentin bonds in humans over 1 to 3 years *Journal of Dental Research* **79(6)** 1385-1391.

6. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M & Van Meerbeek B (2005) A critical review of the durability of adhesion to tooth tissue: Methods and results *Journal of Dental Research* **84(2)** 118-132.

7. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry *Operative Dentistry* **26(Supplement 6)** S119-S144.

8. Burrow MF, Satoh M & Tagami J (1996) Dentin bond durability after three years using a dentin bonding agent with and without priming *Dental Materials* **12(5)** 302-307.

9. Perdigão J, Lambrechts P, Van Meerbeek B, Vanherle G & Lopes AL (1995) Field emission SEM comparison of four post-fixation drying techniques for human dentin *Journal of Biomedical Materials Research* **29(9)** 1111-1120.

10. Robinson G & Gray T (1996) Electron microscopy 2: Practical procedures In: Bancroft JD, Stevens A (eds) *Theory and Practice of Histological Techniques* Chuchill Livingstone New York 585-626.

11. Yoshikawa T, Sano H, Burrow MF, Tagami J & Pashley DH (1999) Effects of dentin depth and cavity configuration on bond strength *Journal of Dental Research* **78(4)** 898-905.

12. Armstrong SR, Keller JC & Boyer DB (2001) The influence of water storage and C-factor on the dentin-resin composite microtensile bond strength and debond pathway utilizing a filled and unfilled adhesive resin *Dental Materials* **17(3)** 268-276.

13. Shirai K, De Munck J, Yoshida Y, Inoue S, Lambrechts P, Suzuki K, Shintani H & Van Meerbeek B (2005) Effect of cavity configuration and aging on the bonding effectiveness of six adhesives to dentin *Dental Materials* **21(2)** 110-124.

14. Kemp-Scholte CM & Davidson CL (1990) Complete marginal seal of Class V resin composite restorations effected by increased flexibility *Journal of Dental Research* **69(6)** 1240-1243.

15. Kemp-Scholte CM & Davidson CL (1990) Marginal integrity related to bond strength and strain capacity of composite resin restorative systems *The Journal of Prosthetic Dentistry* **64(6)** 658-664.

16. Van Meerbeek B, Willems G, Celis JP, Roos JR, Braem M, Lambrechts P & Vanherle G (1993) Assessment by nano-indentation of the hardness and elasticity of the resin-dentin bonding area *Journal of Dental Research* **72(10)** 1434-1442.

17. Perdigão J, Lambrechts P, Van Meerbeek B, Braem M, Yildiz E, Yucel T & Vanherle G (1996) The interaction of adhesive systems with human dentin *American Journal of Dentistry* **9(4)** 167-173.

18. Choi KK, Condon JR & Ferracane JL (2000) The effects of adhesive thickness on polymerization contraction stress of composite *Journal of Dental Research* **79(3)** 812-817.

19. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P & Vanherle G (2003) Four-year water degradation of total-etch adhesives bonded to dentin *Journal of Dental Research* **82(2)** 136-140.

20. Zheng L, Pereira PN, Nakajima M, Sano H & Tagami J (2001) Relationship between adhesive thickness and microtensile bond strength *Operative Dentistry* **26(1)** 97-104.

21. Tay FR, King NM, Chan KM & Pashley DH (2002) How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? *Journal of Adhesive Dentistry* **4(4)** 255-269.

22. Armstrong SR, Vargas MA, Fang Q & Laffoon JE (2003) Microtensile bond strength of a total-etch 3-step, total-etch 2-step, self-etch 2-step, and a self-etch 1-step dentin bonding system through 15-month water storage *Journal of Adhesive Dentistry* **5(1)** 47-56.

23. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Suzuki K & Lambrechts P (2004) Four-year water degradation of a resin-modified glass-ionomer adhesive bonded to dentin *European Journal of Oral Sciences* **112(1)** 73-83.

24. Van Meerbeek B, Yoshida Y, Lambrechts P, Vanherle G, Wakasa K & Nakayama Y (1998) Mechanisms of bonding of a resin-modified glass-ionomer adhesive with dentin *Journal of Dental Research* **77(Special Issue B)** Abstract 2236 p 911.

25. Huang C, Kei LH, Wei SH, Cheung GS, Tay FR & Pashley DH (2002) The influence of hygroscopic expansion of resin-based restorative materials on artificial gap reduction *Journal of Adhesive Dentistry* **4(1)** 61-71.