Effect of long-term water aging on microtensile bond strength of self-etch adhesives to dentin

ALI I. ABDALLA, PHD

ABSTRACT: **Purpose:** To evaluate the effect of water storage on the microtensile dentin bond strength of one total-etch and four self-etching adhesives to dentin. **Methods:** The adhesive materials were: one total-etch adhesive (Admira Bond) and four self-etch adhesives (Clearfil S tri Bond, Hybrid Bond, Futurabond NR, Adhe SE). Freshly extracted human third molar teeth were used. For each tooth, dentin was exposed on the occlusal surface by cutting with an Isomet saw and the remaining part was mounted in a plastic ring using dental stone. After adhesive application, a composite resin (Grandio) was placed in 5-6 mm height to form a crown segment. For each tested adhesive, two test procedures (n=6 teeth) were carried out. Procedure A: the teeth were stored in water for 24 hours, and then sectioned longitudinally, buccolingually and mesiodistally to get rectangular beams of 1 ± 0.1 mm thickness on which a micro-tensile test was carried out. Procedure B: The specimens were stored in water at 37°C for 3 years before sectioning and microtensile testing. During microtensile testing the beams were placed in a universal testing machine and load was applied at cross-head speed of 0.5 mm/minute. **Results:** For the 24-hour water storage groups, there was no significant difference in the bond strength between the different adhesives. After 3 years of water storage, the bond strength of all self-etch adhesives was significantly reduced compared to the control groups (24 hours). In contrast, the bond strength of Admira Bond was not significantly reduced. (*Am J Dent* 2010;23:29-33).

CLINICAL SIGNIFICANCE: Water storage for 3 years significantly reduced the bond strength of tested self-etch adhesives to dentin. The bond produced by the total-etch system was able to resist 3-year water degradation.

✉: Dr. Ali I. Abdalla, Department of Restorative Dentistry, Faculty of Dentistry, University of Tanta, Tanta, Egypt. E-✉: ali_abdalla79@yahoo.com

Introduction

The major shortcoming of contemporary adhesive restoratives is their limited durability *in vivo*.[1] The most cited reasons for failure of adhesive restorations are loss of retention and marginal adaptation.[2,3] Therefore, a valuable approach to prolong the clinical lifetime of adhesives might be to focus on improving the stability of the bond of these biomaterials to tooth tissue. The immediate bonding effectiveness of most current adhesive systems is quite favorable,[4] regardless of the adhesive used. However, when these adhesives are tested in a clinical trial, the bonding effectiveness of some materials appears dramatically low, whereas the bonds of other materials are more stable.[5,6]

The tooth-biomaterial bond may also degrade by exposure of the interface to water and/or human/bacterial enzymes present in saliva. Ingression of water into the hybrid layer[7] and subsequent leaching out of resin components are believed to lead to inefficient *in situ* polymerization[8] and degradation of resin components.[9-11] Also, hydrolysis of hydroxyapatite-depleted or insufficiently resin coated collagen fibrils compromises long-term bonding effectiveness.[12]

Biomaterial-tooth interfaces are subjected to chemical as well as mechanical degradation. Chemically, the most important reactions are hydrolysis and plasticizing of the resin components, which are both related to the ingression of water. Since this ingression is a diffusion-like process, the related degradation mechanisms will also be diffusion-dependent. Hydrolysis can break up covalent bonds, linking the different units of collagen fibrils as well as resinous polymers.[13,14]

The most commonly used artificial aging technique is long-term water storage. The bonded specimens are stored in fluid at 37°C for a specific period. This period may vary from a few months[15] up to 4-5 years[16,17] or even longer. Most studies report significant decreases in bond strengths, even after relatively short storage periods.[18-20] Decrease in bonding effectiveness in this type of study is, first, supposed to be caused by degradation of interface components by hydrolysis (mainly resin and/or collagen). But, as previously mentioned, water can also infiltrate and decrease the mechanical properties of the polymer matrix, by swelling and reducing the frictional forces between the polymer chains, a process known as "plasticization".[21,22] Furthermore, some interface components, such as uncured monomers and break-down products of previous mechanisms, can elute, weakening the bond.[23]

The present study was designed to evaluate the influence of water storage on the microtensile bond strength of one total-etch adhesive and four self-etching adhesives to dentin.

Materials and Methods:

The materials used in this study (Table 1) include a total-etch adhesive: Admira Bond; and four self-etch adhesives; Clearfil S tri bond, Hybrid Bond, Futurabond NR and Adhe SE.

Test methods - Sixty extracted human sound lower molar teeth were collected and stored in 0.5% chloramine solution in water. The teeth were used within 1 month after extraction.

For each tooth, the coronal portion was removed using a low speed diamond saw (Isomet®) with water coolant. The cut dentin surface was then abraded against 600-grit wet silicon carbide papers to produce a uniform smear layer. The remaining apical part of each tooth was mounted in a plastic ring using hard dental stone. The adhesive material (Table 1) was applied to the dentin surface according to manufacturer's

Table 1. Composition of the adhesive systems used in the study.

Material	Component	Composition
Admira Bond[a]	Acid	36% phosphoric acid
	Bond	Acetone, bonding ormocer, dimethacrylate, functionizing methacrylates, initiators, stabilizer
Clearfil S tri Bond[b]	Adhesive	10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, microfiller
Hybrid Bond[c]	Brush	Sodium p-toluenesulfinate, sodium N-phenyl-glycine (NPG-Na)
	Adhesive	Methylmethacrylate (MMA), 4-methacryloxy-ethyltrimetillic acid anhydride tri (2-hydroxy-ethyl)-isocyanurat-triacrylate (THIT), HEMA, acetone, water.
Futurabond NR[a]	Liquid A	Water, ethanol, silicium dioxide
	Liquid B	Acid modified methacrylate (methacrylate ester), HEMA, camphorquinone
Adhe SE[d]	Primer	Dimethacrylate, phosphonic acid acrylate, initiator, stabilizer, water
	Bond	HEMA, dimethacrylate, silicon dioxide, initiator, stabilizer

instructions with 12 teeth for each tested adhesive.

Admira Bond - The dentin surface was etched for 15 seconds with 37% phosphoric acid, rinsed with water spray. Excess water was removed with an air blast for 3 seconds leaving the dentin moist. Admira Bond was applied with a disposable brush, thinned with mild air for 2-3 seconds and light-cured for 20 seconds. The output of the light-curing unit was regularly checked (500 mW/mm^2).

Clearfil S tri Bond - The bond was applied, thinned with a gentle stream of air and light-cured for 20 seconds.

Hybrid Bond - Hybrid Bond was dispensed into the mixing well. A Hybrid Bond brush was dipped into the solution, stirred shortly and then applied to the surface for 20 seconds. The adhesive was thinned with a gentle blast of air for 5 seconds and light-cured for 20 seconds.

Futurabond NR - One drop of liquid A and one drop of liquid B was mixed in the mixing palette for 5 seconds. The material was then applied to the dentin surface, massaged for 20 seconds, dried with air for 5 seconds and light-cured for 10 seconds.

Adhe SE - An adequate amount of Adhe SE primer was applied to wet the dentin surface using a brush, and the primer was brushed onto the surface for 30 seconds. Excess primer was dispersed with a strong stream of air until the mobile liquid film disappeared. The Adhe SE was applied, dispersed with a weak stream of air and cured for 10 seconds.

The resin based composite Grandio[a] was placed in 3-4 layers to a height of 5-6 mm to form a crown segment. Each increment of composite was cured for 60 seconds using a Visulux[f] curing unit.

Two test procedures were carried out for each adhesive including six teeth for each study group:

Procedure A: The teeth were stored in water at 37°C for 1 day, and then microtensile bond strength measurements were carried out ("no water storage").

Procedure B: The teeth were kept in water at 37°C for 3 years and then microtensile bond strength measurements were carried out.

Table 2. Mean bond strength of tested materials (Mean ± SD MPa).

Material	n	Without water storage	Water storage 3 years	Statistical difference
Admira Bond	20	39.7 ± 5.1	32.6± 3.5	NS
Clearfil S tri Bond	20	33.2 ± 4.7	18.4± 2.7	P= 0.03
Hybrid Bond	20	38.5 ± 4.7	22.2± 4.6	P= 0.02
Futurabond NR	20	39.3 ± 4.3	25.9± 3.8	P= 0.02
Adhe SE	20	36.5 ± 5.2	21.3± 2.6	P= 0.04

NS= not significant (P> 0.05).
n = number of beams tested.

Specimen preparation for microtensile bond strength - All samples were sectioned longitudinally, perpendicular to the adhesive interface, buccolingually and mesiodistally with low speed water cooled diamond saw,[e] and then the mounted tooth was rotated 90º and sectioned at its cervical portion to separate the micro specimens. This serial sectioning leads to the formation of numerous rectangular "beams" or "sticks" with approximately 1 - 1.2 mm^2 of cross-sectional area. Only beams from the central portion of the restoration were selected as peripheral beams may not have had the same dentin thickness. Four six-beams were obtained from each tooth and the cross-sectional areas were measured with a digital calliper[g] before testing. For each test procedure, 20 beams were prepared. Each slab was attached to the set-up by its lateral sides and placed in a universal testing machine[h] and tensile load was applied at a cross-head speed of 0.5 mm/minute until failure occurred.

Statistical analysis - The raw data were tabulated and analyzed using two-way ANOVA with the adhesive system and testing procedure as the main factors using Systat[i] statistical software package. When F ratio was significant, a Student-Newman-Keuls multiple comparison test was used.

Fracture surfaces observation - After microtensile testing, the fractured surface of the bonded specimen was inspected under stereomicroscope[j] to evaluate the mode of failure. In addition, some specimens of each material were mounted on aluminum stubs, sputter-coated with gold (20 nm) and observed by using SEM (Philips XL30[k]) operating at 15 kV.

Results

Microtensile bond strength test - The bond strength values of the tested adhesive materials at different storage conditions are shown in Table 2. After 24 hours water storage, there was no significant difference in the bond strength between the different adhesives tested. After 3 years of water storage, the bond strength of Clearfil S tri Bond, Hybrid Bond, Futurabond NR and Adhe SE were significantly (P< 0.05) reduced compared to their 24-hour results. In contrast the values of Admira Bond were not changed significantly (P > 0.05).

Fracture surface evaluation and SEM observation - The fractured pattern of bonded specimens is shown in Tables 3 and 4. At 24 hours water storage, 38% of the samples failed either cohesive in dentin or in composite while 62% of the specimens showed adhesive failure. After water storage for 3 years, 95% of the samples failed adhesively at the interface between adhe-

Table 3. Fracture patterns of bonded specimens (24-hours water storage).

Material	No. of teeth	No. of beams	Cohesive/ dentin	Cohesive/ composite	Adhesive
Admira Bond	6	20	4	6	10
Clearfil S tri Bond	6	20	4	2	14
Hybrid Bond	6	20	4	4	12
Futurabond NR	6	20	4	4	12
Adhe SE	6	20	2	4	14
			18 (18%)	20 (20%)	62 (62%)

Table 4. Fracture patterns of bonded specimens after 3-year water storage.

Material	No. of teeth/slabs	Cohesive/ dentin	Cohesive composite	Adhesive
Admira Bond	6/20	0	3	17
Clearfil S tri Bond	6/20	0	0	20
Hybrid Bond	6/20	0	0	20
Futurabond NR	6/20	0	2	18
Adhe SE	6/20	0	0	20
		0 (0%)	5 (5%)	95 (95%)

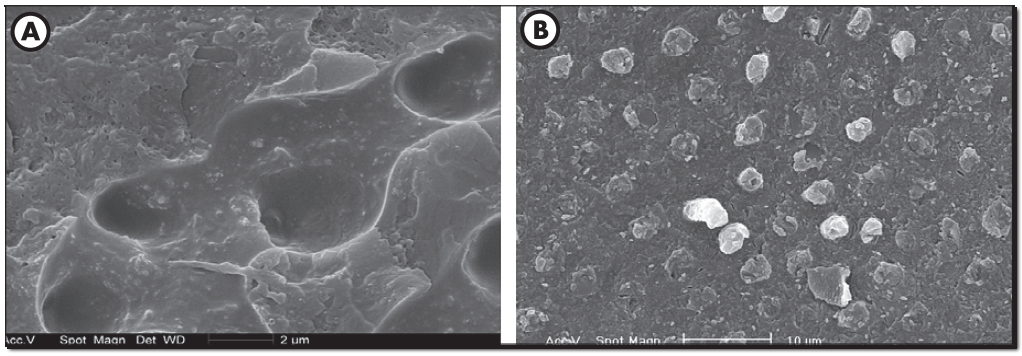

Fig. 1**A**. The fractured surface of Admira Bond specimen after 24 hours of water storage. A dense hybrid layer that consisted of resin enveloped collagen fibrils and resin matrix. **B.** The fractured surface of Admira Bond specimen after 3-year water storage showed good hybridization with resin are infiltrated well into dentin tubules as well as intertubular dentin.

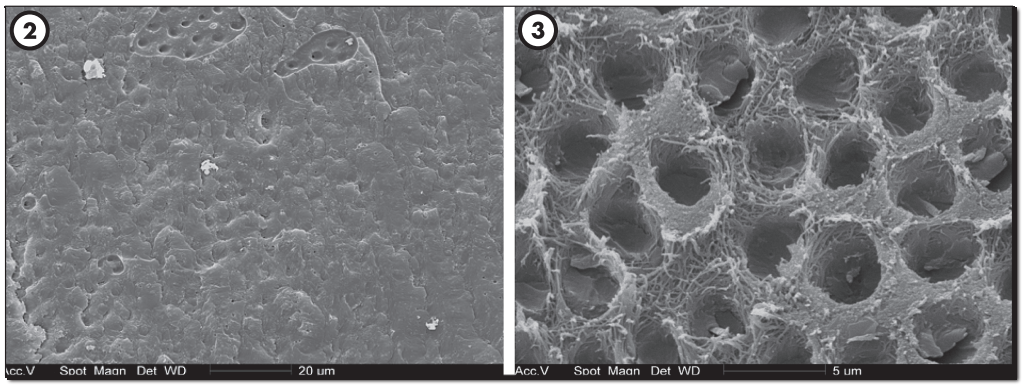

Fig. 2. The fractured surface of Futurabond NR specimen after 24 hours water storage. Resins are evident into collagen mesh and interfibrillar spaces.

Fig. 3. The fractured surface of Clearfil S tri Bond after 3 years of water storage. Resin was lost and collagen fibrils were exposed.

sive and resin composite or between adhesive and dentin, while 5% failed cohesively.

SEM observation of the fractured surface of bonded specimens after 24 hours of water storage showed a dense hybrid layer that consisted of resin enveloped collagen fibrils and resin matrix. (Figs. 1a, 2). After 3 years of water storage, Admira Bond (Fig. 1b) showed good infiltration of resins into the interfibrillar spaces as well as into the dentin tubules. In contrast, all self-etch adhesives showed poor hybridization. Resin seemed to be extracted from the hybrid layer with an increase in the size of the interfibrillar spaces (Fig. 3).

Discussion

In the present study the effect of water storage on the bond strength of four self-etching adhesives and one total-etch adhesive to dentin was evaluated. Samples were prepared in flat dentin surfaces surrounded by enamel margins. In this case, the resin-dentin interface was exposed to water indirectly. This represents a situation in which the restorations were made with margins in enamel. Under a clinical situation, cycling masticatory function has been reported to fatigue the integrity of resin enamel bond, thereby permitting micro- or nanoleakage of the peripheral enamel seal.[24] This in turn could lead to degradation of both resin and exposed collagen fibrils by exposure to water, saliva and enzyme attack.[25] In addition, restorations with margins that extend into the cementum are more susceptible to degradation by water contact.[26]

With the total-etch system, Admira Bond, a bond strength of 39.7 ± 5.1 MPa was found at 24 hours. The primary bonding mechanism of Admira Bond was thought to be diffusion-based and depends on hybridization or infiltration of resin within the exposed collagen mesh as well as into dentin tubules. After *in situ* polymerization, the formed hybrid layer provides micromechanical retention. Accordingly, exposed collagen fi-

brils seemed to be well enveloped with resinous component, which protects them from hydrolysis (Fig. 1a). After 3-year storage, the bond strength was not significantly affected. Again, the optimal dentin hybridization of Admira Bond and the presence of ormocers (organic modified ceramic) in the bonding resin could explain such findings.[27] Ormocers have a calcium complexing function which enhances the bond strength to tooth structure,[28] and reduces the rate of release of residual monomers in comparison with conventional composites[29] which, if not polymerized thoroughly, may be washed out in time. This might explain the existence of resin into the interfibrillar spaces after water storage. SEM observation of the fractured surface of specimens showed a dense hybrid layer that consisted of resin enveloped collagen fibrils and resin matrix (Fig. 1b).

Several investigations[30-33] have attributed the degradation of resin-bond strength after water storage to the disintegration of collagen fibrils and the loss of associated resin in the area of exposed collagen fibrils in the demineralized zone of dentin, which was created by the discrepancy between the depth of acid etching and resin infiltration. They reported that for the total etch system, if infiltration depth is less than the demineralized depth, a zone of hydroxyapatite depleted collagen fibrils is left exposed and unsupported. These naked collagen fibrils will undergo more strain than the overlying well resin infiltrated hybrid layer since the modulus of elasticity of the demineralized dentin collagen matrix is far lower than that of the hybrid layer. Thus the demineralized dentin at the bottom of the hybrid layer would become a weak link in the bonding interface over time.

Clearfil S tri Bond is a one-step mild self-etching adhesive. This adhesive contains 10-MDP as functional monomer dissolved in water to result in a pH around 2. On dentin, Clearfil S tri Bond does not remove the smear layer. It impregnates the smear plugs, fixing it at the tubules. The bonding mechanism of Clearfil S tri Bond was suggested to result from the simultaneous demineralization and infiltration of enamel and dentin to form a continuum in the substrate incorporating the smear plug in the resin tag.[34] The presence of the highly hydrophilic 10-MDP monomer is believed to improve the wetting to moist tooth surface.[35] In spite of such a favorable bonding mechanism, this material seems to be unable to tolerate excess water caused by water storage for 3 years. In this case the bonded dentin surface could not completely prevent fluid movement. Water storage might accelerate degradation of resin-dentin bond. Fig. 3 shows the fractured surface of Clearfil S tri Bond after water storage. Resin was lost and interfibrillar spaces were evident.

Hybrid Bond is a one step self-etching adhesive. Hybrid Bond contains 4-META as an active monomer component. In an aqueous environment, this monomer is converted to the dicarboxylic acid 4-MET, the etching component of Hybrid Bond with pH around 2.[36] Due to the small size of the molecule, 4-MET penetrates well into the etched tooth structures. The amphiphilic monomer wets the exposed collagen network strings and bonds *via* hydrogen bridges. At the same time the collagen coated surfaces are rendered hydrophobic *via* the methacrylate group and are thus prepared to bond to the hydrophobic monomers of the resin composite.[37] Further, the 4-MET offer the advantages of forming ionic bonds to the calcium in apatite.[38] This bonding mechanism which seems to encapsulate the collagen fibrils by the bonding resin may explain such favorable performance of Hybrid Bond at bonding procedure and after 24 hours. However, after 3-year water storage, lower bond strength values have been reported. A possible reason for such findings could be the absence of coupling hydrophobic bonding agent, which made the materials behave as permeable membranes after polymerization. In the absence of more hydrophobic coating in the simplified adhesive system, rapid water sorption can occur *via* the hydrophilic and permeable adhesive layer.[8] In addition, Yoshida *et al*[39] found a lower bonding potential of 4-MET to residual hydroxyapatite around exposed collagen fibrils. This means low chemical bonding efficiency to tooth structure.

Futurabond NR contains polyfunctional adhesive monomers (methyl phosphorus acid ester and carbonic acid modified methacrylate ester). These acid esters when mixed with water produced a pH value of 1.4. Accordingly, the smear layer was mobilized and the hydroxyl apatite was solubilized (demineralized). The resulting collagen network and the open dentin tubules are then penetrated by the hydrophilic bonding agent. Chemical bonding also takes place in the surface of tooth structure due to complexation of the calcium by the adhesive monomers.[40] In addition, Futurabond NR is a nano filled adhesive with the ability of the nano filler to penetrate into dentin tubules. This will form a thicker adhesive layer capable of preventing fluid movement from dentin tubules. Again, such a bonding mechanism may explain the favorable performance of this material when specimens were not stored in water. However, such a mechanism was unable to resist deterioration by water storage for a long period. In this case, the bonded dentin surface could not completely prevent fluid movement. Slow water penetration might accelerate degradation of resin-dentin bond. Figure 2 shows the fractured surface of a specimen of Futurabond NR without water storage. Good hybridization was evident.

Adhe SE is a two-step self-etch adhesive. Its self etching capacity is based on phosphonic acid acrylate. This monomer with a pH 1.4 dissolves the smear layer and smear plugs. Accordingly this system seemed to interact with dentin in a way midway between total-etch adhesive and self-etch ones. Phosphonic acid acrylate was reported to form a weak bond to dentin.[41] Also it has high hydrophilic properties which made it behave as a semipermeable membrane.[42] Under water storage more channels will be formed and this will lead to deterioration of the resin-dentin bond.

Another factor which could contribute to the deterioration of resin-dentin bond after water storage is the fact that the physical properties of the hydrophilic dental adhesives, such as those present in current adhesives, were reduced by 30-40% after 3-6-month water storage.[43] This resulted from the plasticizing effect of water on the mechanical properties of resin. Water sorption swells the polymer chain causing decreased mechanical properties with passive hydrolysis and leaching effect. This passive hydrolysis and leaching effect is the most important mode of degradation of the resin-dentin bond.

This study showed that water storage could significantly affect the durability of the resin-dentin bond for certain adhesives. Both the residual water within the polymerized adhesive and water uptake from the media could deteriorate the adhesive bond.

a. Voco, Cuxhaven, Germany.
b. Kuraray Medical, Tokyo, Japan.
c. Sun Medical, Shiga, Japan
d. Vivadent, Schaan, Liechtenstein.
e. Buehler,Ltd, Lake Bluff, IL, USA
f. 3M ESPE, St. Paul, MN, USA.
g. Mitutoyo, Tokyo, Japan.
h. Instron, Corp., High Wycombe, UK.
i. Systat, San Jose, CA, USA.
j. Olympus, Tokyo, Japan.
k. Philips, Eindhoven, The Netherlands.

*Disclosure statement:*The author declares no conflict of interest.

Dr. Abdalla is Assistant Professor, Department of Restorative Dentistry, Faculty of Dentistry, University of Tanta, Tanta, Egypt.

References

1. Marquezan M, da Silveira BL, Burnett LH Jr, Rodrigues CR, Kramer PF Microtensile bond strength of contemporary adhesives to primary enamel and dentin. *J Clin Pediatr Dent* 2008;32:127-132.
2. Abdalla AI, Elsayed HY, García-Godoy F. Effect of hydrostatic pulpal water pressure on microtensile bond strength of self-etch adhesives to dentin. *Am J Dent* 2008;21:233-238.
3. Osorio R, Pisani-Proenca J, Erhardt MC, Osorio E, Aguilera FS, Tay FR, Toledano M Resistance of ten contemporary adhesives to resin-dentine bond degradation. *J Dent* 2008; 36:163-169.
4. Uekusa S, Tsubota K, Tonegawa M, Tsuchiya H, Iwasa M, Kawamoto R, Takamizawa T, Miyazaki M. Microtensile bond strengths of single-step self-etch adhesive systems to bovine dentin. *J Oral Sci* 2007;49:183-189.
5. Toba S, Veerapravati W, Shimada Y, Nikaido T, Tagami J. Micro-shear bond strengths of adhesive resins to coronal dentin *versus* the floor of the pulp chamber. *Am J Dent* 2003;16 (Sp Is A): 51A-56A.
6. Chigira H, Manabe A, Hasegawa T, Yukitani W, Fujimitsu T, Itoh K, Hisamitsu H, Wakumoto S. Efficacy of various commercial dentin bonding systems. *Dent Mater* 1994;10:363-368.
7. Chappell RP, Eick JD. Shear bond strength and scanning electron microscopic observation of six current dentinal adhesives. *Quintessence Int* 1994;25:359-368.
8. De Munck J, Shirai K, Yoshida Y, Inoue S, Van Landuyt K, Lambrechts P, Suzuki K, Shintani H, Van Meerbeek B. Effect of water storage on the bonding effectiveness of 6 adhesives to Class I cavity dentin. *Oper Dent* 2006;31:456-465.
9. Sano H, Shono T, Sonoda H, Takatsu T, Ciucchi B, Carvalho R, Pashley DH. Relationship between surface area for adhesion and tensile bond strength. Evaluation of a micro-tensile bond test. *Dent Mater* 1994;10:236-240.
10. Nakajima M, Kanemura N, Pereira PN, Tagami J, Pashley DH. Comparative microtensile bond strength and SEM analysis of bonding to wet and dry dentin. *Am J Dent* 2000;13:324-328.
11. El Zohairy AA, de Gee AJ, de Jager N, van Ruijven LJ, Feilzer AJ. The influence of specimen attachment and dimension on microtensile strength. *J Dent Res* 2004;83:420-424.
12. Armstrong SR, Keller JC, Boyer DB. 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. *Dent Mater* 2001;17:268-276.
13. Sano H, Yoshikawa T, Pereira PN, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, *in vivo*. *J Dent Res* 1999;78:906-911.
14. Hashimoto M, Ohno H, Sano H, Tay FR, Kaga M, Kudou Y, Oguchi H, Araki Y, Kubota M. Micromorphological changes in resin-dentin bonds after 1 year of water storage. *J Biomed Mater Res* 2002;63:306-311.
15. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, Pashley DH. Nanoleakage: Leakage within the hybrid layer. *Oper Dent* 1995;20:18-25.
16. Sano H, Yoshikawa T, Pereira PN, Kanemura N, Morigami M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a selfetching primer *in vivo*. *J Dent Res* 1999;78:906-911.
17. Fukushima T, Inoue Y, Miyazaki K, Itoh T. Effect of primers containing N-methylolacrylamide or N-methylolmethacrylamide on dentin bond durability of a resin composite after 5 years. *J Dent* 2001; 29:227-234.
18. Van Meerbeek B, Peumans M, Gladys S, Braem M, Lambrechts P, Vanherle G. Three-year clinical effectiveness of four total-etch dentinal adhesive systems in cervical lesions. *Quintessence Int* 1996;27:775-784.
19. Van Meerbeek B, Perdigão J, Lambrechts P, Vanherle G. The clinical performance of adhesives. *J Dent* 1998;26:1-20.
20. Van Meerbeek B, Yoshida Y. Snauwaert Hellmans L, Lambrechts P, Vanherle G, Wakasa K, Pashley DH. Hybridization effectiveness of a two-step *versus* a three-step smear layer removing adhesive system examined correlatively by TEM and AFM. *J Adhes Dent* 1991:7-23.
21. Gladys S, Van Meerbeek B, Lambrechts P, Vanherle G. Microleakage of adhesive restorative materials. *Am J Dent* 2001;14:170-176.
22. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonocore memorial lecture: Adhesion to enamel and dentin: Current status and future challenges. *Oper Dent* 2003;28:215-235
23. Ajlouni R, Bishara SE, Soliman MM, Oonsombat C, Laffoon JF, Warren J. The use of Ormocer as an alternative material for bonding orthodontic brackets. *Angle Orthod* 2005;75:106-108.
24. Frankenberger R, Tay FR. Self-etch *vs* etch-and-rinse adhesives: Effect of thermo-mechanical fatigue loading on marginal quality of bonded resin composite restorations. *Dent Mater* 2005;21:397-412.
25. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. *In vivo* degradation of resin-dentin bonds in humans over 1 to 3 years. *J Dent Res* 2000;79:1385-1391.
26. Van Dijken JW. Clinical evaluation of three adhesive systems in class V non-carious lesions. *Dent Mater* 2000;16:285-291.
27. Dantas DC, Ribeiro AI, Lima LH, de Lima MG, Guênes GM, Braz AK, Braz R.Influence of water storage time on the bond strength of etch-and-rinse and self-etching adhesive systems. *Braz Dent J* 2008; 19:219-223.
28. van Meerbeek B, Dhem A, Goret-Nicaise M, Braem M, Lambrechts P, Vanherle G. Comparative SEM and TEM examination of the ultra-structure of resin-dentin interdiffusion zone. *J Dent Res* 1993;72:495-501.
29. Kitasako Y, Burrow MF, Nikaido T, Tagami J. The influence of storage solution on bond durability of resin cement. *Dent Mater* 2000;16:1-6.
30. Sato M, Miyazaki M. Comparison of depth of dentin etching and resin infiltration with single step adhesive system. *J Dent* 2005;33:475-484.
31. Tagtekin DA, Yanikoglu FC, Bozkurt FO, Kologlu B, Sur H. Selected characteristics of an Ormocer and a conventional hybrid resin composite. *Dent Mater* 2004;20:487-497.
32. Oliveira SS, Pugach MK, Hilton JF, Watanabe LG, Marshall SJ, Marshall GW Jr. The influence of the dentin smear layer on adhesion: A self-etching primer *vs*. a total-etch system. *Dent Mater* 2003;19:758-767.
33. Abdalla AI, Feilzer AJ. Four-year water degradation of a total-etch and two self-etching adhesives bonded to dentin. *J Dent* 2008;36:611-617.
34. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent Mater* 2001;17:296-308.
35. Misra DN. Adsorption from solutions on synthetic hydoxyapatite: Nonaqueous *vs*. aqueous solvents. *J Biomed Mater Res* 1999;48:848-855.
36. Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. *Crit Rev Oral Biol Med* 2001;12:136-151.
37. Carrilho MRO, Carvalho RM, Tay FR, Pashley DH. Effect of storage media on mechanical properties of adhesive systems. *Am J Dent* 2004; 17:104-108.
38. Gopferich A. Mechanism of polymer degradation and erosion. *Biomaterials* 1996;17:103-117.
39. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J, Van Meerbeek B. Comparative study on adhesive performance of functional monomers. *J Dent Res* 2004;83:454-458.
40. *Interaction of Futurabond NR with Tooth Substrate*. Voco GmbH. Da/EG 040929. 2008;2.
41. Hashimoto M, Ohno H, Sano H, Tay FR, Kaga M, Kudou Y, Oguchi H, Araki Y, Kubota M. Micromorphological changes in resin-dentin bonds after 1 year of water storage. *J Biomed Mater Res* 2002;63:306-311.
42. Carrilho MR, Carvalho RM, Tay FR, Yiu C, Pashley DH. Durability of resin-dentin bonds related to water and oil storage. *Am J Dent* 2005; 18:315-319.
43. Takahashi A, Inoue S, Kawamoto C, Ominato R, Tanaka T, Sato Y, Pereira PN, Sano H. *In vivo* long-term durability of the bond to dentin using two adhesive systems. *J Adhes Dent* 2002;4:151-159.