RESEARCH ARTICLE

Three-month water degradation of resin-dentin interfaces subjected to direct and indirect exposure

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ABSTRACT

Objectives: The aim of this study was to assess the effects direct or indirect water exposure on the 3 months hydrolytic degradation of three dentin bonding agents.

Materials and Methods: The samples were divided in three groups: Clearfil SE Bond, Clearfil S3, Adper Singlebond 2; and the samples were restored with Filtek Z350. Subsequent to the restorative procedures, the specimens of each group were divided into three subgroups (immersed in water deionised): Control (24h-37ºC), Direct Water exposure DWE for 3 months (37º), Indirect water exposure (IWE-3m) with enamel margins for 3 months (37ºC). After the storage the samples were sectioned into sticks and µTBS testing (EZ test) and Scanning Electronic Microscopy assessed the failure mode. The µTBS data were statistically analysed using two-way ANOVA and Tukey's test at $\alpha=0.05\%$. The samples were processed for nanoleakage evaluation immersed in 50 wt% ammoniacal silver nitrate (24 h), rinsed and immersed in a photo-developing solution for 8 h.

Results: After 3 months CSE was the least affected by water degradation regardless the aging strategy. IWE afforded very little variation on µTBS after 3 m. Intense nanoleakage was observed with DWE groups with increases incidence of mixed failures instead

Conclusions: Bonded dentin margins are more prone to hydrolytic degradation than resin-enamel interfaces. The increased nanoleakage and the drop of bond strength showed this.

Keywords: Hydrolytic degradation, bonding, Scanning electron microscopy, dentin, enamel.

INTRODUCTION

The election of the dentin-bonding agent is indeed a critical decision as the bonding effectiveness of them reduces remarkably over time.[1] The long-lasting integrity of bonded restorations may be influenced by several factors such as thermal challenges, chewing load,[2] enzymes[3] and bacterial fluids.[4] The degradation of dentin bonds relies on the hydrolytic breakdown of collagen,[3] polymers[2] and dissolution of inorganic fillers.[5] The exposure of the resin-dentin interface to oral environment and fluids, for instance in class V and deep class II cavities would lead to faster water sorption than similar build-ups with enamel borders[1] which prevents the readily uptake of water.

Water seepage in the dentin has shown an adverse effect on durability of dentin bonding.[6,7] Hydrolytic degradation on dentin is far more active than on enamel.

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Therefore the latter substrate could provide a border protection (indirect dentin bond exposure).[1]

The classic three-step etch-and-rinse adhesives have been replaced by simplified bonding agents, which need few and easy clinical application steps. The self-etch adhesives attain more standardized application among different operators and provide less post-operative sensitivity. However, previous studies observed that the three-step adhesives cause lower permeability and lower reduction of the bond strength over time. [9,10]

Thus, the aim of this in vitro study was to assess the effects direct or indirect water exposure on the 3-month hydrolytic degradation of three dentin bonding agents by scanning electron microscopy (SEM) and microtensile bond strength. The null hypothesis to be tested is that there is no difference in the adhesives' bonding performance after 3 months under direct and indirect water exposure.

MATERIALS AND METHODS

Sample preparation

Forty five human third molars extracted for surgical reasons were utilized in this study. The teeth were stored in 0.5% chloramine/water solution at 4 °C no longer than three months after extraction. After approval of the institutional Ethics Committee (protocol 167/2009) of Piracicaba Dental School the teeth were used in the present investigation.

Deep dentin specimens with remaining tissue thickness of ~0.9 mm[11] were obtained by removing the roots 2.0 mm below cemento-enamel junction (CEJ) and the occlusal crown 2.0 mm above CEJ using a slow-speed water-cooled diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA). The dentin surface of each specimen was wet-polished with a 600-grit SiC (CarbiMet 2; Buehler) paper for 30 s to create a standard smear-layer. The specimens were thoroughly rinsed using deionised water (5 s) and immediately bonded with the tested adhesives.

Experimental design

The dentin specimens were randomly divided into three principal groups (*n*=15) based on the adhesives selected for this study: i) two-step self-etch adhesive (CSE - Clearfil SE Bond; Kuraray Medical, Tokyo, Japan); ii) one-step self-etch adhesive (CS3 - Clearfil S3; Kuraray Medical); iii) two-step etch-and-rinse adhesive (SB - Adper Singlebond 2; 3M ESPE, St. Paul, MN, USA). The composition of each dental bonding agent is detailed in Table 1.

A nano-filled resin composite (Filtek Z350; 3M ESPE) was used to perform the build-up (six layers – 1 mm each). The adhesives and each composite layer were light-cured as *per* manufacturer's recommendations using a quartz-tungsten-halogen lamp (XL-2500; 3M-ESPE).[11,12] The light intensity (600 mW/cm²) was checked using a radiometer (Optilux Radiometer Model 100; SDS Kerr, Donbury, CT, USA).

Subsequent to the restorative procedures, the specimens of each group were divided into three subgroups (*n*=5) based on the aging strategy employed in this study:

1) Control: immersion in deionised water for 24 h (37 ºC), subsequently sectioning into sticks and tested;

2) Direct water exposure (DWE-3m): sectioning into sticks and their immersion in deionised water for 3 months (37 ºC);

3) Indirect water exposure-A (IWE-3m): immersion of the bonded teeth (with enamel margins) in deionised water for 3 months (37 ºC), sectioning into sticks and µTBS testing;

Microtensile Bond Strength (µTBS)

Resin-bonded teeth were sectioned in resin-dentin sticks (0.9X0.9 mm²) suitable for the microtensile bond strength. The sticks from the most peripheral area presenting residual enamel were excluded from the test.

The sticks were glued to a jig with a cyanoacrylate gel (Super Bonder gel, Loctite, Henkel Corp., Rocky Hill, CT, USA) and tested to failure in an universal testing machine (EZ-test; Shimadzu, Kyoto, Japan) with a 500-N load cell (cross-head speed: 1.0 mm/min). The exact cross-sectional area of each tested stick was measured with a digital calliper after fracture. The µTBS results were calculated and expressed in MPa. The values obtained from the sticks of the same resin-bonded tooth were averaged and the mean bond strength was used as one unit for statistical analysis. Five resin-bonded teeth (n=5) were evaluated for each group. The µTBS data were statistically analyzed using two-way ANOVA (the dependent variables are the bonding agents and the aging regimens) and Tukey's test at α=0.05%.

Failure mode

Subsequent to the µTBS testing, the mode of failure of each fractured stick was determined using a stereomicroscope (Olympus Sz 40-50; Tokyo, Japan) at x100 magnification. The fractures were classified as adhesive, mixed, cohesive in composite or cohesive in dentin.

Nanoleakage evaluation

One central stick was selected from each bonded tooth of each subgroup (n=5) during the cutting procedure. They were processed for nanoleakage evaluation as previously described.[13,14] In brief, the

Table 1. Adhesives, chemical compositions, application procedures and lots.

Materials	Composition	Application Procedure	Lot
Clearfil S3 Bond	MDP, BisGMA, HEMA, dimethacrylates, photoinitator	Apply adhesive for 20s. Air-dry for 5s to evaporate solvent. Light cure for 10s.	127A
Clearfil SE Bond	-Primer: MDP, HEMA, water, photoinitator -Bond: MDP, BisGMA, HEMA, TEGDMA, hydrophobics dimethacrylates, photoinitator	Apply primer for 20s, gently air-dry; apply bond. Light cure for 10s.	896A 1321A
Adper Singlebond 2	-Etchant: 37% phosphoric acid -Adhesive: HEMA, BisGMA, TEGDMA, polyalkenoic acid copolymer, dimethacrylates, ethanol, water and camphorquinone	Acid-etch for 15s, rinse with water for 15s leaving the dentine moist. Bond was applied in two coats and gently air-dried. Light cure for 10s.	7KK 9WP

*BisGMA: bisphenol-A-diglycidylmethacrylate; HEMA: hydroxyethylmethacrylate; MDP: 10-methacryloyloxi-decyl-phophate; TEGDMA: triethylene-glycol-dimethacrylate;

sticks were immersed in 50 wt% ammoniacal silver nitrate $[Ag(NH_3)_2NO_3](aq)$ solution in total darkness for 24 h. Subsequently, the specimens were rinsed in H_2O to remove the excess silver nitrate and then immersed in a photo-developing solution for 8 h under UV-light (60cm from the specimens) to reduce silver ions into metallic silver grains along the resin-dentine interface. The silver-impregnated sticks were included in epoxy resin and wet-polished using #600, #1200, #2000 SiC papers and diamond pastes (Buehler) 6, 3, 1, and 0.25 µm. The specimens were ultrasonically cleaned for 20 minutes after each abrasive/polishing step. Finally, they were air-dried, dehydrated for 24h, coated with evaporated carbon and observed using a SEM (JSM-5600LV; JEOL, Tokyo, Japan) in backscattered electron mode with 10mm working distance and 15kV accelerating voltage.

RESULTS

The statistical results showed a significant interaction (p=0.009) of factors (bonding agent and aging strategy). Very few pre-test failures were obtained from each group; these values were excluded from the statistical analysis.

Mean values (and standard deviations) of µTBS outcomes are presented in Table 2. After 3 months CSE was the DBA least affected by water degradation regardless the aging strategy. Indirect water exposure (IWE) afforded very little variation on µTBS after 3 months for all the groups. IWE was statistically similar to the 24h control groups for all DBAs (p>0.05). Nevertheless, the direct water exposure of resin-dentin interfaces induced significant bonding degradation (µTBS reduction) for the simplified adhesives SB and CS3.

The failures' distribution is presented in Figure 2. The adhesive failures were more often with 3-month DWE storage than in control and IWE groups. Contrariwise, the failure modes analysis of IWE and control groups showed less adhesive fractures than the DWE groups with increased incidence of mixed failures instead.

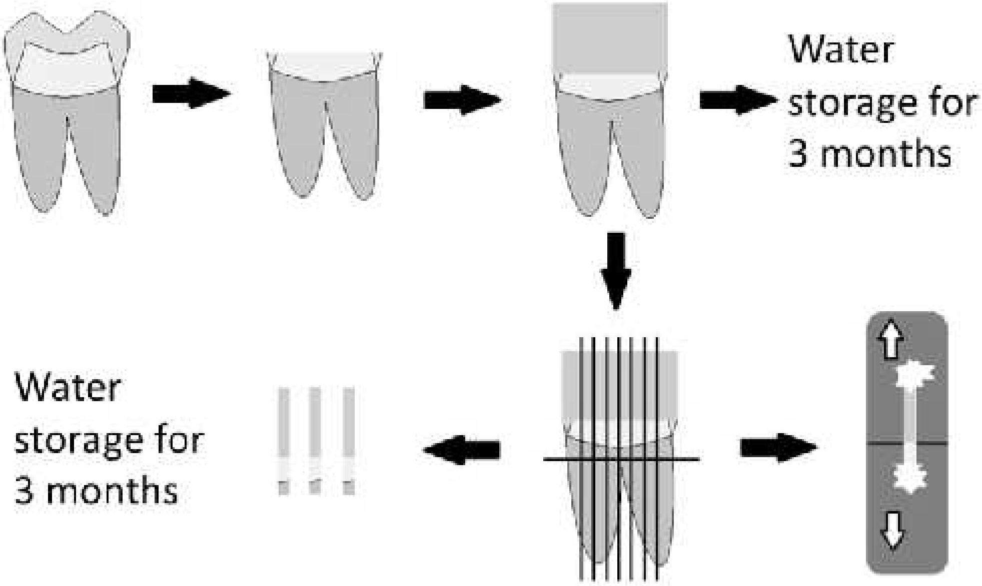

Figure 1. Schematic drawing depicting the different aging strategies after 3 months. The direct water exposure was undertaken by water immersion of resin-dentin sticks whereas the indirect water exposure was realized by the water immersion of bonded teeth.

Table 2. Means (Standard deviations) of µTBS in MPa.

DBA	24h - Control	3 months - DWE	3 months - IWE
Clearfil S3 Bond	42.2 (5.8) $^{A, a}$	33.1 (6.7) $^{B, b}$	41.6 (6.9) $^{A, a}$
Clearfil SE Bond	45.1 (7.8) $^{A, a}$	43.3 (5.9) $^{A, a}$	44.4 (7.3) $^{A, a}$
Adper Single Bond	47.7 (7.1) $^{A, a}$	38.9 (6.9) $^{B, a}$	45.7 (7.9) $^{A, a}$

Same uppercase letter represent no statistical significant difference in the row (p>0.05). Same lowercase letter represent no statistical significant difference in the column (p>0.05).

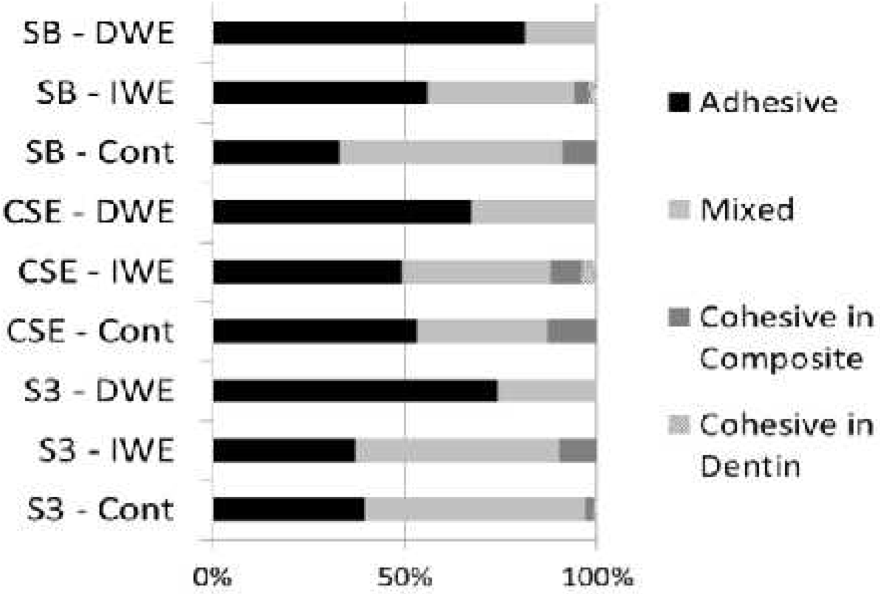

Figure 2. Overview of the failure patterns (%) attained in each group.
Mixed and adhesive failures were most frequently observed. Note that for control and indirect water exposure (IWE) groups the predominant failure pattern was mixed; meanwhile, for simulated pulpal pressure (SPP) and direct water exposure (DWE) groups the predominant pattern was adhesive.

Representative nanoleakage micrographs are shown in Figure 3. The silver uptake of control and IWE groups were very similar in spite of some water trees were found for SB (Figure 3F). Intense nanoleakage was observed with DWE for SB and CS3 with several silver deposits found within the adhesive layer (Figures 3G and 3I).

DISCUSSION

The present investigation assessed the role of bonded enamel borders on the dentin bond integrity over time. By the findings, we may observe that the more durable enamel bond act as a barrier for the hydrolytic degradation of dentin bond with bonding agents that adequately bond to enamel. Nevertheless, the direct water

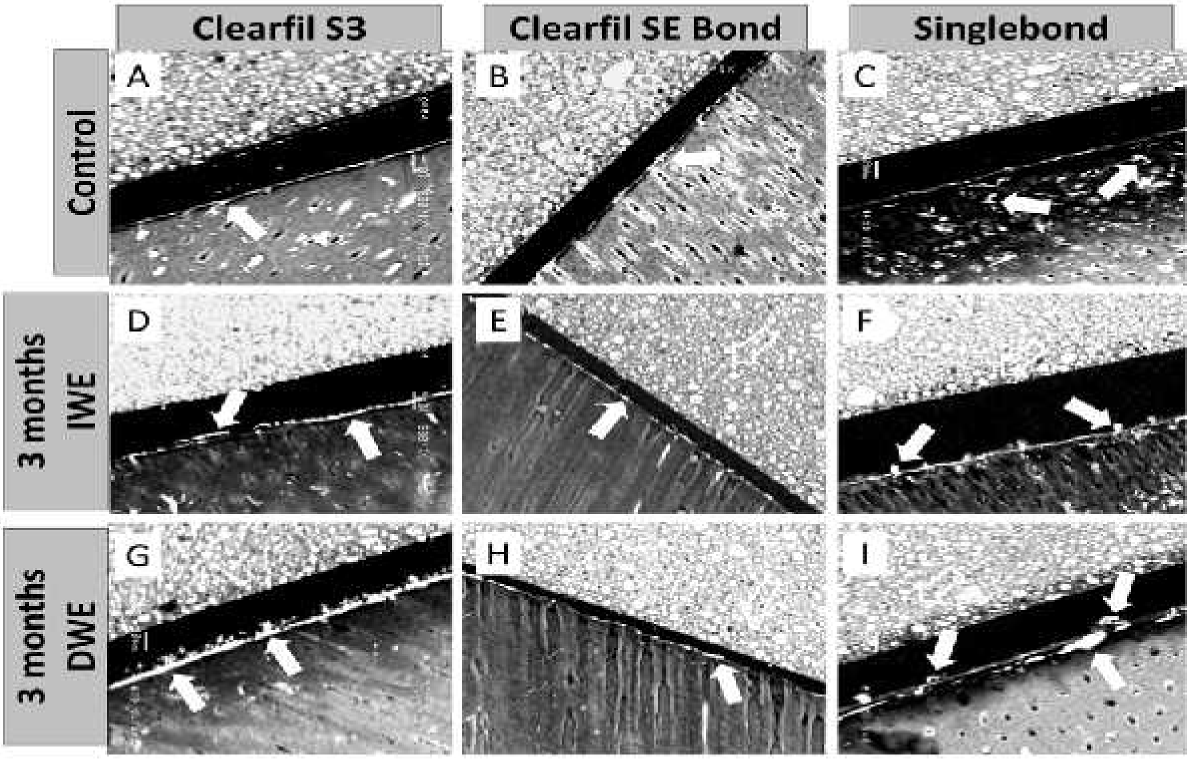

Figure 3. SEM micrographs representing the most common nanoleakage features (silver uptake). The nanoleakage was higher for direct water exposure than for indirect water storage. Clearfil SE Bond presented more resistance against silver uptake than other adhesives. Note the similarity in nanoleakage between controls (figures A, B and C) and indirect water exposure (figures D, E and F). The arrows are evidencing the overall silver deposits in all groups. Some water trees were found in Figures F, G and I for Clearfil S3 and Singlebond.

exposure of resin-dentin interfaces provided significant bonding degradation for two (one-step self-etch and two step etch-and-rinse) of the three adhesives tested and did not influence the degradation of the gold-standard two-step self-etch one (Table 2). These outcomes are in agreement with the findings of Reis et al.[1] and can be attributed to the separate application of a solvent-free hydrophobic resin present in the CSE system which reduces the final percentage of hydrophilic monomers applied in the primer solution and lead to a lower water uptake and hydrolytic degradation. Therefore, the null hypothesis must be partially rejected.

The collagen is a protein, which is denatured by the endogenous proteolytic activity of dentin undertaken by matrix metalloproteinase (MMPs) and cysteine cathepsins.[15] The MMPs are zinc and calcium dependent enzymes present in the saliva and in the dentin. [16, 17] In the hybrid layers, they are able to decrease the bond strength within few months.[18] Both enzymes (cathepsins and MMPs) are activated with the etching procedure during bonding. When they are mineral-depleted, the MMPs are preceded by the pro-MMPs activated by via cysteine-switch mechanism, and the cathepsins suffer an acceleration process due to the released glycosaminoglycans (GAGs), reaching a neutral pH in which they carry on the enzymatic catalyst process.[17]

The hydrophilic monomers also contribute to the rapid hybrid layer degradation, as they are more vulnerable to suffer the breakdown of their ester linkages,[15] also related with the water sorption. [1] This process starts with the disruption of the covalent bonds of the polymer which may be due to unreacted monomers, free-radicals and intrinsic and extrinsic water.[17] Afterwards, the releasing of the resulting oligomers creates nano-voids within the adhesive layer, leading to twofold polymer degradation along with the collagen degradation.[19] The nanoleakage affects the integrity of the hybrid layer. The water trees or spot like silver deposits are some sorts of nanoleakage pattern. This phenomenon permits the diffusion of the degraded oligomers leaving other ligands exposed to water. The nanoleakage may be correlated with the reduced of durability once it traces the water filled specimens.[20,21]

The more striking decrease in μTBS was related to the DWE storage. The hampered dentin bond integrity attained yet more adhesive failures [21,22] (Figure 2). The observation of water trees (Figure 3F) with direct water exposure may be explained by the use of hydrophilic monomers [16] such as HEMA[7] in simplified bonding agents, which induces more water sorption.[23] The separate application of a solvent-free hydrophobic resin in the CSE system decreases the degradation and the incidence of adhesive fractures failures[20] by reducing the final percentage of hydrophilic monomers applied in the primer solution.

In conclusion, the exposure of resin-dentin interfaces (i.e. in Class II or V restoratives) clinically compromises the longevity of the bonded restoratives as the bonded outer enamel margins play a critical role in reducing the dentin bond degradation for the simplified adhesives particularly. The design of the margins of the composite restorations is indeed significant for a long-lasting durability. Bonded dentin margins are more prone to hydrolytic degradation than resin-enamel interfaces. This was shown by the increased nanoleakage and the drop of bond strength.

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