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Hydrolytic degradation of the resin–dentine interface induced by the simulated pulpal pressure, direct and indirect water ageing

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ABSTRACT

Objectives: The aim of this study was to compare the hydrolytic effects induced by simulated pulpal pressure, direct or indirect water exposure within the resin–dentine interfaces created with three "simplified" resin bonding systems (RBSs).

Methods: A two-step/self-etching (CSE: Clearfil SE Bond), one-step/self-etching (S3: Clearfil S3) and etch-and-rinse/self-priming (SB: Single-bond 2) adhesives were applied onto dentine and submitted to three different prolonged (6 or 12 months) ageing strategies: (i) Simulated [illegible] Direct [illegible] tted to [illegible] survey was also performed on resin disks. Results were analysed with two-way ANOVA and Tukey's test ($p < 0.05$).

Results: The μTBS of CS3 and SB dropped significantly ($p < 0.05$) after 6 months of SPP and DWE. CSE showed a significant μTBS reduction only after 12 months of DWE ($p = 0.038$). IWE promoted no statistical change in μTBS ($p > 0.05$) and no evident change in nanoleakage. Conversely, SPP induced a clear formation of "water-trees" in CS3 and SB. WS outcomes were CS3 > SB = CSE.

Conclusion: The hydrolytic degradation of resin–dentine interfaces depend upon the type of the *in vitro* ageing strategy employed in the experimental design. Direct water exposure remains the quickest method to age the resin–dentine bonds. However, the use of SPP may better simulate the *in vivo* scenario. However, the application of a separate hydrophobic solvent-free adhesive layer may reduce the hydrolytic degradation and increase the longevity of resin–dentine interfaces created with simplified adhesives.

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1. Introduction

The resin–dentine interface is the most susceptible part of the adhesive-composite restorations to hydrolytic degradation[1] due to heterogeneity of the bonding structures and questionable stability of hydrophilic polymers contained within the composition of modern ''simplified'' resin bonding systems (RBSs).[2] Nevertheless, the *in vivo* durability of the resin–dentine interface may result superior to that estimated during *in vitro* assessments[3]; indeed, controversial outcomes are often observed in the scientific literature.[3–7] Several laboratory investigations presented remarkable degradation of resin–dentine bonds subsequent to a reasonably short-period of direct water ageing.[4,5] In contrast, further *in vivo* clinical studies performed on resin–dentine specimens created with the same RBSs previously tested *in vitro* showed a longevity of eight,[6] twelve[7] and twenty-two years.[8]

Although, many *in vitro* strategies have been employed to depict differences between adhesives and bonding techniques, some degradation regimens may submit bonds undergoing situations widely different from clinical conditions.[4,9]

The mainly accepted ageing strategy to challenge the durability of the resin–dentine bonds remains the direct exposure of match-stick or slabs in deionised water.[3,9] The water exposure of intact resin-bonded teeth, requires longer periods to contrast differences,[11] although it may resemble a more realistic clinical situation in terms of hydrolytic degradation. In contrast, the hydrolytic effect on smaller resin–dentine specimens directly exposed to water may be achieved in a relatively short period (*i.e.* 3–6 months).[10,12–14]

However, in a clinical situation, except for large class II and V cavities, resin–dentine interfaces are only partially in contact with environmental fluids, since outer resin-bonded enamel has been shown to prevent water uptake.[3,10] In such circumstances, these resin–dentine bonds may come in contact with fluids *in vivo* only *via* pulpal pressure through dentinal tubules.[15,16] Consequently, the use of the simulated pulpal pressure (20 cm H_2O) during the ageing period may be a suitable method for promoting hydrolytic degradation of resin-bonded dentine specimens *via* water seepage and polymer plasticisation.[17] Unfortunately, there is little information regarding the comparison of the hydrolytic effects induced by direct water exposure of tiny-specimens, indirect water exposure of intact bonded teeth and intact bonded-teeth submitted to simulated pulpal pressure.

This investigation aimed at comparing the influence of the simulated pulpal pressure, direct or indirect water exposure on the microtensile bond strengths (μTBS) and nanoleakage of resin–dentine specimens created using three representative simplified RBSs. The water sorption of the tested RBSs was also evaluated to discriminate the differences in the hydrolytic effects induced by the different ageing strategies.

Two null hypotheses were tested: (1) There is no difference between simulated pulpal pressure, direct and indirect water exposure in promoting hydrolytic degradation within the resin–dentine interface after a period of 6 or 12 months; (2) The three tested RBSs have similar attitude to water sorption.

2. Materials and methods

2.1. Sample preparation

One hundred five human third molars extracted for surgical reasons under approval of the institutional Ethics Committee (protocol 167/2009) were used in this study. The teeth were stored in 0.5% chloramine/water solution at 4 °C no longer than 2 months after extraction.

Deep dentine specimens with remaining tissue thickness of ~0.9 mm[18] were obtained by removing the roots 2 mm below cemento-enamel junction (CEJ) and the occlusal crown 2 mm above CEJ using a slow-speed water-cooled diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA). The pulpal tissue was removed with small surgical tweezers without altering or scratching the pre-dentine surface along the walls of the pulpal chamber. The dentine 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 RBSs.

2.2. Experimental design

The dentine specimens were randomly divided into three principal groups ($n = 35$) based on the RBSs selected for this study: (i) self-etching/two-step adhesive (CSE – Clearfil SE Bond; Kuraray Medical, Tokyo, Japan); (ii) self-etching/one-step

Table 1 – Adhesives used, batches, chemical compositions and application protocols.

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

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

adhesive (CS3 – Clearfil S3; Kuraray Medical); (iii) total-etching/self-priming adhesive (SB – Adper Singlebond 2; 3M-ESPE, St. Paul, MN, USA). The composition of each RBS is shown in Table 1.

A nanofilled resin composite (Filtek Z350; 3M ESPE) was used to perform the build-up (six layers – 1 mm each). The RBSs and each composite layer were light-cured as *per* manufacturer's recommendations using a quartz–tungsten–halogen lamp (XL-2500; 3M-ESPE) with a pulpal pressure of 0 cm H_2O.[18,19] The light intensity (>600 mW/cm^2) was checked using a photo-radiometer (Optilux Radiometer Model 100; SDS Kerr, Donbury, CT, USA).

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

1) Control: immersion in deionised water for 24 h (37 °C) and subsequently sectioned into sticks (1 mm^2);
2) Direct water exposure-A (DWE-6m): immersion in deionised water (H_2O) for 24 h (37 °C), sectioned into sticks and finally stored in H_2O for 6 months (37 °C);
3) Direct water exposure-B (DWE-12m): immersion in H_2O for 24 h (37 °C), sectioned into sticks and finally stored in water for 12 months (37 °C);
4) Indirect water exposure-A (IWE-6m): immersion in H_2O for 6 months (37 °C) and finally sectioned into sticks;
5) Indirect water exposure-B (IWE-12m): immersion in H_2O for 12 months (37 °C) and finally sectioned into sticks;
6) Simulated pulpal pressure-A (SPP-6m): submitted to 20 cm H_2O of simulated pulpal pressure, immersed in H_2O for 6 months (37 °C) and finally sectioned into sticks;
7) Simulated pulpal pressure-B (SPP-12m): submitted to 20 cm H_2O of simulated pulpal pressure and immersed in H_2O for 12 months (37 °C) and finally sectioned into sticks. Fig. 1 presents a schematic representation of the ageing strategies employed in this experimental design.

The simulation of the hydrostatic pulpal pressure was accomplished as previously described.[18] Briefly, the bonded teeth were covered with two coats of nail varnish at resin–enamel border to avoid water seepage through this margin; hence, the passage of water was possible only through dentinal tubules. The specimens were glued sideways on the lid of a cylindrical receptacle. The pulp chamber and the receptacle were filled with H_2O, sealed and turned upside down to create a 20 cm water column similarly to the classic method of SPP.[18,19]

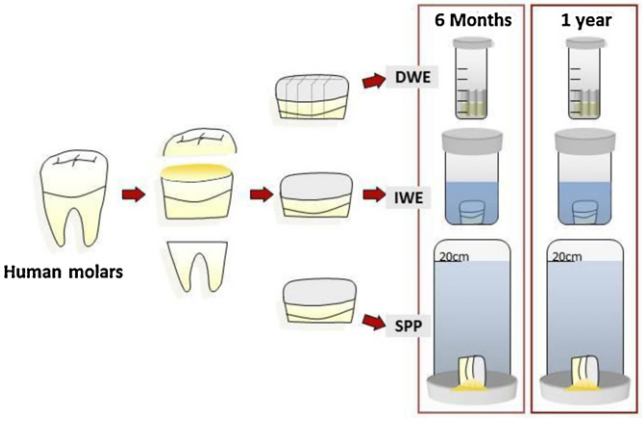

Fig. 1 – Schematic drawing depicting the different ageing strategies after 6 and 12 months. "DWE" means direct water exposure, "IWE" means indirect water exposure and "SPP" means simulated pulpal pressure.

2.3. Microtensile bond strength (μTBS)

Resin-bonded teeth were sectioned in small resin–dentine sticks (1 mm^2) suitable for the microtensile bond strength. The sticks from the most peripheral area presenting residual enamel were excluded from the test. The exact cross-sectional area of each tested stick was measured with a high precision digital calliper.

The sticks were glued to a jig with a cyanoacrylate gel (Super Bonder gel; Loctite Henkel, Rocky Hill, CT, USA) and tested in universal testing machine (EZ-test; Shimadzu, Kyoto, Japan) with a 500-N load cell (cross-head speed: 1.0 mm/min). The μTBS results were calculated and expressed in MPa. The value (MPa) attained 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 analysed using two-way ANOVA (adhesive and ageing regimen) and Tukey's test at $\alpha = 0.05\%$.

2.4. Failure mode and SEM ultra-morphological analysis

Subsequent to the μTBS testing, the mode of failure of each single fractured stick was determined using a binocular stereomicroscope at ×100 (Olympus Sz 40-50; Tokyo, Japan). Subsequently, five paired representatives fractured sticks, exhibiting the most frequently observed failure pattern and μTBS values close to the group mean (MPa) were processed for scanning electron microscopy (SEM). In brief, the fractured specimens were paired, mounted in aluminium stubs, dehydrated over night and finally. gold-coated (Balzers SCD 050 sputter coater; B.U.A., Fürstentum, Germany). The SEM ultra-morphological analysis was executed through SEM, (JSM-5600LV; JEOL, Tokyo, Japan), at 15 kV and 20 mm work distance. The fractures were classified as adhesive, mixed, cohesive in composite or cohesive in dentine.[19,20]

2.5. Nanoleakage evaluation

Two central sticks were selected from the teeth of each subgroup ($n = 14$) and processed for nanoleakage assessment as previously described.[21] In brief, the sticks were immersed in 50 wt% ammoniacal silver nitrate $[Ag(NH_3)_2]NO_3$ (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 (60 cm 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 min after each abrasive/polishing

step. Finally, they were air-dried, dehydrated over night, coated with carbon and observed using a SEM (JSM-5600LV; JEOL, Tokyo, Japan) in backscattered electron mode.

2.6. Water Sorption assessment

Adjunctive water sorption evaluation was conducted according to a protocol previously described,[22–24] following the method in ISO 4049 except for specimen dimension. For solvated adhesives (SB and S3), solvent was removed before preparing the specimens using a 3 bar air-stream for 30 min, while for the CSE, only the solvent-free bond resin was employed in this assessment. Ten disc-shaped specimens with 7 mm diameter and 1 mm thickness were prepared for each adhesive using a standard polyvinylsiloxane mould. The light-curing procedure was executed using the quartz–tungsten–halogen lamp (XL-2500; 3M-ESPE) for 120 s. The specimens were weighed on an analytical balance (JK-180: Chyo, Tokyo, Japan) every 5 min up to the stabilisation of the mass (~10 min).

The specimens were subsequently stored in a silica-containing desiccator at 37 °C and weighed after 24 h intervals up to the stabilisation of the constant mass (*M1*) (variation less than 0.2 mg in three weigh measures). To calculate de volume (*V*) of the specimens (mm^3), the thickness and diameter were measured with a digital calliper (±0.01 mm). The specimens were immersed in 1.5 mL of distilled water at 37 °C and weighed after 14 days storage (*M2*). Subsequently, the specimens were dried in the desiccator and weighed daily until a final constant mass was obtained (*M3*). Water sorption (WS) was calculated using the equation: $WS = M2 - M3/V$.[22,23] Data was statistically analysed by one-way ANOVA and Tukey's test at $\alpha = 5\%$.

3. Results

The mean (±SD) of the μTBS outcomes are shown in Fig. 2. The statistical results generated by the comparison between ageing regimens and μTBS showed a significance interaction ($p = 0.006$) (Fig. 4). Premature failures were rare and no more than one pre-test failure was attained in each group; these values were excluded from the statistical analysis.

Overall, the resin–dentine specimens of SB and CS3 groups were more affected by the hydrolytic degradation than CSE. The degradation of the resin–dentine interface induced by the IWE was not substantial in all groups (Fig. 2); no significant differences in μTBS was found in both indirect water exposure (IWE-6 months; $p = 0.93$) (IWE-1 year; $p = 0.81$) compared to the control.

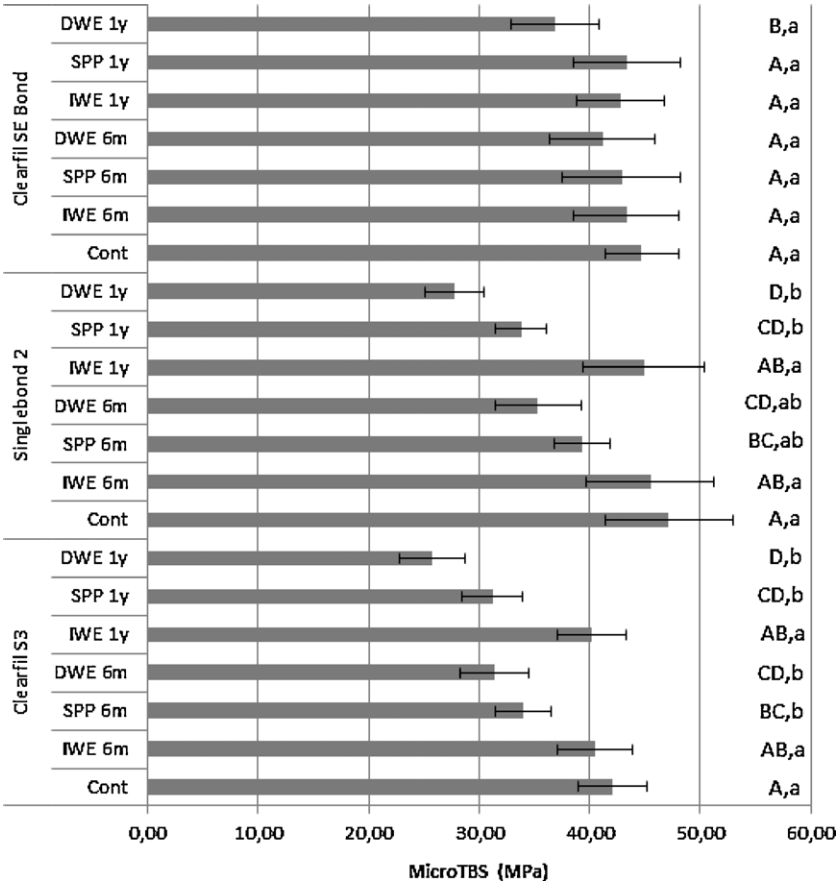

Fig. 2 – Graphic showing the outcomes of microtensile bond strength (MPa) and outcomes of two-way ANOVA and Tukey's test. Different capital letters show statistically significant difference among ageing strategies ($p < 0.05$). Different low case letters present significant difference among the bonding agents for the same ageing regime ($p < 0.05$). "Cont" = control group; "IWE" = ageing by indirect water exposure; "DWE" = ageing by direct water exposure; "SPP" = ageing under simulated pulpal pressure (20 cm H_2O).

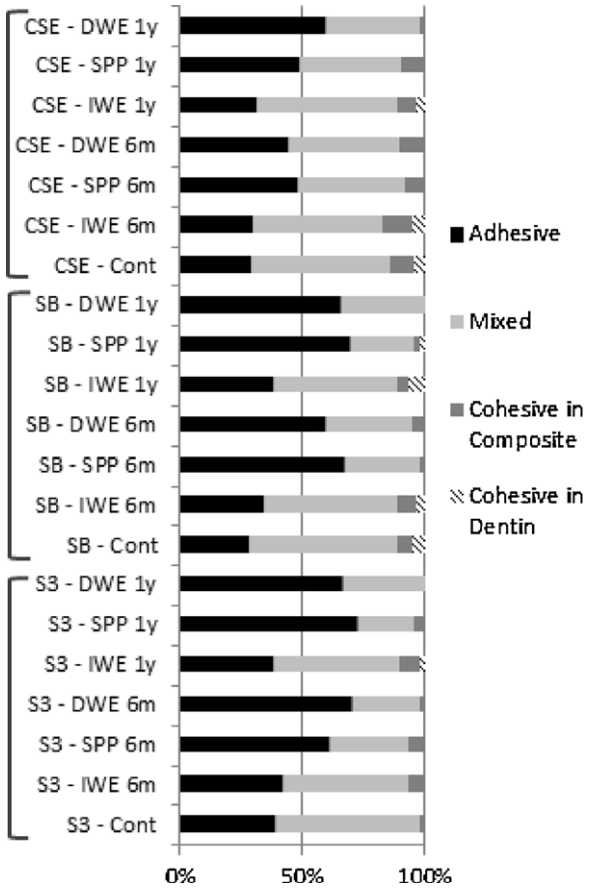

Fig. 3 – Overview of the failure patterns (%) attained in each group. Mixed and adhesive failures were most frequently observed. Note that for control and indirect water exposure (IWE) groups the predominant failure pattern was mixed; meanwhile, for simulated pulpal pressure (SPP) and direct water exposure (DWE) groups the predominant pattern was adhesive.

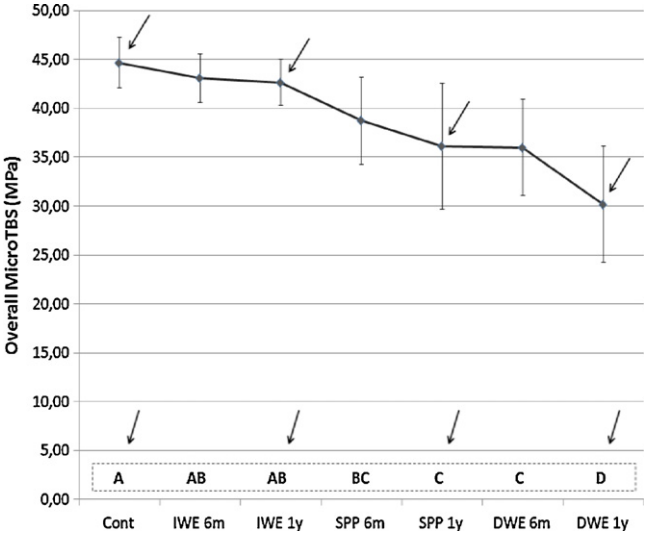

Fig. 4 – Overall averaged microtensile bond strengths (MPa) for all adhesives with standard deviations within each degradation regime, showing the progression of degradation (drop in bond strength) for each strategy. "Cont" means control group, "IWE" means ageing by indirect water exposure, "DWE" means ageing by direct water exposure and "SPP" means ageing by 20 cm H_2O simulated pulpal pressure. The statistical results are shown above the group indications at horizontal axis. Different letters indicate statistically significant difference ($p < 0.05$). Note the outcomes of ageing strategies after one year in comparison with control (arrows). Direct water exposure displayed a higher degradation rate than simulated pulpal pressure. The latter showed a higher degradation rate than indirect water exposure, which presented no difference from control.

The dentine-bonded specimens created with CSE showed a significant drop in μTBs only after 12 months of DWE ($p = 0.038$). Conversely, the μTBS results of the specimens of the groups CS3 and SB dropped significantly ($p < 0.001$) both when submitted to SPP and DWE challenge for 6 months. Significant differences ($p < 0.001$) were also found between the control and simulated pulpal pressure (SPP-6 months and SPP-1 year) and between the control and direct water exposure (DWE-6 months and DWE-1 year). However, the degradation rate of DWE was higher than SPP rate.

Analysis of the failure mode presented predominantly mixed failures (Fig. 3) for control and IWE groups (Fig. 7A1–B2). Remnants of partial cohesive fracture in resin composite were frequently observed along with partial adhesive failure (Fig. 7A1–A3). Contrariwise, with DWE and SPP the most predominant failure mode attained during the μTBS testing was adhesive (Fig. 3); some voids were created by the hydrostatic pulpal pressure (Fig. 7C1 and C2). The specimens of the DWE groups failed principally at the hybrid layer both after 6 months and 12 months (Fig. 7D1 and D2). The percentages for the failure patterns can be observed in Fig. 3.

The nanoleakage patterns observed during the SEM analysis are illustrated in Fig. 5. The silver uptake within the resin–dentine interfaces stored in water for 6 and 12 months (IWE) was similar to that observed in the control groups; except for SB which showed presence of silver deposits at the bottom of hybrid layer (Fig. 5B2 and E2). The highest silver uptake was observed after DWE for SB and S3, with intense silver deposits filling hybrid and adhesive layers. Moreover, evident water trees reaching the adhesive layer could be observed only under SPP.

Water sorption analysis showed statistical difference among adhesives ($p < 0.001$). CS3 (104.8 ± 11.2 μg/mm^3) presented higher ($p < 0.001$) water sorption than CSE (75.7 ± 3.4 μg/mm^3) and SB (76.1 ± 5.8 μg/mm^3). No difference was found between CSE and SB ($p = 0.996$). The mean water sorption for each adhesive with standard deviations is depicted in Fig. 6.

4. Discussion

The results of this study demonstrated that the three tested ageing strategies employed in this study induced remarkable differences in terms of hydrolytic degradation within the

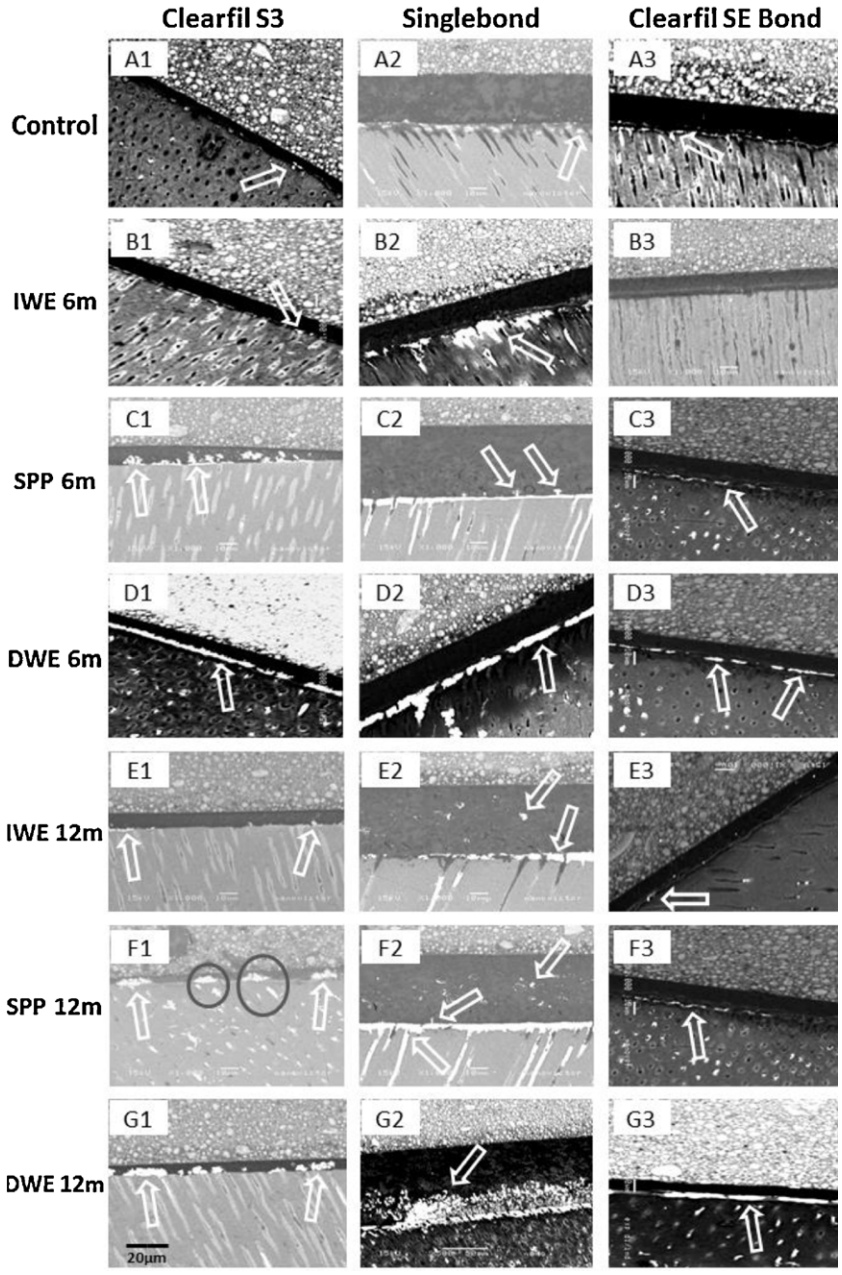

Fig. 5 – SEM micrographs representing the most common nanoleakage features (silver uptake). The nanoleakage was higher for direct water exposure than for other methods of water storage. Clearfil SE Bond presented more resistance against silver uptake than other RBSs; however, after one year of direct water exposure (G3), large silver deposits were observed. Note the similarity in nanoleakage between controls (figures A1–A3) and indirect water exposure (figures B1–B3 and E1–E3). The arrows are evidencing the overall silver deposits in all groups. Water trees were found under simulated pulpal pressure (C1, C2 and F2) for Clearfil S3 and Singlebond. Continuing silver impregnation from dentinal tubules to adhesive layers (grey circles) was observed under simulated pulpal pressure (C2, F1 and F2). Direct water exposure provided a random pattern of nanoleakage with some water trees (G1) and large silver deposits in hybrid (D1, D2 and G3) and adhesive layers (G2).

resin–dentine interfaces. Therefore, the first null hypotheses that there is no difference between simulated pulpal pressures, direct or indirect water exposure in promoting degradation within the resin–dentine interface created using simplified RBSs after a 6 or 12 months ageing period must be rejected.

The ability of simplified RBSs to absorb water plays an important role in hydrolytic degradation of resin–dentine bonds[22] as well as their bonding approach (*i.e.* self-etching or total-etching) [25] and application mode (*e.g.* one-step or multi-step). In terms of water sorption, this study has shown that CS3 had the highest ability to absorb water while, CSE and SB showed similar attitude to water sorption ($p = 0.996$). Hence, the second null hypotheses that three tested RBSs have similar attitude to water sorption must be also rejected.

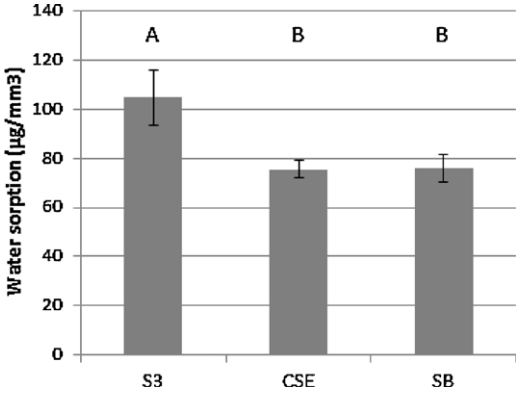

Fig. 6 – Graphic showing the outcomes of water sorption survey and outcomes of one-way ANOVA and Tukey's test. Different letters over the columns indicate statistically significant difference ($p < 0.05$). "S3" = Clearfil S3, "CSE" = Clearfil SE Bond and "SB" = Singlebond 2.

The resin–dentine specimens created using the SB and CS3 were characterised by a significant μTBS drop ($p < 0.001$) both after 6 months of DWE and SPP challenge (Fig. 1). Conversely, no significant difference was attained subsequent to indirect water exposure (IWE) both in SB ($p = 0.93$) and CS3 ($p = 0.81$). The specimens of the CSE group showed significant drop in μTBS ($p < 0.001$) only when submitted to DWE for 12 months while, the IWE caused no drop in the μTBS results ($p > 0.001$).

In terms of nanoleakage, the highest silver uptake was observed within the resin–dentine interfaces of the specimens in SB and CS3 groups after DWE. The silver deposits within the hybrid and adhesive layers appeared more intense after 12 months of DWE.

Whereas the indirect water storage (IWE: 6 and 12 months) of the specimens of the CSE and CS3 groups caused similar silver uptake compared to control groups (24 h). The specimens in the SB group showed the presence of silver deposits at the bottom of hybrid layer (Fig. 5B2 and E2) after prolonged DWE challenge (6 and 12 months) and evident water trees which propagated into the adhesive layer due to the effect of SPP (Fig. 5C2 and F2). These results were supported by the analysis of the failure mode (SEM) which showed that DWE and SPP induced predominantly an adhesive fracture (Fig. 3) characterised by the presence of several micro-porosities and droplet-like voids (Fig. 7C1 and C2).

This study has demonstrated that the main factor which affects the hydrolytic degradation rate of the resin–dentine interface and the longevity of the resin-composite restorations *in vitro* is related to the type of strategy used to age the specimens.[9] The commonly-used ageing strategy to challenge

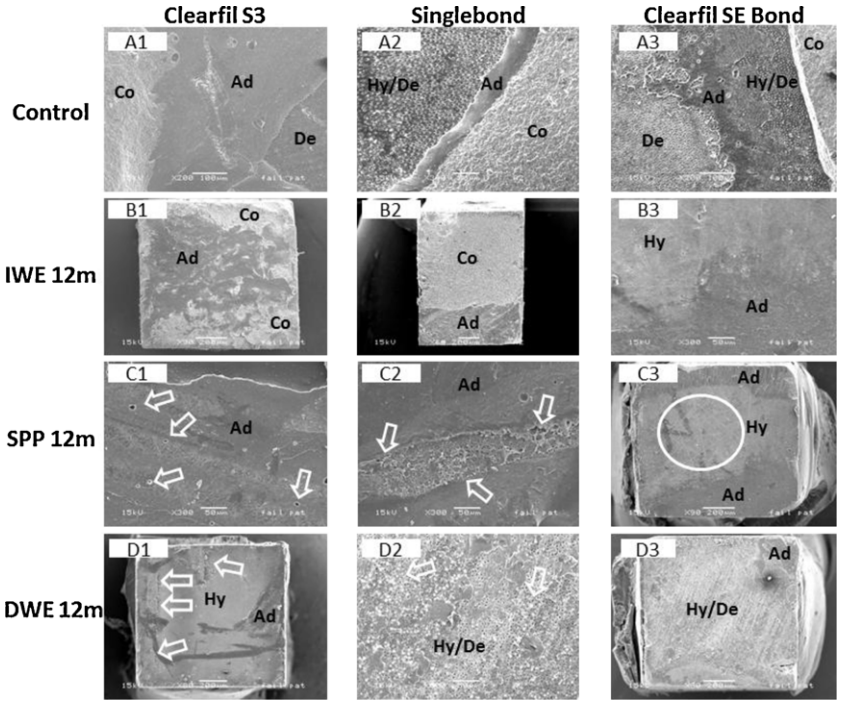

Fig. 7 – SEM micrographs representing the most common features observed in the failure pattern analysis. For control and indirect water exposure (IWE) groups, the predominant failure pattern was mixed between resin composite and adhesive/hybrid layers. This pattern may be observed in A1, A2, A3, B1 and B2. Figure B3 shows an adhesive fracture in hybrid and adhesive layers of Clearfil SE Bond. For this adhesive, this pattern was commonly seen after all ageing strategies (C3 and D3). In figure C3, the scratches of silicon carbide abrasion before bonding were incorporated to hybrid layer and could be depicted (circle). The ageing under simulated pulpal pressure (SPP) provided intense polymer degradation for Singlebond (arrows in C2) and for Clearfil S3 with the formation of voids into adhesive layer created by hydrostatic pressure (arrows in C1). Ageing by direct water exposure (DWE) led to severe polymer (arrows in D1) and collagen hydrolysis (arrows in D2) at hybrid layers created with Singlebond and Clearfil S3. Co: resin composite; Ad: adhesive layer; Hy: hybrid layer; De: dentine.

the resin–dentine bonds *in vitro* is that where tiny sticks (*i.e.* ~1 mm) or slabs (*i.e.* 1–2 mm) are subjected to direct water exposure (DWE).[9,10,12] However, this investigation have highlighted that the use of a positive simulated pulpal pressure (PP: 20 cm H_2O) may be a suitable method to challenge *in vitro* the resin–dentine in a short-term and in a more clinically realistic manner.[2,16,26] Indeed, in a clinical *in vivo* scenario, the resin–dentine interfaces may not be in direct contact with water, since bonded enamel usually acts as a protective barrier against water diffusion.[3] However, the tubular structure of dentine and the pulp blood circulation may provide constant hydrostatic water pressure at the resin–dentine interface.[16,26] In particular, this water uptake is more evident in deep dentine, which may supply excessive amounts of water to light-cured adhesives after the vasoconstrictions effect of local aesthetic solutions.[17,18] It is important to take into account that the pulpal pressure, which is under sympathetic control,[27] may increase due to other factors such as the presence of specific proteins which influence the osmotic pressure[28] and to the lymphatic vessels which are dilated in inflammed dental pulp[29] particularly with deep carious cavities.[27] Therefore, in the case of inflamed pulps especially, the pulpal pressure is high regardless the presence of anaesthetics and vasoconstrictor[27] and should be implemented during the bonding procedures.

The presence of simulated physiological pulpal pressure through hybrid and adhesive layers may provide more polymer hydrolysis and plasticisation jeopardising the long-term durability of resin resin–dentine interfaces.[2,17,18] Hence, the ageing strategy based on the use of simulated pulpal pressure (PP) is therefore considered a reliable and an effective approach to challenge the resin–dentine bonds in a more relevant clinical situation.[2,16]

Carvalho et al.[3] stated that resin–dentine bonds may degrade at a much faster rate in laboratory studies than real clinical situations. This faster degradation rate is due to DWE storage as observed in the present results (Figs. 1 and 3) as well as in previous investigations.[12–14] Conversely, an ageing strategy based on the use of PP may induce a slower bond degradation compared to DWE,[30] but faster than IWE (Fig. 3). The hydrolytic features promoted by the PP (Fig. 4) may also be encountered in previous clinical investigations.[31,32]

Further ageing strategies may be found in literature; for instance, thermocycling[33,34] and/or mechanical cycling load,[34,35] and immersion in artificial saliva[33] or in proteolytic agents to accelerate bonding degradation is short-term (some hours).[35] Moreover, the durability of resin–dentine interface is influenced by the degradation effects of specific proteolytic enzymes such as matrix-metalloproteinases (MMPs) and cathepsins.[36] This factor may contribute to the relatively short-term degradation in laboratory studies especially when immersed in saline solutions.[37]

However, the c-factor is a further important aspect to consider when undertaking experiments regarding the degradation and durability of resin–dentine interfaces as it may influence the polymerisation stress and reduction of the bond strength.[20] Moreover, high c-factors may also increase the risk for hydrolytic degradation within resin–dentine interface in presence of simulated pulpal pressure.[20] Once again, an ageing strategy based on the use of simulated pulpal pressure or indirect water exposure may be a more appropriate strategy to evaluate the real performance of resin-composite restorations *in vitro*. However, the results attained in this study showed that it is possible to realise a faster ageing of the resin–dentine interface when using the simulated pulpal pressure, although it was employed only after the bonding procedures in order to reduce the number of variables involved in the experiment and comparing the hydrolytic effects of the pulpal pressure on the resin–dentine interface to direct and indirect water exposure.

Furthermore, as the dentinal fluid is mainly constituted by water (~98 wt%) but it also contains proteins and inorganic/ionic molecules which may influence the osmotic pressure of the dentine-pulp complex, a serum fluid should be used in future studies to better simulate a clinical scenario of pulpal pressure and dentinal fluids at the bonding substrate.[38]

In conclusion, direct water exposure displayed the fastest degradation, while simulated pulpal pressure induced an intermediate degradation rate. The indirect water exposure showed a very low degradation rate. The Clearfil SE Bond presented highest degradation resistance, thus, the separate application of a hydrophobic solvent-free adhesive resin should be recommended.[39,40] However, many alternative bonding approaches have been advocated to improve the longevity performance of simplified adhesives, such as double adhesive application and/or the use of more hydrophobic bond layers.[40,41] Although these procedures had shown great improvements in bonding, they convert these simplified DBAs into multi-step adhesives. Further clinical procedures to improve the performance of 1-SEAs are: (1) agitation during RBS application[42]; (2) use of a warm air-stream[43]; (3) extended drying time to increase solvent evaporation.[43]

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