Full Length Article

Susceptibility of contemporary single-bottle self-etch dentine adhesives to intrinsic water permeation

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ABSTRACT

Objectives: To evaluate the effect of intrinsic water permeation on the microtensile bond strengths of different adhesive systems to dentine and the quality of resin-dentine interfaces.
Methods: Ninety-six non-carious human third molars were divided into 4 groups: Clearfil S³ Bond Plus (CSBP; Kuraray); Clearfil S³ Bond (C3S; Kuraray); iBond Self-Etch (IB; Heraeus-Kulzer) and Prime & Bond NT (PB, control etch-and-rinse adhesive, Dentply-Sirona). For each adhesive, specimens from one subgroup (N = 10) were bonded using zero pulpal pressure, while specimens from the other subgroup (N = 10) were bonded using 15 cm water pressure (PP). Each bonded tooth was sectioned into 1 × 1 mm sticks and stressed to failure. Data were analysed using two-way ANOVA and Holm-Sidak pairwise comparisons to examine the effects of "adhesive", "pulpal pressure" and their interaction on bond strength (α = 0.05). Representative fractured sticks were examined by SEM. The remaining tooth slabs in each subgroup were used for TEM and CLSM.
Results: Microtensile bond strengths (mean ± SD; in MPa) were: 33.4 ± 6.9 (CSBP), 33.2 ± 4.7 (CSBP-PP), 35.0 ± 8.6 (C3S), 25.5 ± 7.3 (C3S-PP), 18.4 ± 4.0 (IB), 16.5 ± 6.9 (IB-PP), 28.2 ± 5.5 (PB), 20.5 ± 7.2 (PB-PP). "Adhesive-type" (P < 0.001), "pulpal-pressure" (P < 0.001) and their interactions (P < 0.001) significantly affected bond strength results. No difference between no-PP and PP subgroups was found for CSBP and IB (P > 0.05). Water droplets were identified along the resin-dentine interface for IB, IB-PP and C3S-PP.
Conclusion: IB exhibits water sensitivity when bonding is performed with/without pulpal pressure. C3S exhibits water sensitivity when bonding is performed with pulpal pressure. CSBP does not exhibit water sensitivity when bonding is performed with/without pulpal pressure.

Clinical significance: Intrinsic water permeation during bonding procedures significantly affects bond strength results and the resin-dentine interface of contemporary single-bottle self-etch dentine adhesive systems.

1. Introduction

Water is the most common substance that exists in nature [1]. It has been coined the "universal solvent" because water is capable of dissolving more substances than any other liquid [2]. Because of this property, water is responsible for infiltration and enlargement of cracks in concrete blocks [3], decomposition of wood [4] and initiating oxidation of iron-containing substances [5,6]. Water is also the major component of the dentinal fluid [7–9]. Free and bound water constitutes approximately 10% of the total weight of mineralised dentine

[10]. When dentine is demineralised with 32–37% phosphoric acid, apatite crystallites are completely dissolved along the top 5–10 μm of the mineralised dentine, with the spaces replaced by free water. In demineralised dentine, unbound (free water) constitutes 75–79% of the total water, while the remaining 21–25% is bound water [11]. Although it is necessary to replace the unbound water with adhesive components during dentine bonding, replenishment of the intrinsic moisture lost by evaporation by pulp pressure often results in water transudation along the dentine-adhesive interface [12].

Water plays an antagonistic role in dentine bonding. In dentine

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demineralised by strong acids, water keeps the collagen matrix suspended and fully expanded by preventing the formation of interpeptide hydrogen bonds between adjacent collagen fibrils [13]. Water is an intrinsic component of self-etch adhesives and keeps acidic resin monomers in their ionised states for demineralisation of the smear layer and the underlying intact dentine [14,15]. Although water is initially necessary for dentine bonding, excess water that is not removed during the polymerisation of an adhesive compromises the integrity of the resin-dentine interface [16]. When polar solvents are evaporated from adhesives without incorporating 2-hydroxylethyl methacrylate as a co-solvent, water-insoluble dimethacrylate resin monomers undergo phase separate into hydrophobic-rich domains when they come into contact with residual water; this results in an adhesive layer with non-uniform swelling/water-sorption characteristics and mechanical properties [17]. Excessive moisture adversely affects solvent evaporation [18] and adhesive resin polymerisation [19]. Water provides a medium for enzymes to function. Hydrolysis of the resinous and collagenous components of the hybrid layer results in the degradation of resin-dentine bonds [20,21], which, in turn, compromises the clinical longevity of resin composite restorations [22]. With the advent of newer single-bottle self-etch adhesive systems, it is important for clinicians to appreciate whether bonded resin-dentine interfaces created by these new systems are less susceptible to intrinsic water permeation.

Accordingly, the objective of the present study was to identify whether intrinsic water permeation affects the microtensile bond strengths and the quality of resin-dentine interfaces using recently commericialized single-bottle one-step self-etching and compared with the results achieved with a time-tested etchand-rinse adhesive. The null hypothesis tested was that bonding in the absence or presence of simulated pulpal pressure has no effect on the bond strength and the quality of the resin-dentine interface for the adhesives systems examined.

2. Materials and methods

Ninety-six recently extracted, non-carious human third molars were collected after receiving the patients' informed consent under a protocol approved by the Augusta University Human Assurance Committee. These teeth were stored at 4 °C in 0.9% sodium chloride solution supplemented with 0.02% sodium azide to prevent bacteria growth. The teeth were divided into four groups and randomly assigned to one of the four groups:

Group I: Clearfil S[3] Bond Plus (Kuraray Noritake Dental Inc., Tokyo, Japan), a single-bottle, one-step, self-etching adhesive;

Group II: Clearfil S[3] Bond (Kuraray Noritake Dental Inc.), a single-bottle, one-step, self-etching adhesive;

Group III: iBond Self Etch (Heraeus-Kulzer, Hanau, Germany); a single-bottle, one-step, self-etching adhesive; and

Group IV: Prime & Bond NT (Caulk Division, Dentsply Sirona, York, PA, USA), a two-step etch-and-rinse adhesive.

The composition of these adhesives and their application procedures are listed in Table 1. Each group was subdivided into two equal subgroups of 10 teeth each. Specimens in first subgroup were bonded using zero pulpal pressure. Specimens in second subgroup were bonded using 15 cm water pressure. For each tooth, a flat coronal dentine surface was prepared perpendicular to the longitudinal axis of the tooth using a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) in the presence of water-cooling. The dentine surface was wet-polished with 320 grit silicon carbide paper for 1 min to create standardized smear layer. A second section was made 1 mm below the cementoenamel junction to create a crown segment containing the top of the pulp chamber. The distance between the pulp horn and dentin surface was measured to closest 0.01 mm, and specimens with dentin thickness less than 1 mm thick were discarded. The cut root surface of the crown segment was attached to a 14 × 14 × 5 mm piece of polymethyl methacrylate using a cyanoacrylate glue (Zapit, Dental Ventures of American, Corona, CA, USA). The polymethyl methacrylate plate was penetrated by an 18-gauge stainless steel tubing that ended flush with the upper surface of the plate.

After filling the pulp chamber with isotonic saline, a 40 cm length of polyethylene tubing was attached *via* the 18-gauge tubing to a syringe barrel containing isotonic saline. The height of the column of isotonic saline was adjusted to 15 cm above the exposed dentine surface. This arrangement enabled one to apply simulated physiological pulpal pressure to dentine during bonding (Fig. 1). For the no pulpal pressure subgroup, the polyethylene tubing was clamped to deliver a simulated pulpal pressure of 0 cm water pressure. Each tooth was bonded with one of the four adhesives by following meticulously the instructions supplied by the respective manufacturer. After evaporation of the adhesive solvent, each adhesive was polymerised for 20 s using a quartz-tungsten-halogen light curing unit with an output intensity of 600 mW/cm^2. This was followed by incremental placement of two 2-mm thick layers of a universal nano-hybrid resin composite (Clearfil Majesty Esthetic, Kuraray Noritake Dental Inc.) that were light-cured separately for 40 s each.

The additional 16 teeth were used for transmission electron microscopy (TEM). The teeth in each subgroup (N = 2) were bonded in the manner described previously using the respective adhesive. Clearfil Protect Liner F (Kuraray Noritake Dental Inc.), a flowable resin composite with nanoscopical silica fillers, was used for buildup so that specimens could be sectioned without damaging the diamond knife by chunky inorganic fillers that are usually incorporated in hybrid resin composites.

2.1. Microtensile bond testing

After storage in deionised water at 37 °C for 24 h, each tooth was vertically sectioned into 1 mm-thick serial slabs using the Isomet saw with water cooling. The two central slabs were sectioned into 1 × 1 (± 0.2) mm wide sticks, each containing the adhesive joint in the centre of the stick. The two longest sticks with an average dentin thickness of 2–2.5 mm from each slab were randomly selected for tensile testing, yielding 4 sticks per tooth. Thus, the specimens for microtensile testing in each subgroup comprised 4 sticks × 10 teeth = 40 sticks to enable valid statistical comparisons to be performed [23].

The "non-trimming" version of the microtensile technique was employed for bond strength evaluation [24]. Each stick was secured *via* cyanoacrylate glue to a testing jig and stressed to failure under tension using a universal tester (Vitrodyne V1000, Liveco Inc., Burlington, VT, USA) at a cross-head speed of 1 mm/min. After bond testing, the two ends of the fractured stick were removed and examined under a surgical microscope to determine the mode of failure. Failure modes were classified as adhesive failure (*i.e.* failure along the adhesive interface), mixed failure (*i.e.* failure within the adhesive joint and with attached resin composite or dentine) or cohesive failure (*i.e.* failure within the resin composite or dentine). Beams that failed prematurely during specimen preparation were not included in the statistical analysis.

The mean strength of the four beams in each tooth was used to compile the mean microtensile bond strength for that tooth. Analysis was performed using tooth number as the statistical unit (N = 10 teeth). Because the normality (Shapiro-Wilk test) and equal variance assumptions (modified Levene test) of the tensile strength data appeared to be violated, the data were logrithymically transformed to enable a two-factor analysis of variance to be performed. The analysis was used to determine the effects of "adhesive" and "pulpal pressure" and the effect of the interaction of those two factors on the microtensile bond strength results. All pairwise comparisons were performed using the Holm-Sidak procedure. Statistical significance was set at α = 0.05.

Table 1
Adhesives used in the present study.

Adhesive	Classification	Composition	Application
Clearfil S³ Bond Plus	single-bottle one-step self-etching adhesive	MDP, HEMA, BisGMA, dimethacrylates, water, ethanol, new photoinitiator, NaF, CQ, SiO₂	Apply for 10 s; dry with mild air pressure. Air-thin the adhesive surface with mild air pressure for more than 5 s until the liquid no longer moves. Light-cure for 10 s.
Cleariл S³ Bond	single-bottle one-step self-etching adhesive	MDP, HEMA, BisGMA, water ethanol, photo-initiator, CQ, SiO₂	Apply for 20 s; dry with strong air pressure; light-cure for 10 s.
iBond Self Etch	single-bottle one-step self-etching adhesive	UDMA, 4-MET, glutaraldehyde, acetone, water, stabiliser, photoinitiator	Apply 3 consecutive times, rub for 30 s, air-dry until adhesive stops moving, then thoroughly air-dry for 5 s. Light-cure for 20 s.
Prime & Bond NT (PB) (Caulk/ Dentsply, Milford, DE, USA)	two-step etch-and-rinse adhesive	Etchant: 34% phosphoric acid Adhesive: PENTA, BisGMA, TEGDMA, acetone, SiO₂, CQ	Etch 15 s, rinse 10 s, leave dentine moist, apply adhesive for 20 s, evaporate solvent for 5 s, light-cure for 10 s.

Abbreviations: 4-MET: 4-methacryloyloxyethyl trimellitic acid; BisGMA: bis-phenyl A diglycidyl methacrylate; CQ: d,l-camphorquinone; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxy-decyl-dihydrogen-phosphate; PENTA: dipentaerythritol penta acrylate monophosphate; SiO₂: silica nanoparticles; TEGDMA: triethylene glycol dimethacrylate; UDMA: urethane dimethacrylate.
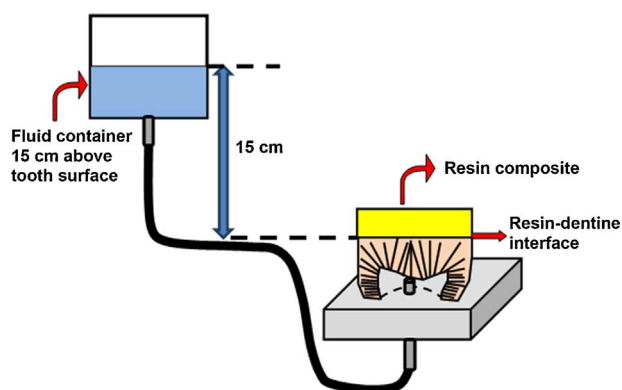

Fig. 1. Schematic of bonding to coronal dentine using simulated physiologic intrapulpal pressure and specimen preparation for the non-trimming version of the microtensile bond testing procedure.

2.2. Scanning electron microscopy of fractured interfaces

The dentine side of four fracture beams from each subgroup with either adhesive or mixed failures and with bond strengths that are closest to the mean bond strength of the respective subgroup were selected for detailed fractographic analysis using scanning electron microscopy (SEM). The specimens were mounted on aluminum stubs, coated with gold/palladium and examined with a field emission scanning electron microscope (XL-30 FEG; Philips, Eindhoven, The Netherlands) operated at 10 KeV.

2.3. Transmission electron microscopy of resin-dentine interfaces

Two 1 mm-thick slabs derived from the bonded teeth designated for transmission electron microscopy were examined for each of the eight subgroups. Each slab was completely demineralised in formic acid-sodium formate (pH 2). After demineralisation of the bonded slabs for 7 days at 25 °C, the specimens were fixed in Karnovsky's fixative (2.5 wt % glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L cacodylate buffer, pH 7.3), rinsed in 0.1 mol/L sodium cacodylate buffer and post-fixed with 1% osmium tetroxide. The slabs were progressively dehydrated through an ascending ethanol series (50–100%). The absolute ethanol was replaced with propylene oxide as a transitional medium. Specimens were subsequently embedded in pure epoxy resin. Ninety nanometre-thick sections were prepared and collected with single-slot carbon- and formvar-coated copper grids, stained with 2% uranyl acetate and Reynold's lead citrate and examined using a JEM-1230 TEM (JEOL, Tokyo, Japan) at 110 kV.

2.4. Confocal laser scanning microscopy of resin-dentine interfaces

Two remaining tooth slabs from each subgroup were used for confocal laser scanning microscopy (CLSM). Each non-demineralised tooth slab was ultrasonicated for 5 min and immersed in 0.1 wt% rhodamine B (MilliporeSigma, St. Louis, MO, USA) dissolved in phosphate-buffered saline (pH 7.4). After 24 h, the dye-infiltrated slabs were rinsed with deionised water and examined using a CLSM (LSM 510 META; Carl Zeiss, Thornwood, NY) that was coupled with a helium neon gas laser (80% of 543 nm excitation, 1.2 mW). Images were captured at 5 μm beneath the polished surface to avoid superficial specimen preparation artefacts.

2.5. Tracer-infused water-rich zones within the resin-dentine interfaces

Six additional non-demineralised tooth slabs from each adhesive subgroup were wet-polished with 2000-grit silicon carbide paper and coated with nail varnish applied 1 mm from the bonded interface. The slabs were immersed in 50 wt% ammoniacal silver nitrate solution (pH 9.5) for 24 h The tracer-infused slabs were immersed in a photo-developing solution for 8 h under a fluorescent light to convert the tracer into metallic silver. Each slab was then gently wet-polished with 1200-grit silicon carbide papers to remove the silver-rich surface layer.

For SEM examination, three tooth slabs from each subgroup were air-dried, coated with gold-palladium and examined with the XL-30 SEM at 30 kV. Imaging was performed using a combination of 70% backscattered electron mode and 30% secondary electron mode. The other three slabs from each subgroup were processed using the TEM embedding protocol described in Section 2.3. After section, the 90 nm thick sections were examined unstained using the JEM-1230 TEM at 110 kV.

3. Results

Microtensile bond strength data of the 8 subgroups derived from the four adhesives are shown in Table 2. Two-factor analysis of variance of the bond strength data indicated that the effect of the factor "adhesive" on microtensile bond strength was highly significant ($p < 0.001$). The effect of "pulpal pressure" on microtensile bond strength was highly significant ($p < 0.001$). The interaction of these two factors was also highly significant ($p < 0.001$).

Table 2
Summary of microtensile bond strength data.

Adhesive	Without pulpal pressure[a],[*]	With pulpal pressure[a],[**]
Clearfil S^3 Bond Plus	33.4 ± 6.9 ^{A,¶}	33.2 ± 4.7 ^{1,¶}
Clearfil S^3 Bond	35.0 ± 8.6 ^{A}	25.5 ± 7.3 ^{2}
iBond Self Etch	18.4 ± 4. ^{C,¶}	16.5 ± 6.9 ^{4,¶}
Prime & Bond NT	28.2 ± 5.5 ^{B}	20.5 ± 7.2 ^{3}

For each adhesive, subgroups denoted by "¶" indicate no statistical significance between bonding in the absence or presence of simulated intrapulpal pressure.

[a] Values represent mean ± standard deviation (in MPa).

[*] For adhesives that were bonded in the absence of pulpal pressure, subgroups with different upper case letter superscripts are significantly different (p < 0.05).

[**] For adhesives that were bonded in the presence of pulpal pressure, those with different low case letter superscripts are significantly different (p < 0.05).

Pairwise comparisons of the adhesives bonded without pulpal pressure showed that the microtensile bond strength of Clearfil S^3 Bond Plus was not significantly different from that of Clearfil S^3 Bond (p > 0.05). Both of these adhesives exhibited significantly higher bond strength that Prime & Bond NT, which in turn, had a higher bond strength than that exhibited by iBond Self Etch (p < 0.05). Pairwise comparisons of the adhesives bonded in the presence of simulated intrapulpal pressure showed that the microtensile bond strengths of the four adhesives were, in descending order, Clearfil S^3 Bond Plus > Clearfil S^3 Bond > Prime & Bond NT > iBond Self Etch (p < 0.05).

For Clearfil S^3 Bond Plus, there was no difference in the bond strength of the adhesive in the absence or presence of simulated intrapulpal pressure (p > 0.05). A similar result was identified for iBond Self Etch (p > 0.05) although the bond strength of this adhesive for the two subgroups were significantly lower than those of Clearfil S^3 Bond Plus. For both Clearfil S^3 Bond and Prime and Bond NT, significantly higher microtensile bond strengths were observed when those adhesives were bonded in the absence of simulated pulpal pressure (p < 0.05).

Distribution of failure modes identified by examination with the surgical microscope is presented in Fig. 2. For Clearfil S^3 Bond Plus, Clearfil S^3 Bond and Prime & Bond NT, the predominant failure mode was mixed failure, irrespective of whether bonding was performed in the absence or presence of simulated intrapulpal pressure. For iBond Self Etch bonded in the absence of pulpal pressure, the predominant failure mode was also mixed failure. However, in the presence of simulated intrapulpal pressure, the predominant mode of failure for iBond Self Etch was adhesive failure along the surface of dentine.

Fractographic examination of representative specimens that were stressed to failure under tension is collective represented in Fig. 3. Of all the specimens examined, adhesive failure was only served in the specimens bonded with iBond Self-Etch under simulated pulpal pressure. Nevertheless, voids in the fracture adhesive, probably representing

entrapped water droplets, could be identified along the fracture adhesive layer of Clearfil S^3 Bond, iBond Self Etch and Prime & Bond NT specimens that were bonded with simulated pulpal pressure.

Transmission electron microscopy of stained specimens depicted the thickness of the hybrid layers created by the self-etching adhesives an the control etch-and-rinse adhesive (Fig. 4). Based on the thickness of the hybrid layers identified in Clearfil S^3 Bond Plus, Clearfil S^3 Bond and iBond Self Etch, these new adhesives may be classified as mildly aggressive self-etching adhesives [25]. Voids, probably representing entrapped water droplets, could be identified above the hybrid layers of Clearfil S^3 Bond, iBond Self Etch and Prime & Bond NT specimens that were bonded with simulated pulpal pressure (Fig. 4B–D, right column).

Representative confocal laser scanning microscopy images of the rhodamine-stained specimens are collectively represented in Fig. 5. Similar to the TEM images, water-rich zones could be identified within the resin-dentin interface of Clearfil S^3 Bond, iBond Self Etch and Prime & Bond NT specimens that were bonded with simulated pulpal pressure (Fig. 5B–D, right column), as well as in iBond Self Etch specimens bonded without the application of simulated pulpal pressure (Fig. 5C, left column). Water-rich zones were absent in specimens bonded using Clearfil S^3 Bond Plus, irrespective of whether they were bonded with or without pulpal pressure (Fig. 5A).

Nanoleakage within the resin-dentine interface of the bonded specimens could be identified with both SEM (Fig. 6) and TEM (Fig. 7) after infusion of silver nitrate and its subsequent reduction to metallic silver. Practically speaking, none of the specimens was completely devoid of nanoleakage within the hybrid layer. At the TEM level, water trees, which provide a record of water movement during adhesive application and polymerisation [16], could be identified in the resin-dentine interface of specimens bonded by the three self-etch adhesives, even when bonding was performed in the absence of pulpal pressure. For specimens bonded with of Clearfil S^3 Bond, iBond Self Etch or Prime & Bond NT, additional silver-filled, roughly spherical water droplets could be seen in specimens bonded under simulated pulpal pressure.

4. Discussion

Previous studies indicated that both single-bottle etch-and-rinse adhesives and one-step self-etching adhesives behave as permeable membranes after polymerisation due to the high osmolality and increase in hydrophilic components of these adhesives, and the lack of a relative impermeable hydrophobic layer to protect these adhesives from water transudation from the dental pulp during adhesive polymerisation [26,27]. Even in the absence of simulated pulpal pressure, water transudation from the tubular orifices is ultrastructurally manifested as water channels (*i.e.* water trees) along the adhesive-dentine interface. When polymerisation is delayed, some of the water permeates the adhesive to form water droplets between the resin composite and the adhesive. Entrapment of water droplets along the dentine-adhesive interface and the adhesive-composite interface results in the incorporation of stress raisers along a bonded interface [28]. This results in lowering of the microtensile bond strength when these specimens are under stress. Water transudation across these adhesives is considerably more severe when bonding is performed under simulated pulpal pressure [22,29]. Hence, the null hypothesis that "bonding in the absence or presence of simulated pulpal pressure has no effect on the bond strength and the quality of the resin-dentine interface for the adhesives systems examined" has to be rejected.

The results of the present study indicate that with the exception of the Clearfil S^3 Bond Plus self-etching adhesive and the iBond Self Etch, the microtensile bond strength of the other adhesives decreased significantly when bonding was performed under simulated pulpal pressure. Although the microtensile bond strength of iBond Self Etch did not decline when bonding was performed under simulated pulpal pressure, one has to exercise caution in interpreting the results. This is because
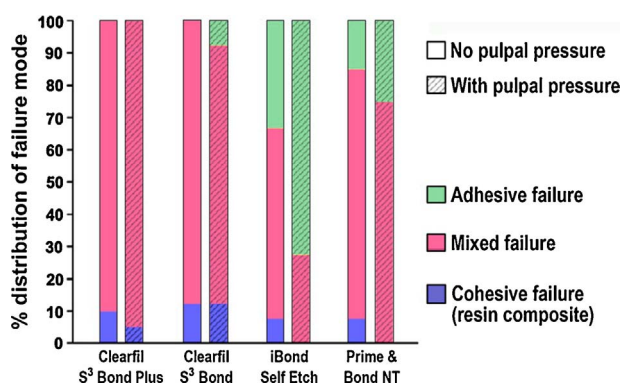

Fig. 2. Distribution of failure modes in the eight adhesive subgroups.

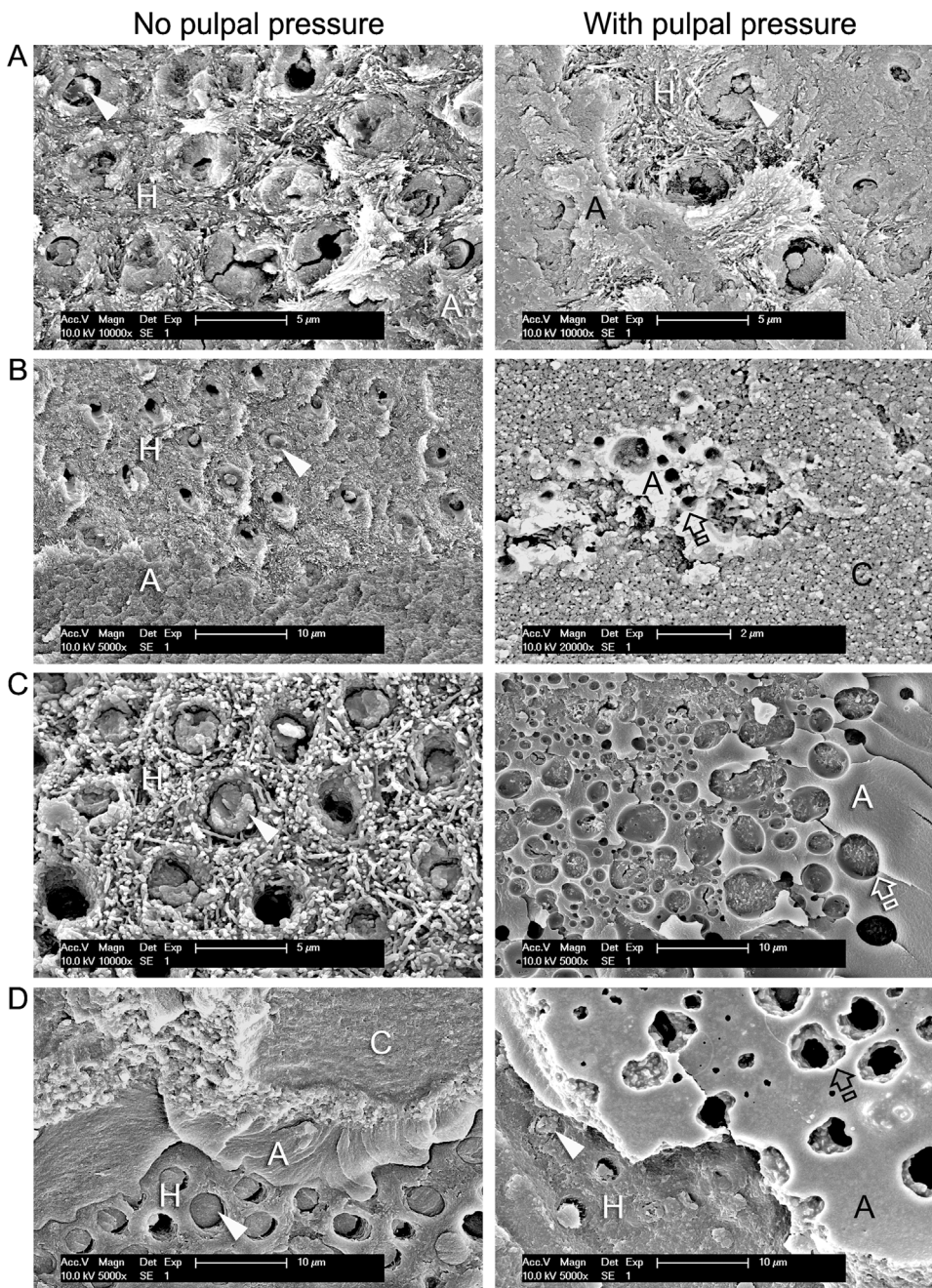No pulpal pressure   With pulpal pressure

Fig. 3. Scanning electron microscopy images of adhesive and mixed failure modes along the resin-dentine interface of specimens that had been stressed to failure under tension. *Left column*: specimens bonded without pulpal pressure. *Right column*: specimens bonded with the application of 15 cm water pressure. **A.** Clearfil S³ Bond Plus. **B.** Clearfil S³ Bond. **C.** iBond Self Etch. **D.** Prime & Bond NT. Abbreviations – H: hybrid layer; A: adhesive; C: resin composite; Solid arrowhead: fractured resin tags within dentinal tubules; Open arrow: voids within the adhesive that probably represent water trapped within the uncured adhesive prior to polymerisation.

when bonding was performed without pulpal pressure, the bond strength of iBond Self Etch was already significantly lower than the other three adhesives (Table 2). Although no statistical analysis was performed on the failure modes, there is a tendency for more adhesive failure to occur in all adhesives with the exception of Clearfil S³ Bond Plus (Fig. 2).

When the microtensile bond strength data was examined together with the results of fractographic analysis, it became apparent that the decrease in microtensile bond strength associated with bonding of Clearfil S³ Bond and Prime & Bond NT under simulated pulpal pressure was caused by the presence of water-induced voids along the resin-dentine interface. For iBond Self Etch, fractographic analysis revealed that the adhesive was very susceptible to water transudation even in the absence of simulated pulpal pressure during bonding. This may be seen by the presence of innumerous water-induced voids along the resin-dentine interface, particularly in those specimens that exhibited adhesive failure. When bonding of iBond Self Etch was performed under

the influence of simulated pulpal pressure, every fractured interface demonstrated the presence of those water-induced voids. Thus, the absence of significant difference in the iBond Self-Etch subgroups is not due to the insensitivity of that adhesive to water transudation. On the contrary, iBond Self Etch is extremely sensitive to water transudation even in the absence of simulated pulpal pressure during bonding.

The SEM fractographic results are further supported by TEM and CLSM imaging of the resin-dentine interfaces, as well as the SEM and TEM nanoleakage results. In terms of aggressiveness, the three self-etching adhesives examined may be classified as mild or ultra-mild based on their self-etching potential. Depending on the thickness of the original smear layer, these adhesives produced very thin hybrid layers ranging from 0.5 to 1 μm thick irrespective of whether simulated pulpal pressure was employed during bonding. The etch-and-rinse adhesive Prime and Bond NT produced a 4–6 μm thick hybrid layer when phosphoric acid was used as the dentine etchant, with complete dissolution of the smear layer and the smear plugs. Even for this time-
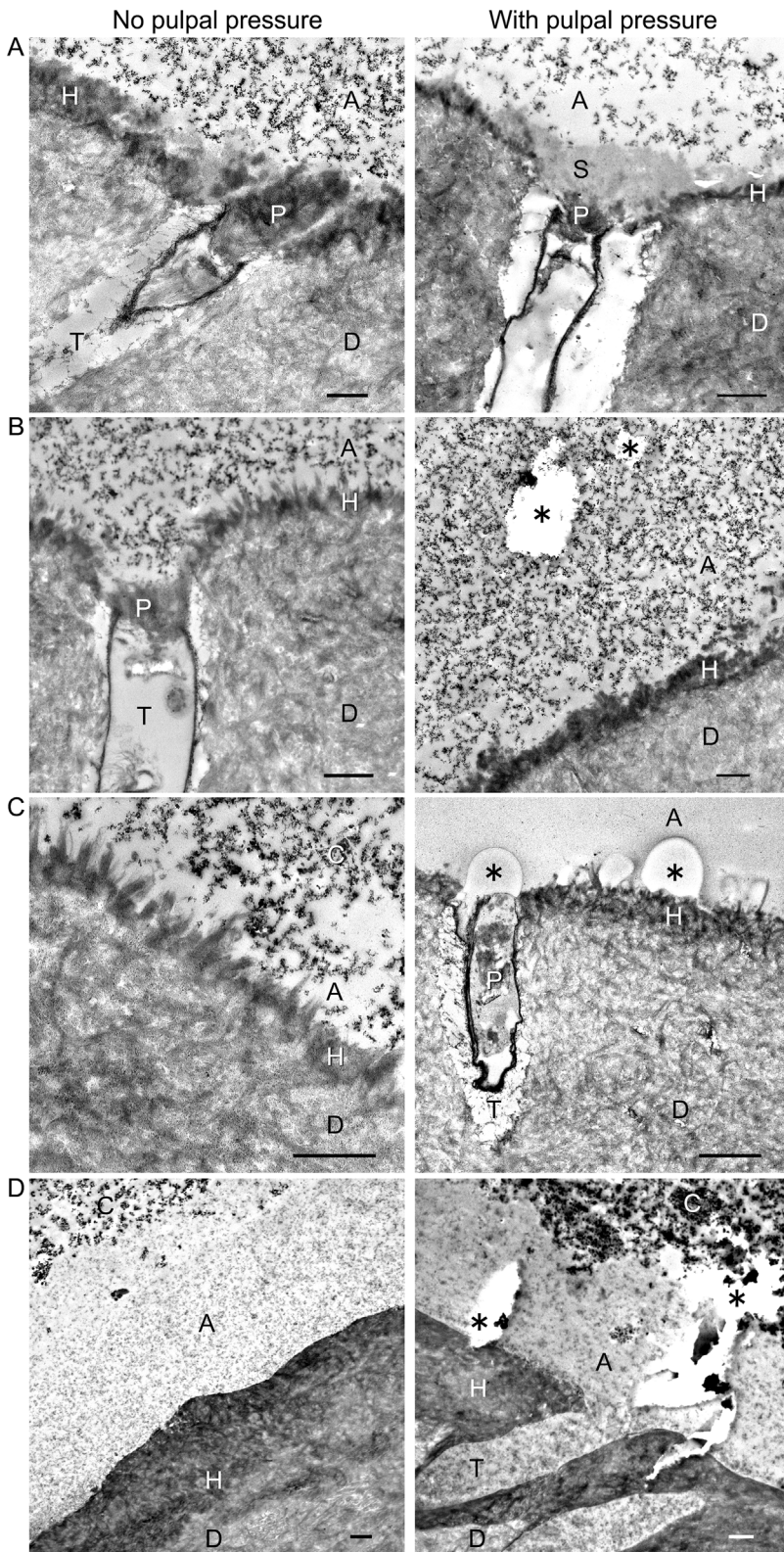
Fig. 4. Transmission electron microscopy inages of the resin-dentine interface in completely demineralised and stained intact bonded specimens. *Left column*: specimens bonded without pulpal pressure. *Right column*: specimens bonded with the application of 15 cm water pressure. **A**. Clearfil S³ Bond Plus. **B**. Clearfil S³ Bond. **C**. iBond Self Etch. **D**. Prime & Bond NT. Scale bars = 1 μm. Abbreviations – H: hybrid layer; A: adhesive; C: resin composite; D: intertubular dentine; T: dentinal tubule; S: smear layer remnants; P: smear plug; Asterisk: voids within the adhesive that probably represent water trapped within the uncured adhesive prior to polymerisation.

tested etch-and-rinse adhesive, decrease in bond strength and deterioration of the quality of the resin-dentine interface occurred when bonding was performed using simulated pulpal pressure. With the exception of Clearfil S³ Bond Plus, all the other three adhesives exhibited evidence of water entrapment within the resin-dentin interface when bonding was performed under simulated pulpal pressure.

The adhesion-decalcification concept suggests that chemical adhesion of a self-etching acidic resin monomer to an aptatite-containing substrate is considerably more stable when the calcium salt produced by that resin monomer is less soluble in water [30]. Inoue et al. reported that an adhesive containing the functional monomer 10-methacryloyloxy-decyl-dihydrogen-phosphate (10-MDP), which forms insoluble calcium salts with hydroxyapatite, showed no signs of degradation in bond strength and interfacial ultrastructure [31].
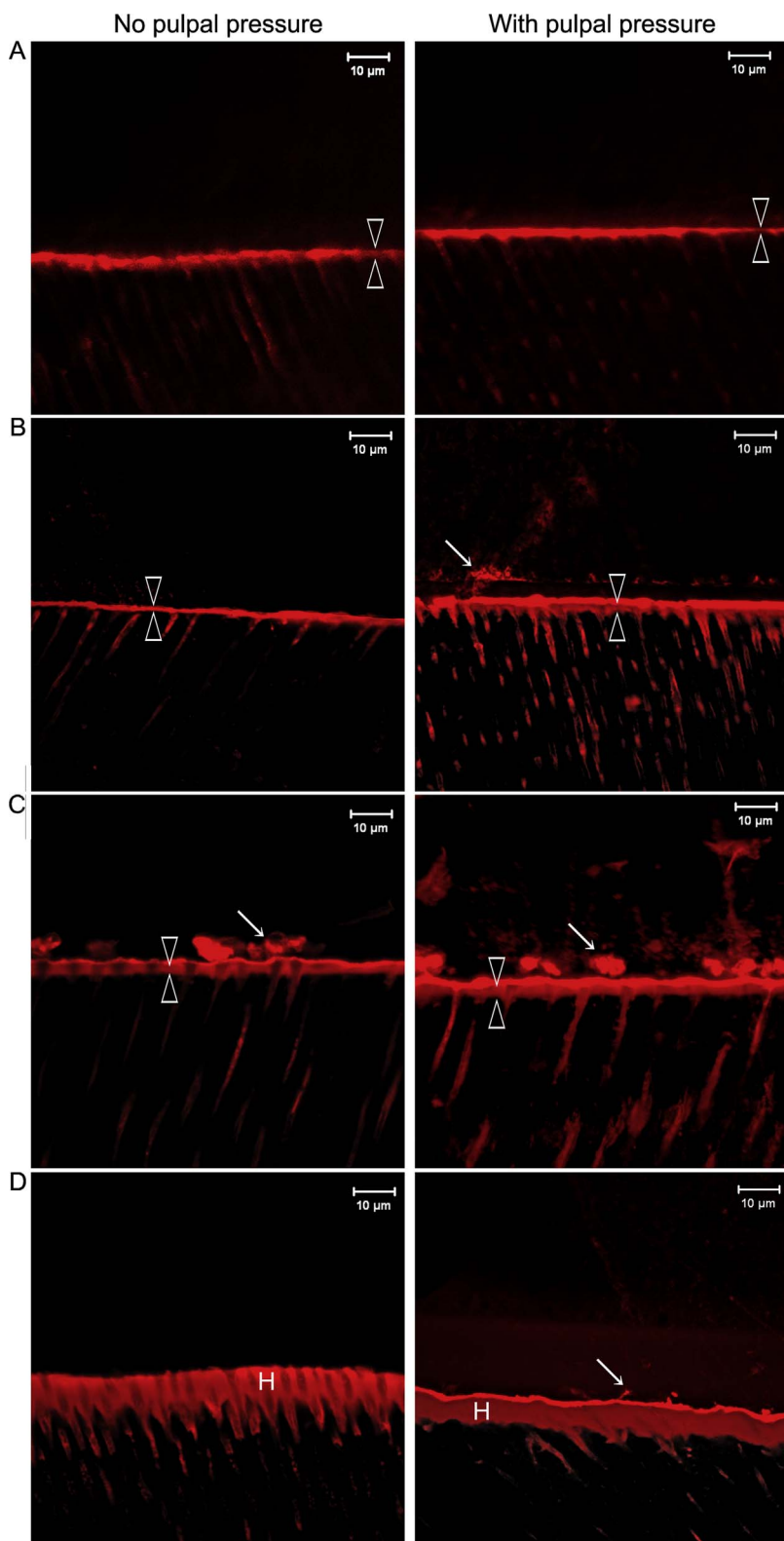
Fig. 5. Rhodamine-stained confocal laser scanning microscopy images of the resin-dentine interface in non-demineralised bonded specimens. *Left column*: specimens bonded without pulpal pressure. *Right column*: specimens bonded with the application of 15 cm water pressure. **A.** Clearfil S[3] Bond Plus. **B.** Clearfil S[3] Bond. **C.** iBond Self Etch. **D.** Prime & Bond NT. Scale bars = 10 µm. Abbreviations: Between open arrowheads: hybrid layer in self-etch adhesive; H: hybrid layer in etch-and-rinse adhesive; Arrows: voids within the adhesive and along the hybrid layer surface that probably represent water trapped within the uncured adhesive prior to polymerisation.

However, the results of the present work indicate that Clearfil S[3] Bond is adversely affected by intrinsic water permeation, while Clearfil S[3] Bond Plus is not. Because both adhesives contain 10-MDP, it is likely that other components of an adhesive system can influence the susceptibility of the adhesive to intrinsic water permeation. The photo-initiator incorporated in Clearfil S[3] Bond Plus is presumably more hydrophilic, enabling a better degree of conversion of the adhesive resin

monomers [32]. This probably results in the production of a denser polymer matrix during polymerization that resists fluid movement. Nevertheless, more research on the long-term performance of Clearfil S[3] Bond Plus is warranted.

It is rather surprising that the susceptibility of contemporary self-etch adhesive systems to intrinsic water permeation has not improved much since the report of the water-tree phenomenon in 2005 [16]. Self-
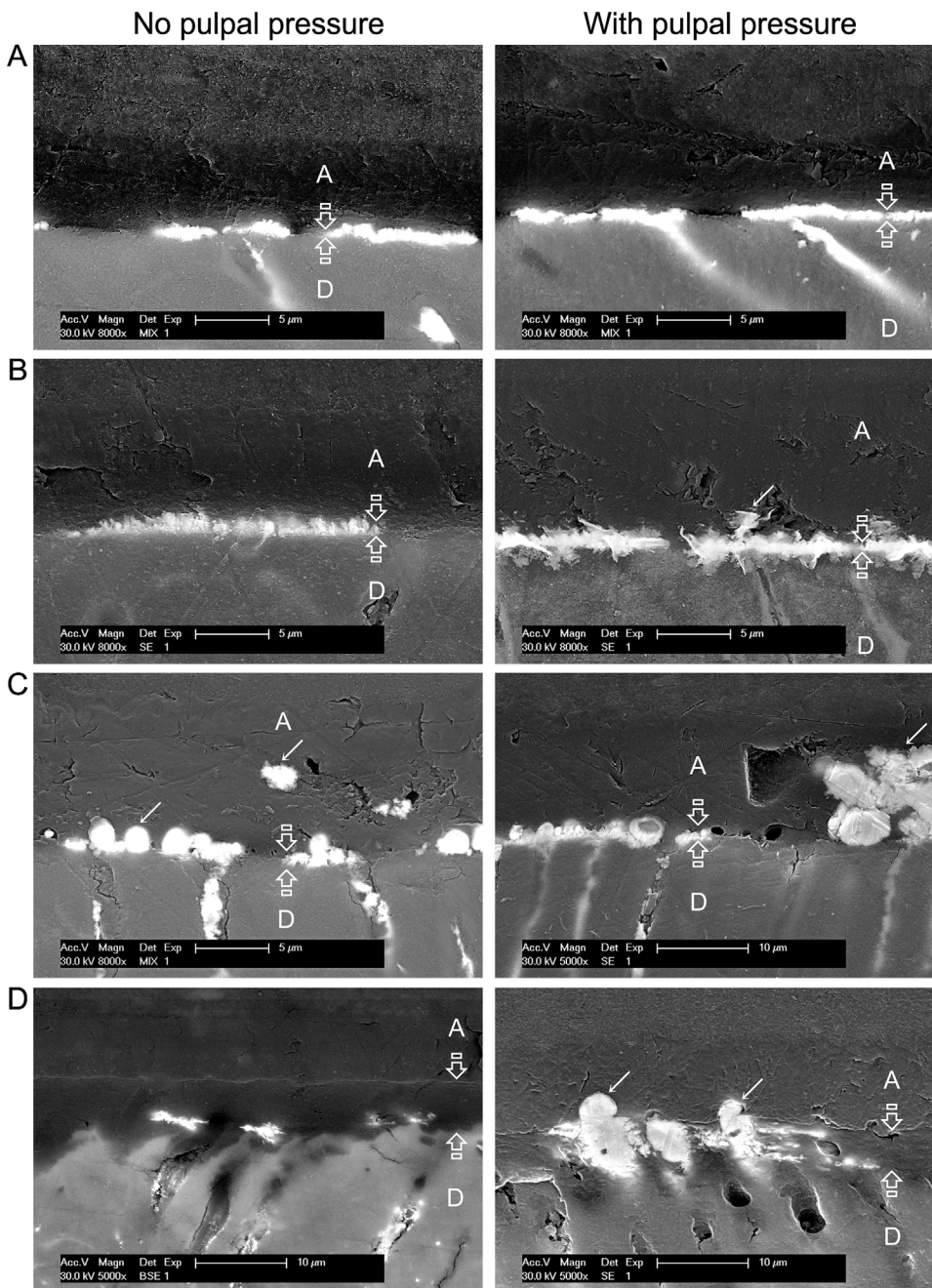
Fig. 6. Scanning electron microscopy images of silver-infused water-rich zones within the resin-dentine interface of non-demineralised bonded specimens. *Left column*: specimens bonded without pulpal pressure. *Right column*: specimens bonded with the application of 15 cm water pressure. **A.** Clearfil S^3 Bond Plus. **B.** Clearfil S^3 Bond. **C.** iBond Self Etch. **D.** Prime & Bond NT. Abbreviations – A: adhesive; D: intertubular dentine; between open arrows: hybrid layers; Arrows: voids within the adhesive and along the hybrid layer surface that probably represent water trapped within the uncured adhesive prior to polymerisation. Similar to the nanoleakage within the hybrid layer; these voids are impregnated with ammoniacal silver nitrate and were reduced to silver. The less extensive "water-trees" along the surface of the hybrid layer are less clearly depicted by scanning electron microscopy.

etch adhesives that produce acceptable bonding minimal waiting time [33] and with the incorporation of photoinitiators that are more compatible with the resin monomer polymerisation in a wet bonding environment [32] may perform better against the simulated pulp pressure challenge. In future studies, one may consider retention of the smear plugs with the use of the new chelate-and-rinse concept [34] to reduce transudation of water or dentinal fluid from dentinal tubules, as well as eliminate water movement through the resin-dentine interface during adhesive application. Using this chelate-and-rinse extrafibrillar calcium chelation technique, collagen fibrils with retained intrafibrillar minerals will not collapse upon air-drying [34]. This may also help eliminate the problems associated with the use of phosphoric acid for etching dentine during water-wet bonding of etch-and-rinse adhesives. These problems include suboptimal polymerisation of resin monomer components or phase separation of adhesive resin monomers during polymerisation in a wet environment [35].

5. Conclusion

Within the limits of the present *in vitro* study, it may be concluded that intrinsic water permeation during bonding procedures significantly affects bond strength results and the resin-dentine interface of contemporary single-bottle self-etch dentine adhesive systems. iBond Self Etch exhibits water sensitivity when bonding is performed with or without pulpal pressure. Clearfil S^3 Bond exhibits water sensitivity when bonding is performed with pulpal pressure. By comparison, Clearfil S^3 Bond Plus exhibits the least sensitivity to intrinsic water permeation when bonding is performed with or without pulpal pressure.

Acknowledgements
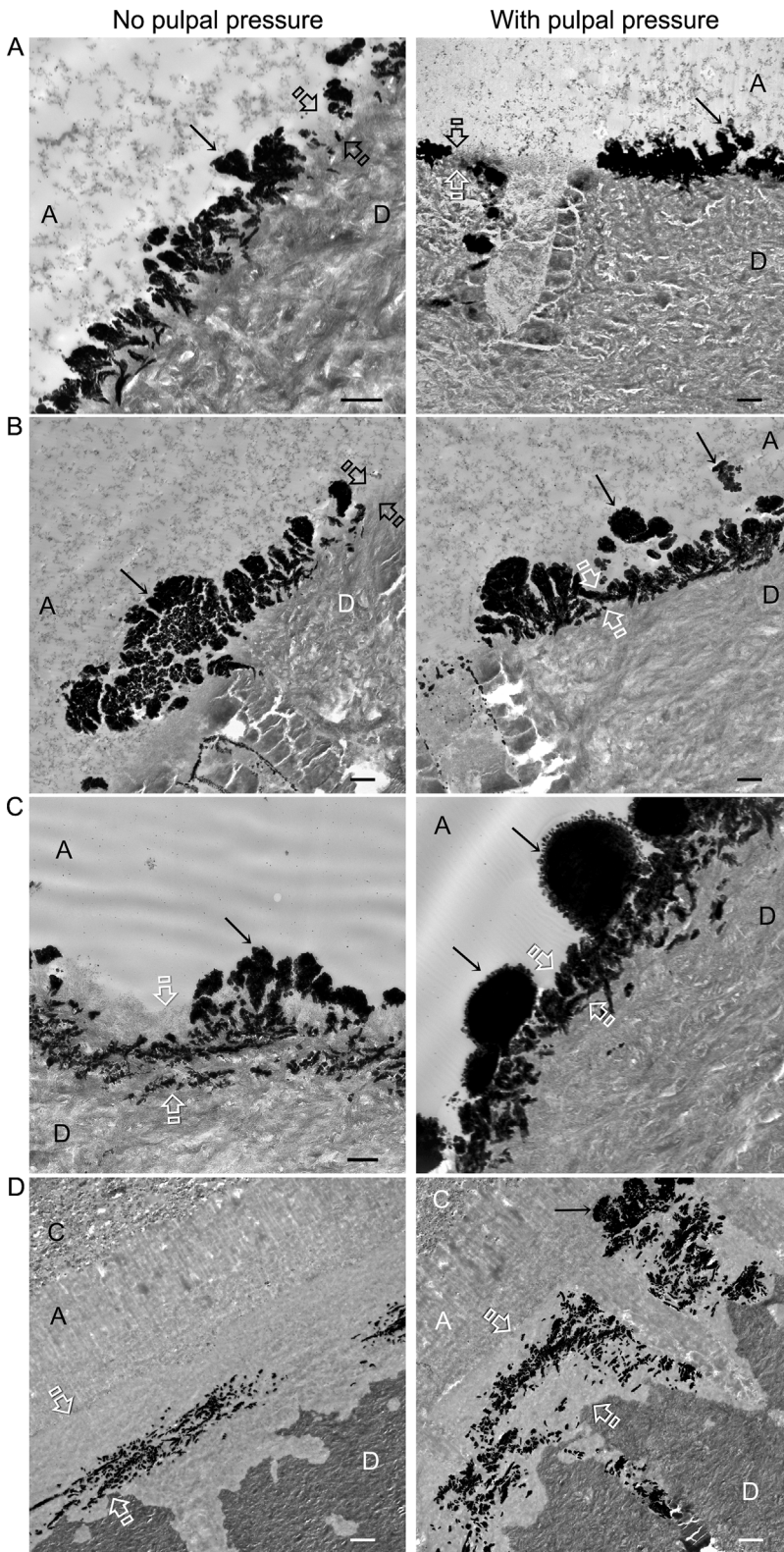
No pulpal pressure   With pulpal pressure

Fig. 7. Transmission electron microscopy images of silver-infused water-rich zones within the resin-dentine interface of non-demineralised bonded specimens. *Left column*: specimens bonded without pulpal pressure. *Right column*: specimens bonded with the application of 15 cm water pressure. **A**. Clearfil S³ Bond Plus. **B**. Clearfil S³ Bond. **C**. iBond Self Etch. **D**. Prime & Bond NT. Scale bars: 500 nm (**A–C**) and 2 μm (**D**). Abbreviations: Between open arrows: hybrid layer; A: adhesive; C; resin composite; D: intertubular dentine; Arrows: silver-impregnated "water trees" and more extensive water droplets trapped within the adhesive later prior to polymerisation of the adhesive.

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