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Defying ageing: An expectation for dentine bonding with universal adhesives?

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ABSTRACT

Objectives: The present study evaluated the long-term dentine bonding effectiveness of five universal adhesives in etch-and-rinse or self-etch mode after 12 months of water-ageing.
Methods: The adhesives evaluated included All-Bond Universal, Clearfil Universal Bond, Futurabond U Prime&Bond Elect and Scotchbond Universal. Microtensile bond strength and transmission electron microscopy of the resin-dentine interfaces created in human coronal dentine were examined after 24 h or 12 months.
Results: Microtensile bond strength were significantly affected by bonding strategy (etch-and-rinse *vs* self-etch) and ageing (24 h *vs* 12 months). All subgroups showed significantly decreased bond strength after ageing except for Prime&Bond Elect and Scotchbond Universal used in self-etch mode. All five adhesives employed in etch-and-rinse mode exhibited ultrastructural features characteristic of collagen degradation and resin hydrolysis. A previously-unobserved inside-out collagen degradation pattern was identified in hybrid layers created by 10-MDP containing adhesives (All-Bond Universal, Scotchbond Universal and Clearfil Universal Bond) in the etch-and-rinse mode, producing partially degraded collagen fibrils with intact periphery and a hollow core. In the self-etch mode, all adhesives except for Prime&Bond Elect exhibited degradation of the collagen fibrils along the thin hybrid layers. The three 10-MDP containing universal adhesives did not protect surface collagen fibrils from degradation when bonding was performed in the self-etch mode.
Conclusions: Despite the adjunctive conclusion that bonds created by universal adhesives in the self-etch bonding mode are more resistant to decline in bond strength when compared with those bonds created using the etch-and-rinse mode, bonds created by universal adhesives are generally incapable of defying ageing.

1. Introduction

Since the dawn of human civilisation, the quest for defying ageing to remain forever young has been a recurrent motif in literary, metaphysical and scientific pursuits [1–5]. With the advent of contemporary consumerism, this motif is evident not only in the ubiquity of cosmetics and enhancing surgery, but is also expressed in everyday examples of social and behavioural endeavours, from diets and exercises to stem cell rejuvenation therapy [6]. In the grand scheme of things, dentine bonding has evolved from the era of emerging technology trigger, through the stage of inflated expectations and trough of disillusionment, to the slope of enlightenment [7,8], wherein the ability to "defy ageing and remain forever young (or at least more durable)" has transcended from being a metaphor, to become the spur for research scientists to continue the legacy initiated by Dr. Michael Buonocore 60 years ago [9].

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The ultimate goal of bonding with dentine adhesives is to achieve long-term seal of the tooth-restorative interface. Although the progress in dentine bonding technology over the past six decades has been phenomenal, durable adhesion to tooth structures in the oral environment faces both physical and chemical challenges, with detrimental changes being amplified upon ageing of the bonds [10–12]. As a vital and dynamic substrate, the high organic and water content of dentine impose colossal challenges to stable long-term adhesion. Dentine exhibits variability in structural and chemical composition that may be further altered by age of the subject and the pathological condition of the teeth [13].

Universal adhesives are the latest innovation marketed by dental manufacturers for bonding of dental materials to tooth substrates [14]. These multimodal adhesives may be used in etch-and-rinse mode, self-etch mode or selective-etch mode, depending on the clinician's preference. They may also be used for bonding to indirect resin composite restorations. Some of these adhesives also incorporate monomer components that enable them to bond to zirconia and silica-based ceramics [15]. Some manufacturers have incorporated 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) into their adhesives as a means of chemical bonding to apatite via nanolayering of 10-MDP-calcium salts [16]. However, what is notably missing from the composition of most of these adhesives are elements that have been considered successful for prevention of bond degradation [17,18]. Although recent studies reported that universal adhesives applied using either the etch-and-rinse or the self-etch mode produces excellent immediate bond strength to various bonding substrates [19–22], limited information is available on the durability of bonds produced by these adhesives on dentine.

Accordingly, the objective of the present study was to evaluate the long-term bonding effectiveness of five universal adhesives bonded to human coronal dentine in the etch-and-rinse mode or self-etch mode after 12 months of water-ageing. Three 10-MDP-containing and two 10-MDP-free universal adhesives were included in the study. The null hypotheses tested were: (1) water-ageing has no effect on the microtensile bond strengths of universal adhesives to dentine when used in the etch-and-rinse mode; (2) water-ageing has no effect on the microtensile bond strengths of universal adhesives to dentine when used in the self-etch mode.

2. Materials and methods

Two hundred non-carious human third molars were collected based on a protocol approved by the Human Assurance Committee of the Georgia Regents University. The teeth were stored at 4 °C in

Table 1
Universal adhesives: composition and application procedures for the two dentine bonding modes.

Adhesive	pH	Composition	Self-etch strategy	Etch-and-rinse strategy
All-Bond Universal (Bisco Inc.)	3.2	2-HEMA, 10-MDP, Bis-GMA, ethanol, water, initiators,	1. Apply two separate coats of adhesive with agitation for 10–15 s per coat 2. Evaporate solvent by thorough air-drying for at least 10 s. No visible movement of adhesive. 3. Surface should have a uniform glossy appearance. If not, repeat steps 1 and 2 4. Light cure for 10 s	1. Etch for 15 s 2. Rinse thoroughly 3. Remove excess water by blotting surface with an absorbent pellet or high volume evacuation for 1–2 s, leaving the preparation visibly moist 4. Apply adhesive as for the self-etch mode
Clearfil Universal Bond (Kuraray Noritake Dental Inc.)	2.3	2-HEMA, 10-MDP, Bis-GMA, hydrophilic aliphatic dimethacrylate, colloidal silica, dl-camphorquinone, silane coupling agent, ethanol, water, accelerators, initiators	1. Apply adhesive and rub it in for 10 s 2. Dry the entire cavity wall by blowing mild air for more than 5 s until adhesive shows no movement. Use a vacuum aspirator to prevent the adhesive from scattering 3. Light cure for 10 s	1. Apply phosphoric acid etching gel for 15 s, then rinse and dry 2. Apply adhesive as for the self-etch mode
Futurabond U (Voco GmbH)	2.3	2-HEMA, Bis-GMA, HEMA, acidic adhesive monomer, urethane dimethacrylate, catalyst, silica nanoparticles, ethanol	1. Activate single dose adhesive package 2. Apply adhesive to the cavity surface using the Voco Single Tim brush and rub adhesive in for 20 s 3. Dry adhesive with dry, oil-free air for at least 5 s 4. Light cure for 10 s	1. Etch dentine using phosphoric acid for 15 s 2. Aspirate acid, rinse with water for 15 s 3. Remove excess moisture with a gentle stream of air to produce a silky matte surface. Do not overdry 4. 4. Apply adhesive as for the self-etch mode
Prime&Bond Elect (Dentsply Caulk)	2.5	Mono-, di- and trimethacrylate resins, PENTA, diketone, stabilisers organic phosphine oxide, cetylamine hydrofluoride, acetone, water, self-cure activator	1. Apply generous amounts of adhesive to thoroughly wet all tooth surfaces 2. Agitate applied adhesive for 20 s. Re-apply to coat preparation for the entire 20 s period 3. Remove excess solvent by gentle drying with clean, dry air for at least 5 s 4. Light cure for 10 s	1. Apply etchant for 15 s 2. Rinse thoroughly for at least 15 s 3. Remove rinsing water completely by blowing gently with air syringe or by blot-drying with cotton pellet 4. Apply adhesive as for the self-etch mode
Scotchbond Universal (3M ESPE, USA)	2.7	2-HEMA, 10-MDP, dimethacrylate resins, Vitrebond^{TM} copolymer, silane, filler, ethanol, water, initiators	1. Apply the adhesive or adhesive mixture to the prepared tooth and rub in for 20 s 2. Gently air-dry the adhesive for 5 sec for the solvent to evaporate 3. Light cure for 10 s	1. Apply etchant for 15 s 2. Rinse thoroughly with water and dry with water- and oil-free air or with cotton pellets; do not overdry 3. Apply adhesive as for the self-etch mode

Abbreviations: 2-HEMA, 2-hydroxyethyl methacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol A glycidyl methacrylate; HEDMA, 1,6-hexanediol dimethacrylate; PENTA, dipentaerythritol penta acrylate monophosphate.

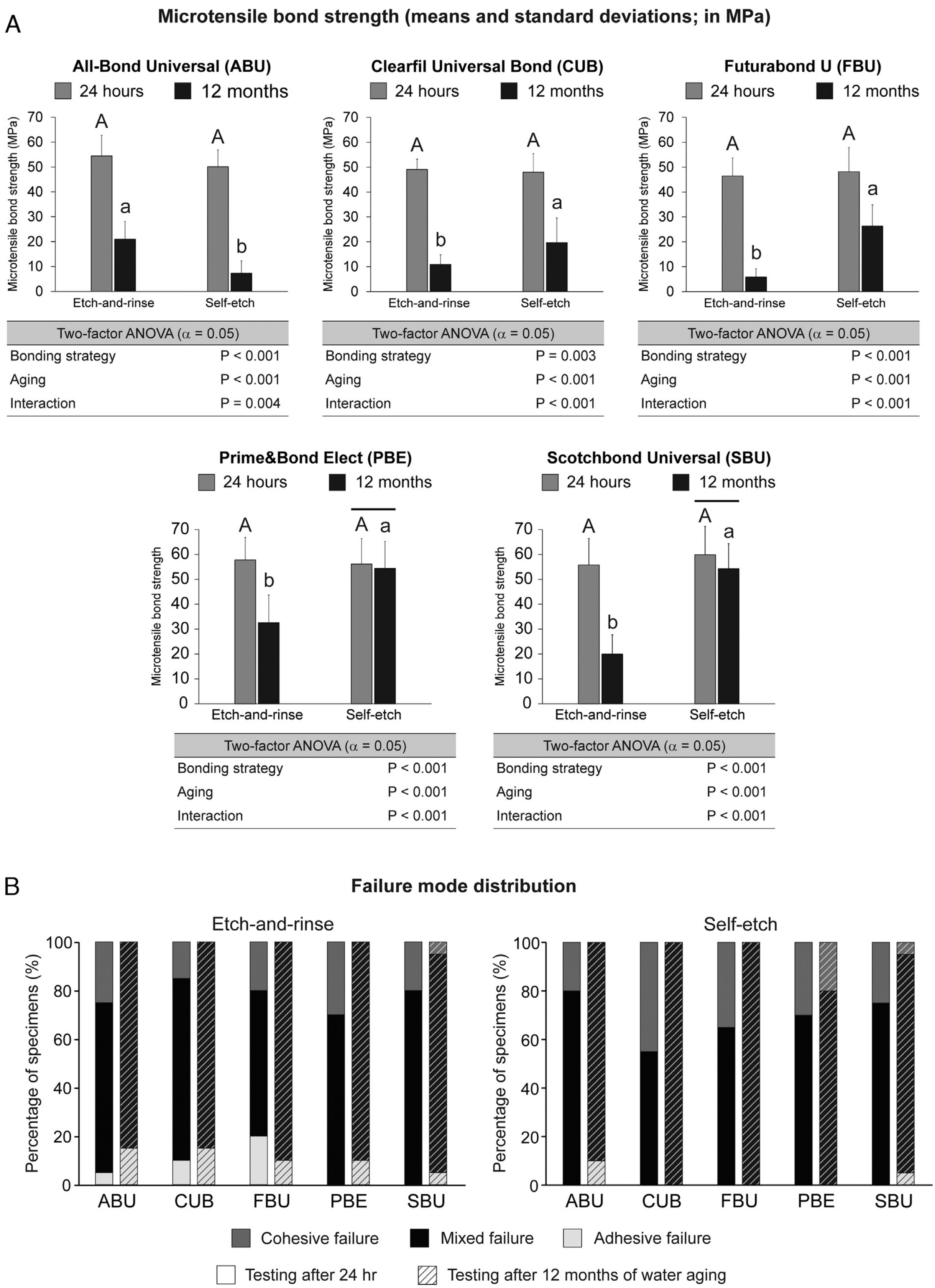

Fig. 1. The effects of bonding strategy and ageing on the bond strength of the five universal adhesives to coronal dentine. (A) Microtensile bond strength data for each adhesive was analysed separately using two-factor ANOVA using tooth as the statistical unit ($N = 10$). Table beneath each chart shows p-values for the two factors and their interactions. For pairwise comparisons within "bonding strategy", columns labelled with the same upper-case letters (etch-and-rinse mode) or lower-case letters (self-etch mode) are not significantly different ($P > 0.05$). For pairwise comparisons within "ageing", columns demarcated with horizontal bars are not significantly different ($P > 0.05$). (B) Failure mode distribution in the subgroups.

0.9% NaCl containing 0.02% sodium azide to prevent bacterial growth, and used within six months after retrieval. A flat bonding surface was prepared on the mid-coronal dentine of each tooth by removal of occlusal third of the crown with a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling. The exposed dentine surfaces were polished with 400-grit silicon carbide paper under water irrigation for 1 min. All dentine surfaces were examined under an optical microscope to ensure the absence of residual enamel or pulpal exposure.

2.1. Bonding procedures

The dentine disks were randomly assigned to five universal adhesives groups (*i.e.* 40 teeth per adhesive): All-Bond Universal (Bisco Inc., Schaumburg, IL, USA), Clearfil Universal Bond (Kuraray Noritake Dental Inc., Tokyo, Japan), Futurabond U (Voco GmbH, Cuxhaven, Germany), Prime&Bond Elect (Dentsply Caulk, Milford, DE, USA) and Scotchbond Universal (3M ESPE, St. Paul, MN, USA). The composition of each adhesive is listed in Table 1. Each adhesive was applied to dentine in the etch-and-rinse mode or the self-etch mode (*i.e.* 20 teeth/adhesive/bonding mode). For bonding in the etch-and-rinse mode, dentine surfaces were etched with 35% phosphoric acid (Select Etch, Bisco, Inc.) for 15 s, rinsed with water for 15 s and blot-dried with lint-free tissue, leaving the bonding surface moist. Each adhesive was applied according to the respective manufacturer's instructions (Table 1). For bonding in the self-etch mode, each adhesive was applied on the dentine surface according to the respective manufacturer's instructions (Table 1). After light-curing of each adhesive, two 2 mm thick layers of a resin composite (TPH, Dentsply Caulk) were placed on the bonded dentine. Each layer was polymerised for 40 s using a light-emitting diode light-curing unit with an output intensity of 1000 mW/cm^2 (Valo, Ultradent Products, Inc., South Jordan, UT, USA).

One-half of the specimens from each adhesive-etching mode subgroup ($N = 10$) were used for bond strength evaluation and ultrastructural examination with storage in deionised water at 37 °C for 24 h. The other half of the specimens from each adhesive–etching mode subgroup ($N = 10$) were stored in the NaCl/NaN_3 solution at 37 °C for 12 months prior to bond strength evaluation and ultrastructural examination. These baseline specimens served as the control, so that the 12-month aged bonded specimens from each adhesive/bonding mode may be compared with data derived from the 24-h unaged control specimens.

2.2. Microtensile bond strength testing

At each designated testing period, the bonded specimens were vertically sectioned into 0.9 mm thick resin–dentine slabs. The central slab derived from each tooth was saved for ultrastructural examination. The two slabs adjacent to the central slab of each tooth were further sectioned into 0.9 × 0.9 mm sticks. The four longest sticks from those two slabs were selected, yielding 4 sticks per tooth for microtensile bond strength (μTBS) testing. Each stick was attached to a testing jig with cyanoacrylate adhesive (Zapit; Dental Ventures of America, Corona, CA, USA) and stressed to

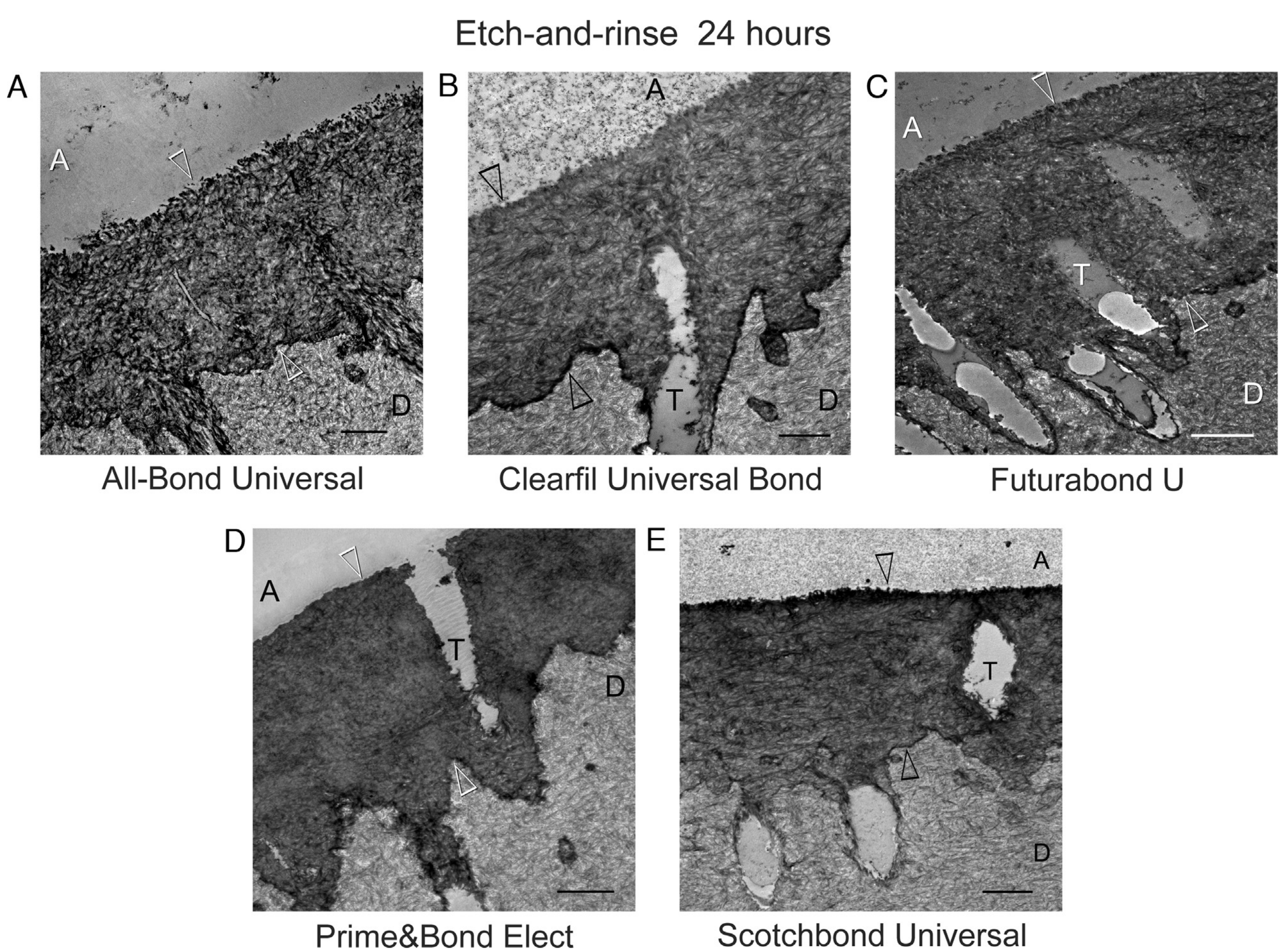

Fig. 2. Representative TEM images of the five universal adhesives bonded to coronal dentine in the etch-and-rinse mode and examined after 24 h (bars = 2 μm). A: adhesive; T: dentinal tubule; D: dentine; Between open arrowheads: stained hybrid layer. No degradation was observed in all specimens ($N = 10$).

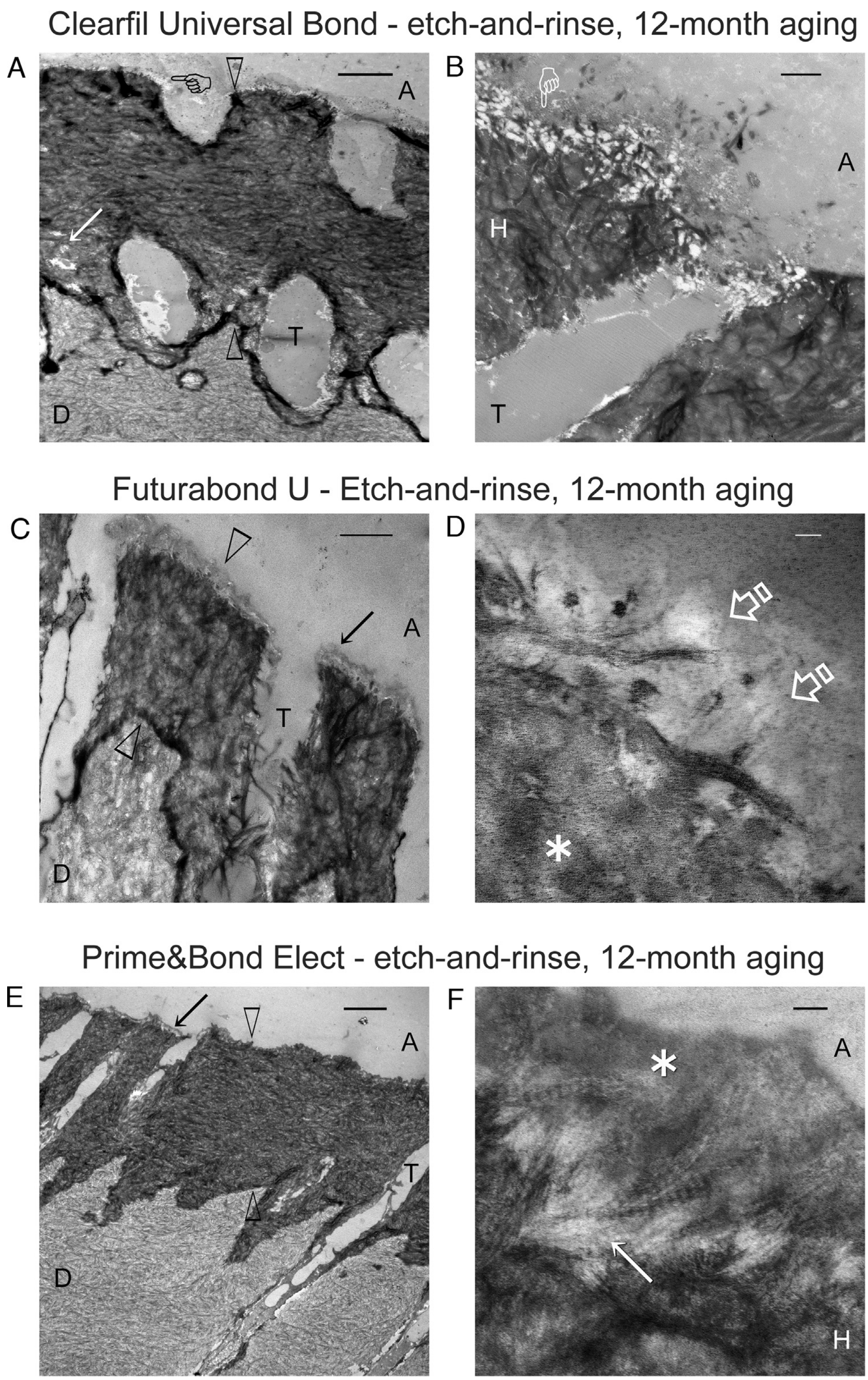

Fig. 3. Representative TEM images of Clearfil Universal Bond (A and B), Futurabond U (C and D) and Prime&Bond Elect (E and F) specimens that had been aged for 12 months. A: adhesive; T: dentinal tubule; D: dentine. (A) Low magnification of the partially-degraded hybrid layer (between open arrows) in Clearfil Universal Bond (bar = 2 μm) where degradation occurred at the top (pointer) and basal portions (arrow). (B) Higher magnification (bar = 200 nm) of the top of the hybrid layer (H) with loss of stained resin and collagen fibrils (pointer). (C) Low magnification of the partially-degraded hybrid layer (between open arrows) in Futurabond U (bar = 2 μm) with degradation present at the top of the hybrid layer (arrow). (D) Higher magnification (bar = 200 nm) of the top of the hybrid layer where there was loss of stained resin components (open arrows). Ultrastructural features of collagen fibrils could not be distinguished within the subsurface part of the hybrid layer (asterisk). (E) Low magnification of the partially-degraded hybrid layer (between open arrows) in Prime&Bond Elect (bar = 2 μm) with degradation present at the top of the hybrid layer (arrow). (F) Higher magnification (bar = 200 nm) of the top of the hybrid layer where structural features of collagen fibrils could not be discerned (asterisk). Within the subsurface part of the hybrid layer, some of the collagen fibrils have degraded into microfibrillar strands (arrow).

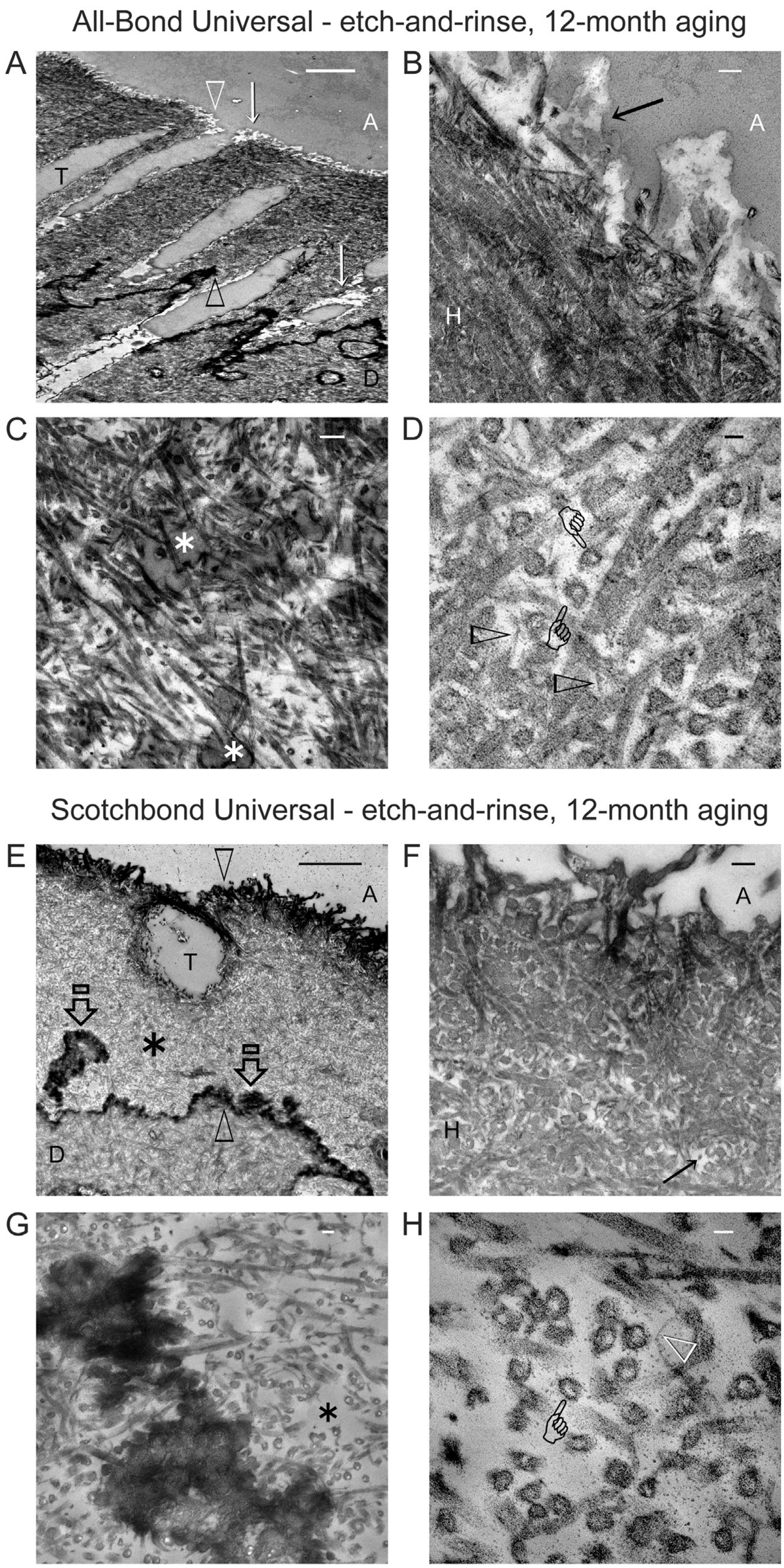

Fig. 4. Representative TEM images of All-Bond Universal (A–D) and Scotchbond Universal (E–H) specimens that had been aged for 12 months. A: adhesive; T: dentinal tubule; D: dentine. (A) Low magnification of the partially-degraded hybrid layer (between open arrows) in All-Bond Universal (bar = 2 μm). Degraded regions are indicated by arrows. (B) Degradation (arrow) along the top of the hybrid layer H is manifested as loss of resin and partial degradation of collagen fibrils (bar = 200 nm). (C) Higher magnification of the degradation (arrow) along the base of the hybrid layer (bar = 200 nm). Retained stained adhesive resin is demarcated by the asterisks. (D) High magnification of the areas devoid of stained resin in "C" (bar = 50 nm), showing thinning of some collagen fibrils (open arrows) and degradation in the center of some of the wider diameter fibrils (pointers). (E) Low magnification of the partially-degraded hybrid layer (between open arrows) in Scotchbond Universal (bar = 2 μm). The bulk of the hybrid layer was palely-

failure under tension in Geraldeli testing jigs attached to a universal tester (Vitrodyne V1000, Liveco Inc., Burlington, VT, USA) that was run at a crosshead speed of 1 mm/min. The tensile force at failure was recorded and divided by the cross-sectioned area of each stick to yield the tensile bond strength in megaPascals (MPa).

Statistical analysis was performed using the tooth as the statistical unit. The mean μTBS obtained from the 4 sticks of each tooth was used to represent the bond strength of that tooth. Data that did not satisfy the normality and equal variance assumptions for parametric statistical analyses were non-linearly transformed prior to the use of those testing methods. The data were analysed using two-factor analysis of variance, to determine the effects of bonding strategy (*i.e.* etch-and-rinse *vs* self-etch) and specimen ageing (*i.e.* baseline *vs* 12-month aging), and the interaction of these two factors on the bond strength results derived from each adhesive. Because each adhesive was compositionally distinct, it was not the intent of the present study to perform a "battle of the bonds" [23] by making comparisons among adhesives produced by different manufacturers. Post-hoc pairwise comparisons were performed using the Holm–Sidak statistic. For all analyses, statistical significance was set at $\alpha = 0.05$.

The two ends of a fractured stick were removed from the testing jig and examined under a stereoscopic microscope at 40× magnification to determine the mode of failure. Failure modes were classified as adhesive failure (failure along the adhesive interface), mixed failure (failure within the adhesive joint together with failure within the resin composite or dentine), or cohesive failure (failure within the resin composite or dentine).

2.3. Transmission electron microscopy (TEM)

The ten central slabs from each adhesive subgroup (*i.e.* etch-and-rinse and self-etch at baseline or 12 months) were processed for TEM. Each slab was completely demineralised in 0.1 M formic acid/sodium formate (pH 2.5). The end-point of demineralisation was determined by adding a 10% potassium oxalate solution to the demineralisation solution, which formed a white precipitate if calcium ions were present.

Complete demineralised slabs were fixed in Karnovsky's fixative, post-fixed in 1% osmium tetroxide, dehydrated in an ascending ethanol series (50–100%), immersed in propylene oxide as a transition medium, and embedded in pure epoxy resin. One epoxy resin-embedded block was prepared from each processed slab for TEM examination (*i.e.* $N = 10$/adhesive/etching mode). Ninety nanometre-thick epoxy resin-embedded sections were cut, stained with 2% aqueous uranyl acetate and Reynold's lead citrate, and examined using a JEM-1230 TEM (JEOL, Tokyo, Japan) operated at 110 keV. For the aged specimens, a section was classified as containing a degraded interface when features of degradation was identified anywhere along the hybrid layer of that section.

3. Results

Microtensile bond strengths of the five adhesives are presented separately in Fig. 1A. For each adhesive, μTBS was significantly affected by the bonding strategy as well as whether aging was performed ($P <$ at least 0.005). The interaction of those two factors was also highly significant. For pairwise comparisons within the factor bonding strategy, each adhesive showed no significant difference in the 24-h μTBS irrespective of whether the adhesive was used in the etch-and-rinse mode or the self-etching mode ($P > 0.05$). After aging for 12 months, the μTBS of All-Bond Universal was significantly higher when used in the etch-and-rinse mode ($P < 0.001$), while the μTBSs of the other 4 universal adhesives were significantly higher when used in the self-etch mode ($P < 0.001$ for all comparisons). For pairwise comparisons within the factor ageing, all five adhesives showed significant decline in μTBSs, by at least 50%, when the respective adhesive was used in the etch-and-rinse mode ($P < 0.001$ for all comparisons). All-Bond Universal, Clearfil Universal and Futurabond U exhibited significant decline in μTBSs, by at least 50%, when the respective adhesive was used in the self-etch mode ($P < 0.001$ for all pairwise comparisons). Both Prime&Bond Elect ($P = 0.591$) and Scotchbond Universal ($P = 0.083$), when used in the self-etch mode, showed no significant differences between the 24-h and 12-month μTBSs. For all adhesive subgroups, mixed failure was the predominant failure mode when the non-trimming version of the μTBS test was employed (Fig. 1B).

Stained resin-dentin interfaces produced by the application of the five universal adhesives in the etch-and-rinse mode are shown in Fig. 2. For each adhesive, the hybrid layer appeared electron-dense and was about 5 μm thick. All five adhesives exhibited variable degrees of degradation within the hybrid layer. For Clearfil Universal Bond (Fig. 3A and B; degradation seen in 8 out of 10 sections), Futurabond U (Fig. 3C and D; 10/10) and Prime&Bond Elect (Fig. 3E and F; 5/10), degradation was predominantly found along the surface and the basal portion of the hybrid layer. Degradation was manifested as disappearance of stainable adhesive resin, loss of structural integrity of the collagen fibrils and dissolution of the degraded microfibrils.

The aforementioned degradative features could also be identified, albeit more extensively, when All-Bond Universal (Fig. 4A–D; 8/10) and Scotchbond Universal (Fig. 4E–H; 10/10) were used in the etch-and-rinse mode. An example of an interface with fairly extensive degradation along the entire width of the hybrid layer is shown in Figs. 4E–H. In this Scotchbond Universal example, the bulk of the hybrid layer appeared palely-stained except for isolated islands along the basal portion that remained heavily-stained. A unique, previously unreported collagen degradation pattern was observed in both All-Bond Universal (Fig. 4D) and Scotchbond Universal (Fig. 4H) specimens. This inside-out pattern of collagen degradation is characterised by the absence of stainable intra-fibrillar components in the centre of fibrils that were cut in cross sections. Similar hollow collagen fibrils could also be identified in specimens bonded with Clearfil Universal Bond (not shown).

Fig. 5 contains TEM images of the five universal adhesives bonded to coronal dentine in the self-etch mode and examined after 24 h or after 12 months of water-ageing. Prior to ageing, electron-dense, stained hybrid layers that were less than 500 nm thick could be identified from resin-dentine interfaces produced by all the adhesives (left column). After ageing, loss of collagen fibrillar integrity could be observed in All-Bond Universal (8/10), Clearfil Universal Bond (7/10), Futurabond U (10/10) and Scotchbond Universal (3/10) (right column). Prime&Bond Elect was the only adhesive that did not exhibit loss of collagen integrity (0/10) when applied to dentine in the self-etch mode.

4. Discussion

Significant lowering of μTBSs was observed for all the five universal adhesives when they were used in the etch-and-rinse mode. Hence, the first null hypothesis has to be rejected. Because

stained (asterisk) except for the surface and islands in the basal portion (open arrows). (F) Higher magnification (bar = 200 nm) of the degradation (arrow) along the top of the hybrid layer H. The subsurface part of the hybrid layer shows wider interfibrillar spaces with some thinned-down collagen fibrils (arrow). (G) Thinning of collagen fibrils and widened interfibrillar spaces (asterisk) were much more extensive at the base of the palely-stained part of the hybrid layer (bar = 100 nm). (H) High magnification of "G" (bar = 50 nm) showing a similar inside-out degradation pattern in the wider diameter collagen fibrils (pointer) and substantially thinned-down fibrils (open arrow).

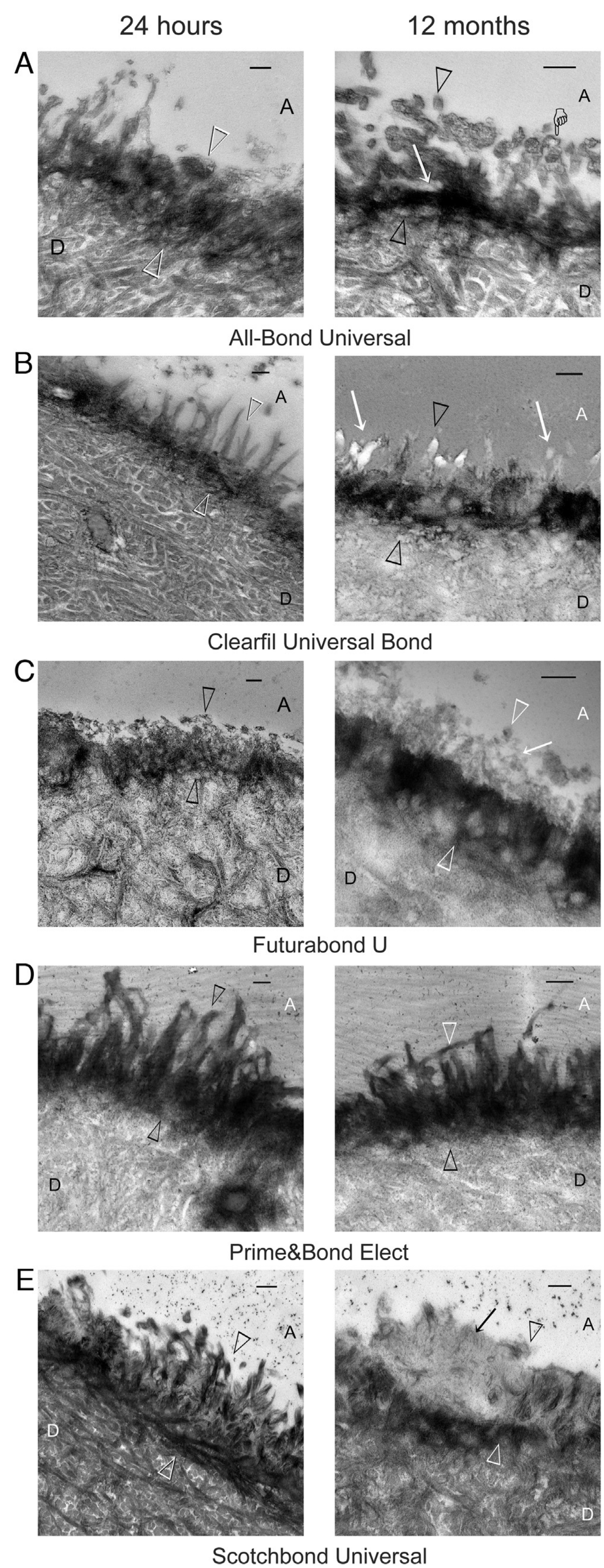

Fig. 5. Representative TEM images of the five universal adhesives bonded to coronal dentine in the self-etch mode and examined after 24 h (left column) or 12 months (right column). Bars = 200 nm. A: adhesive; T: dentinal tubule; D: dentin; Between open arrows: thin hybrid layer created by the adhesives in the self-etch mode. (A) All-Bond Universal; (B) Clearfil Universal Bond; (C) Futurabond U; (D) Prime&Bond Elect. (E) Scotchbond Universal. In the right column, regions within the hybrid layer

significant lowering of μTBSs was found in three of five adhesives when they were used in the self-etch mode, the second null hypothesis also has to be rejected.

Based on the TEM results, the decline in dentine μTBSs that occurred after 12 months of water ageing may be attributed to bond deterioration. For bonding in the etch-and-rinse mode, the variable degrees of resin leaching and collagen degradation that occurred at the basal portion and/or top of the hybrid layers probably represented the initial stages of the more extensive degradation seen in human dentine bonded *in vivo* with two-step etch-and-rinse adhesives within a similar aging period [24–26]. Possible reasons why more severe degradation was identified in hybrid layers bonded *in vivo* include increased dentine permeability during bonding to vital teeth [27], differences in the extent of polymerisation shrinkage stress relief between *in vivo* bonding and restoration of cavities with more complex geometry and *in vitro* bonding to planar surfaces [28], presence of salivary esterases that account for *in vivo* resin hydrolysis [29], and environmental challenges derived from thermal and masticatory stresses [30] and dental plaque biofilms [31].

The inside-out collagen fibril degradation pattern seen in specimens bonded with All-Bond Universal and Scotchbond Universal provides important evidence to the long-unanswered question with respect to whether resin monomers can infiltrate into the intrafibrillar water compartments of demineralised collagen [32]. Those hollow collagen fibrils provided indirect evidence that resin monomers are capable of infiltrating into collagen fibrils. Because resin infiltration was incomplete at the base of the thick demineralised collagen matrices, the pH-and-water-activated dormant endogenous enzymes that were trapped by the peripheral resin envelope caused degradation of the collagen molecules from the inside-out. Because the peripheral resin envelope protected the fibrillar surface from degradation, hollow collagen fibrils were produced. Bertassoni et al. [33] opined that small adhesive resin monomers such as triethyleneglycol dimethacrylate are incapable of occupying the intermolecular spaces between collagen molecules. Contrary to this opinion, Takahashi et al. [34] utilised a modified gel-filtration chromatography technique to demonstrate that demineralised collagen fibrils behave as molecular sieves, enabling replacement of the intrafibrillar water by adhesive resin monomers in a size-dependent manner. Molecules smaller than 1000 Da can freely diffuse into collagen water, while molecules larger than 10,000 Da are excluded. The authors discovered when 2-hydroxyethyl methacrylate 2-hydroxhethyl methacrylate (HEMA) and Bis-GMA were solvated in an ethanol/water mixed solvent, HEMA infiltrate more easily into collagen fibrils than Bis-GMA. Hiraishi et al [35]. investigated the binding interaction of resin monomers with atelocollagen and found that 10-MDP has relatively stable hydrophobic interaction with collagen. On the contrary, HEMA does not interact with collagen [36]. For the 10-MDP containing adhesives, interaction of MDP with HEMA in solution may produce aggregates that reduce the hydrophobicity of MDP and compromise MDP-collagen interaction, leaving collagen fibrils not completely infiltrated by MDP or HEMA [37]. We speculate that when these 10-MDP containing universal adhesives are used in the etch-and-rinse mode, resin monomer infiltration into the demineralised collagen network is inconsistent, resulting in different extents of monomer distribution within the collagen fibrils. Because 10-MDP containing etch-and-rinse adhesives had not been utilised in the authors' *in vivo* hybrid layer degradation studies, these hollow degraded collagen fibrils were identified for the first time in the present work.

For bonding in the self-etch mode, All-Bond Universal, Clearfil Universal Bond and Futurabond U exhibited significant decreases in μTBS after 12-month ageing. For Prime&Bond Elect and that exhibited degradation are demarcated by arrows. No ultrastructural features of degradation were observed in the Prime&Bond Elect specimens.

Scotchbond Universal, the decreases in μTBS were not significantly different. When examined with TEM, except for Prime&Bond Elect, the other four universal adhesives demonstrated different degree of degradation. Theoretically, universal adhesives applied in self-etch mode can condition and prime dentine simultaneously. However, nanoleakage identified in 24-h specimens produced by these universal adhesives [22] supports that hybrid layer degradation can occur in resin-dentine interfaces created by some universal adhesives in the self-etch mode. Universal adhesives are different from each other in solvents, acidity and ingredients. Water is an indispensable component in these adhesives. Remnant water that cannot be fully removed from the evaporated adhesive due to either decrease in vapour pressure caused by the addition of water-soluble adhesive resin monomers [38] or the presence of an osmotic gradient [39] provides the hydrolytic fuel for hydrolysis of polymeric resins and enzymatic degradation of collagen fibrils. Prime&Bond Elect uses acetone as organic solvent, while the other universal adhesives contain ethanol. Acetone has higher vapour pressure compared than ethanol, resulting in rapid solvent evaporation and less retention of residual water due to less lowering of the vapour pressure in organic solvent-water mixtures. This may explain why collagen degradation was not identified in hybrid layers prepared using Prime&Bond Elect in the self-etch mode.

Another important feature is that the 10-MDP containing universal adhesives (All-Bond Universal, Clearfil Universal Bond and Scotchbond U) did not exhibit better performance than 10-MDP free universal adhesives. The inclusion of 10-MDP in a dentine adhesive has been suggested to be responsible for better bonding durability [40]. Because of the purported hydrolytic stability of 10-MDP-Ca salts, nanolayering is thought to be acid-resistant, protecting the partially demineralised collagen from degradation. We could not observe nanolayering in the present study because all the TEM work was performed on completely demineralised specimens. Because of the composition difference among different universal adhesives, the concept of nanolayering protection of collagen degradation should be tested in further studies using experimental self-etch primers containing only 10-MDP that can create nanolayering and 10-MDP analoguess that are incapable of creating nanolayering (Tian et al., unpublished results).

Within the limits of the present study 12-month water-ageing *in vitro* study, it may be concluded that bonds created by the five universal adhesives in the self-etch mode are in general, more durable than those created in the etch-and-rinse mode. Nevertheless, with the exception of bonds created by Scotchbond Universal and Prime&Bond Elect in the self-etch mode, bonds created by these five universal adhesives are ephemeral and are incapable of defying ageing. This preliminary conclusion waits validation in *in vivo* degradation studies.

Conflict of interest

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References

[1] B. Dylan, Forever Young. http://www.azlyrics.com/lyrics/bobdylan/foreveryoung.html, 1974 (assessed 11.04.15).
[2] A.O. Rice, Interview with the Vampire, Ballentine Books, New York, 1991.
[3] O. Rank, Narcissism and the double, in: E. Berman (Ed.), Essential Papers on Literature and Psychoanalysis, New York University Press, New York, 1993, pp. 122–138.
[4] O. Wilde, The Picture of Dorian Gray, Dover Publications, Inc., New York, 1993.
[5] Editors of Scientific American, Forever Young: The Science of Aging, Scientific American, New York, 2013.
[6] I.M. Conboy, M.J. Conboy, A.J. Wagers, E.R. Girma, I.L. Weissman, T.A. Rando, Rejuvenation of aged progenitor cells by exposure to a young systemic environment, Nature 433 (2005) 760–764.
[7] J. Fenn, M. Raskino, Mastering the Hype Cycle: How to Choose the Right Innovation at the Right Time (Gartner), Harvard Business Press, Watertown, MA, 2008.
[8] C. Mason, E. Manzotti, Induced pluripotent stem cells: an emerging technology platform and the Gartner hype cycle, Regen. Med. 4 (2009) 329–331.
[9] D.T. Zero, How the introduction of the acid-etch technique revolutionized dental practice, J. Am. Dent. Assoc. 144 (2013) 990–994 Spec No: 47S-51S.
[10] J. De Munck, K. Van Landuyt, M. Peumans, A. Poitevin, P. Lambrechts, M. Braem, et al., A critical review of the durability of adhesion to tooth tissue: methods and results, J. Dent. Res. 84 (2005) 118–132.
[11] L. Breschi, A. Mazzoni, A. Ruggeri, M. Cadenaro, R. Di Lenarda, E. De Stefano Dorgio, Dental adhesion review: aging and stability of the bonded interface, Dent. Mater. 24 (2008) 90–101.
[12] Y. Liu, L. Tjäderhane, L. Breschi, A. Mazzoni, N. Li, J. Mao, et al., Limitations in bonding to dentin and experimental strategies to prevent bond degradation, J. Dent. Res. 90 (2011) 953–968.
[13] J. Perdigão, Dentin bonding-variables related to the clinical situation and the substrate treatment, Dent. Mater. 26 (2010) e24–e37.
[14] W.L. Rosa, E. Piva, A.F. Silva, Bond strength of universal adhesives: a systematic review and meta-analysis, J. Dent. 43 (2015) 765–776.
[15] B. Seabra, S. Arantes-Oliveira, J. Portugal, Influence of multimode universal adhesives and zirconia primer application techniques on zirconia repair, J. Prosthet. Dent. 112 (2014) 182–187.
[16] Y. Yoshida, K. Yoshihara, N. Nagaoka, S. Hayakawa, Y. Torii, T. Ogawa, Self-assembled nano-layering at the adhesive interface, J. Dent. Res. 91 (2012) 376–381.
[17] L. Tjäderhane, F.D. Nascimento, L. Breschi, A. Mazzoni, I.L. Tersariol, S. Geraldeli, Strategies to prevent hydrolytic degradation of the hybrid layer—a review, Dent. Mater. 29 (2013) 999–1011.
[18] L. Tjäderhane, Dentin bonding: can we make it last? Oper. Dent. 40 (2015) 4–18.
[19] M. Hanabusa, A. Mine, T. Kuboki, Y. Momoi, A. Van Ende, B. Van Meerbeek, Bonding effectiveness of a new 'multi-mode' adhesive to enamel and dentine, J. Dent. 40 (2012) 475–484.
[20] M.A. Muñoz, I. Luque, V. Hass, A. Reis, A.D. Loguercio, N.H. Bombarda, Immediate bonding properties of universal adhesives to dentine, J. Dent. 41 (2013) 404–411.
[21] A. Wagner, M. Wendler, A. Petschelt, R. Belli, U. Lohbauer, Bonding performance of universal adhesives in different etching modes, J. Dent. 42 (2014) 800–807.
[22] C. Chen, L.N. Niu, H. Xie, Z.Y. Zhang, L.Q. Zhou, K. Jiao, Bonding of universal adhesives to dentine—old wine in new bottles? J. Dent. 43 (2015) 525–536.
[23] M. DeGrange, L. Hitmi, D. Bouter, S. Gonthier, F. Basset, J. Bijaoui, Efficiency of new enamel-dentin bonding systems: assessment by general practitioners, in: J.-F. Roulet, N.H.F. Wilson, M. Fuzzi (Eds.), Advances in Operative Dentistry. Challenges of the Future, vol. 2, Quintessence Publishing Co., Inc., Chicago, 2001, pp. 173–183.
[24] J. Hebling, D.H. Pashley, L. Tjäderhane, F.R. Tay, Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo, J. Dent. Res. 84 (2005) 741–746.
[25] M.R. Carrilho, S. Geraldeli, F. Tay, M.F. de Goes, R.M. Carvalho, L. Tjäderhane, et al., In vivo preservation of the hybrid layer by chlorhexidine, J. Dent. Res. 86 (2007) 529–533.
[26] M.G. Brackett, N. Li, W.W. Brackett, R.J. Sword, Y.P. Qi, L.N. Niu, et al., The critical barrier to progress in dentine bonding with the etch-and-rinse technique, J. Dent. 39 (2011) 238–248.
[27] D.H. Pashley, R.M. Carvalho, Dentine permeability and dentine adhesion, J. Dent. 25 (1997) 355–372.
[28] A.J. Feilzer, A.J. De Gee, C.L. Davidson, Setting stress in composite resin in relation to configuration of the restoration, J. Dent. Res. 66 (1987) 1636–1639.
[29] Y. Finer, J.P. Santerre, Salivary esterase activity and its association with the biodegradation of dental composites, J. Dent. Res. 83 (2004) 22–26.
[30] M.R. Carrilho, F.R. Tay, D.H. Pashley, L. Tjäderhane, R.M. Carvalho, Mechanical stability of resin–dentin bond components, Dent. Mater. 21 (2005) 232–241.
[31] M.M. Mutluay, K. Zhang, H. Ryou, M. Yahyazadehfar, H. Majd, H.H. Xu, On the fatigue behavior of resin-dentin bonds after degradation by biofilm, J. Mech. Behav. Biomed. Mater. 18 (2013) 219–231.

[32] D.H. Pashley, N. Nakabayashi, Characterization of the hybrid layer, in: D.H. Pashley, N. Nakabayashi (Eds.), Hybridization of Dental Hard Tissues, Quintessence Publishing Co., Ltd., Tokyo, 1998, pp. 66.

[33] L.E. Bertassoni, J.P. Orgel, O. Antipova, M.V. Swain, The dentin organic matrix—limitations of restorative dentistry hidden on the nanometer scale, Acta Biomater. 8 (2012) 2419–2433.

[34] M. Takahashi, M. Nakajima, J. Tagami, D.L. Scheffel, R.M. Carvalho, A. Mazzoni, et al., The importance of size-exclusion characteristics of type I collagen in bonding to dentin matrices, Acta Biomater. 9 (2013) 9522–9528.

[35] N. Hiraishi, N. Tochio, T. Kigawa, M. Otsuki, J. Tagami, Monomer–collagen interactions studied by saturation transfer difference NMR, J. Dent. Res. 92 (2013) 284–288.

[36] N. Hiraishi, N. Tochio, T. Kigawa, M. Otsuki, J. Tagami, Molecular level evaluation on HEMA interaction with a collagen model, Dent. Mater. 31 (2015) 88–92.

[37] N. Hiraishi, N. Tochio, T. Kigawa, M. Otsuki, J. Tagami, Role of 2-hydroxyethyl methacrylate in the interaction of dental monomers with collagen studied by saturation transfer difference NMR, J. Dent. 42 (2014) 484–489.

[38] E.L. Pashley, Y. Zhang, P.E. Lockwood, F.A. Rueggeberg, D.H. Pashley, Effects of HEMA on water evaporation from water-HEMA mixtures, Dent. Mater. 14 (1998) 6–10.

[39] F.R. Tay, D.H. Pashley, B.I. Suh, N. Hiraishi, C.K. Yiu, Water treeing in simplified dentin adhesives—déjà vu? Oper. Dent. 30 (2005) 561–579.

[40] K. Yoshihara, Y. Yoshida, S. Hayakawa, N. Nagaoka, Y. Torii, et al., Self-etch monom er-calcium salt deposition on dentin, J. Dent. Res. 90 (2011) 602–606.