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Bonding to enamel using alternative Enamel Conditioner/etchants

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ABSTRACT

Objective. Enamel bond durability of three new alternative etchants combined with three representative adhesives was determined.

Methods. The 'immediate' and 'aged' micro-tensile bond strength (μTBS) of the 3-step etch&rinse adhesive OptiBond FL ('O-FL', Kerr), 2-step self-etch (SE) adhesive Clearfil SE Bond 2 ('C-SE2', Kuraray Noritake) and universal adhesive Adhese Universal ('ADU', Ivoclar Vivadent) were measured when bonded to enamel following either a proprietary organic acid-containing Enamel Conditioner ('EC', Shofu), a phosphoric-acid monomer-containing Multi Etchant ('ME', Yamakin: 10-methacryloyloxy tetraethylene glycol dihydrogenphosphate or MTEGP), or a metal salt-based ZON etchant ('ZON', Ivoclar Vivadent: $ZrO(NO_3)_2$). All alternative etchants were used in replacement of phosphoric acid, the latter (K-Etchant, Kuraray Noritake) also used with O-FL and ADU, in addition to C-SE2 that was solely used in SE mode (controls). The enamel-etching patterns and de-bonded fracture surfaces were examined by SEM, while the interfaces with enamel were ultra-morphologically characterized by TEM.

Results. No statistically significant difference in immediate and aged μTBS, obtained by combining the three alternative etchants with the three adhesives, was recorded as compared with the respective controls, except for ME combined with O-FL. Upon aging, significant reduction in μTBS was recorded for the ME/C-SE2 and ME/ADU combinations. The

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percentage of adhesive failures increased with aging. SEM revealed similar etching patterns produced by EC and ZON as by classic phosphoric-acid etching, for which also numerous micro-resin tags at the adhesive-enamel interface were disclosed.

Significance. Durable bonding to enamel was generally obtained for all etchant/adhesive combinations with the exception of the ME/O-FL combination.

1. Introduction

Since the early development of dental adhesive techniques in 1955, phosphoric acid has standardly been employed to condition enamel (and dentin) for bonding resin-based materials to tooth tissue [1]. Phosphoric-acid etching is very effective on enamel because of enamel's mainly mineral content, facilitating bonding as opposed to dentin, to which due to its hydrophilic and wet nature is much more challenging to bond [2,3]. In fact, phosphoric-acid etching modifies the enamel surface topography, substantially increasing its surface energy to the direct benefit of enamel's wettability [4,5]. Following relatively short phosphoric-acid etching, bonding to enamel is simple and based principally on diffusion, driven by capillary forces, and subsequent micro-mechanical interlocking of resin into the micro-retentive etch pits produced by etching [6]. Although classic phosphoric-acid etching is an established technique to durably bond to enamel, phosphoric acid is not always considered the best dentin pre-treatment, by which research opportunities remain to develop new alternative etchants for etching both enamel and dentin.

New alternative etchants were recently introduced on the market. Enamel Conditioner ('EC'; Shofu, Kyoto, Japan) contains proprietary organic acids, while Multi Etchant ('ME'; Yamakin, Osaka, Japan) contains the acidic functional monomer methacryloyloxy tetraethylene glycol dihydrogen phosphate (MTEGP) [7] with bonding potential not only to enamel/dentin but also to zirconia. In addition, an experimental metal salt-based enamel/dentin etchant, referred to as 'ZON', has been developed by Ivoclar Vivadent (Schaan, Liechtenstein).

In general, the extent and depth of the etching pattern produced by etchants, providing micro-mechanical interlocking, primarily determines the bonding receptiveness of enamel [8–10]. Up to our knowledge, limited data in literature is available up to date regarding the etching capability of organic acids, as also potential chemical interaction of the acidic functional monomer MTEGP (in ME) with enamel hydroxyapatite and the activity of metal ions provided with the metal salt-based ZON etchant remain to be explored. Therefore, comparing the bonding effectiveness to enamel of representative adhesives applied following the application of new alternative etchants with that following classic phosphoric-acid etching is timely and highly significant.

Nowadays, universal adhesives are rapidly gaining popularity in routine clinical practice, as they allow the dentist to choose for either an etch-and-rinse (E&R) or self-etch (SE) bonding mode, this depending on the actual clinical conditions [11,12]. Numerous studies have revealed that enamel etching with phosphoric acid remains recommended for universal adhesives [13–16]. Even for bonding to primary enamel, having a lower mineral content as compared to enamel of permanent teeth [17], phosphoric-acid etching of enamel is preferred [18]. The abovementioned alternative etchants were therefore combined in this study with the representative universal adhesive Adhese Universal ('ADU'; Ivoclar Vivadent), as well as with OptiBond FL ('O-FL'; Kerr, Orange, CA, USA), representing a 3-step E&R adhesive ('3E&Ra'), and with Clearfil SE Bond 2 ('C-SE2'; Kuraray Noritake, Tokyo, Japan), representing a 2-step SE adhesive ('2SEa'), the latter still recommended to be used on enamel following a selective enamel-etching protocol. C-SE2 is an optimized version of its predecessor Clearfil SE Bond (Kuraray Noritake) that together with O-FL are considered in recent literature as gold-standard SE and E&R adhesives, respectively, this based on favorable both laboratory and long-term (13-year) clinical research data [19,20].

In light of the above considerations, the purpose of this study was to investigate whether the bond strength and bond durability to enamel of the three representative adhesives, when applied after the three alternative etchants, were equivalent to those obtained when enamel received classic phosphoric-acid etching prior to the application of the adhesives investigated. The null hypotheses tested were that (1) the bonding effectiveness to enamel of the three adhesives combined with one of the alternative etchants did not significantly differ from that obtained when enamel was etched with phosphoric acid, and (2) the enamel bond durability did not decrease upon substantial aging.

2. Materials and methods

A total of 92 carious-free human third molars were collected (following informed consent approved by the Commission for Medical Ethics of KU Leuven under the file number S57622). The molars were stored in 0.5% chloramine T/water solution at 4 °C and used within three months after extraction.

2.1. *pH of etchants*

The pH of all etchants (EC, ME, ZON and K-Etchant) was measured using a digital pH meter (pH 3110, Wissenschaftlich-Technische Werkstätten, Weilheim, Germany), equipped with the electrode MiniTrode (Hamilton Bonaduz, Bonaduz, Switzerland), after calibration with pH-4.00 and pH-7.00 standard solutions. Five measurements were made for each etchant, upon which the mean pH was calculated.

Table 1 – List of different etchants investigated.

Etchant	Code	Composition[a]	Application procedure[b]	pH	Lot number
Enamel Conditioner (Shofu, Kyoto, Japan)	**EC**	Organic acid, tackifier, colorant, others	**1.** Apply EC on the prepared enamel surface for 10 s; **2.** Remove EC by washing thoroughly, followed by drying with oil-free air; **3.** Continue with the bonding procedures.	0.63	031707
Multi Etchant (Yamakin, Tokyo, Japan)	**ME**	Purified water, phosphoric acid monomer (MTEGP[c]), tackifier, colorant	**1.** Apply ME on the prepared enamel surface for at least 20 s; **2.** Wash thoroughly until the color of the etchant can no longer be seen and dry with oil-free air; **3.** Continue with the bonding procedures.	0.82	01071726
Experimental 'ZON' etchant (Ivoclar Vivadent, Schaan, Liechtenstein)	**ZON**	$ZrO(NO_3)_2$, water, glycerol, fumed silica, polyethylene oxide	**1.** Apply ZON on the prepared enamel surface and allow it to interact without agitation for 30 s; **2.** Thoroughly rinse off the etchant with water spray and dry with oil-free air; **3.** Continue with the bonding procedures.	0.56	CD1-054-1
K-Etchant Syringe (Kuraray Noritake, Tokyo, Japan)	**K-Etchant**	Phosphoric acid, polyethylene glycol, colloidal silica, water, pigment	**1.** Apply K-Etchant on the prepared enamel surface for 15 s; **2.** Rinse for 15 s and gentle air-dry; **3.** Continue with the bonding procedures.	0.21	AH0046

[a] According to technical information provided by the respective manufacturer.
[b] According to the respective manufacturer's instructions.
[c] Methacryloyloxy tetraethylene glycol dihydrogen phosphate.

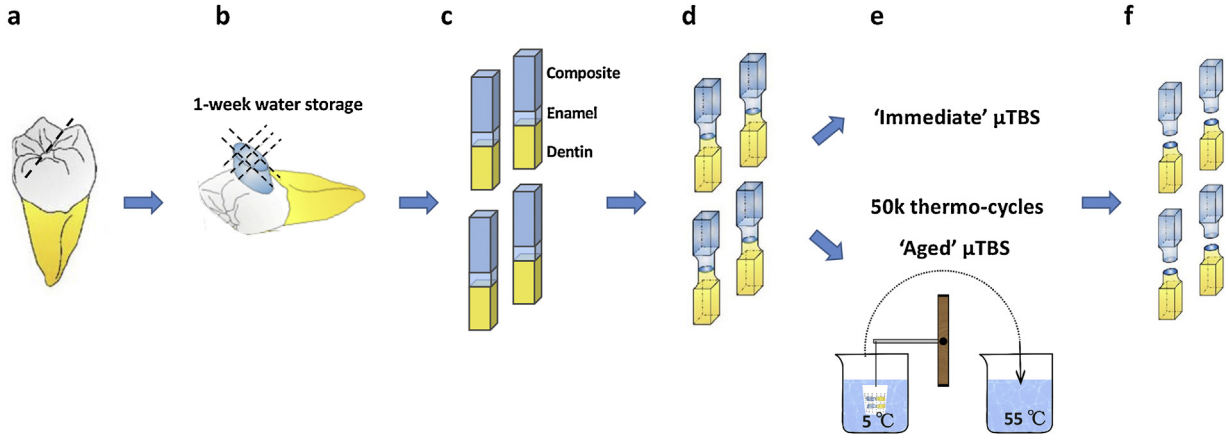

Fig. 1 – Schematic illustrating specimen-preparation methodology for micro-tensile bond strength (μTBS) testing.

2.2. *Tooth preparation*

Flat bur-cut buccal/lingual enamel surfaces were prepared using a high-speed medium-grit (107 μm) diamond bur (882, Komet, Lemgo, Germany), mounted in the MicroSpecimen Former (University of Iowa, Iowa, IA, USA), to produce standardized smear layers. All enamel surfaces were carefully verified for absence of dentin using a stereo-microscope (Stemi 2000-CS, Zeiss, Oberkochen, Germany). Next, the crown was divided in two halves in mesio-distal direction using a slow-speed diamond saw (Micracut 151, Metkon, Bursa, Turkey).

2.3. *Scanning electron microscopy (SEM) of etched enamel surfaces*

Bur-cut enamel of 8 extracted human molars was exposed to the alternative etchants EC, ME, and ZON, and to the classic phosphoric-acid gel K-Etchant (n = 2 per etchant), all applied strictly following the respective manufacturer's instructions, as detailed in Table 1. For ME, enamel surfaces of four additional molars were etched, thoroughly water-rinsed and air-dried, and additionally exposed to acetone (VWR, Leuven, Belgium) to remove any remaining monomer that may not have been removed by simple water rinsing. Two of these additional enamel surfaces were immersed in acetone

Table 2 – List of adhesives used in this study.

Adhesive	Experimental application procedure[a]	Control application[a]
OptiBond FL (Kerr, Orange, CA, USA)	**1.** Upon etching with either EC, ME or ZON, apply OptiBond FL Prime on enamel with a light scrubbing motion for 15 s; **2.** Gently air-dry for 5 s; **3.** Apply OptiBond FL Adhesive on enamel with a light scrubbing motion for 15 s and air-thin for 3 s to achieve a uniformly thin coating; **4.** Light-cure using a high-power LED light-curing unit for 10 s.	Etch-and-rinse (E&R) control **1.** Etch enamel with K-Etchant Syringe (Kuraray Noritake) for 15 s, rinse for 15 s and gently air-dry; **2.** Apply OptiBond FL Prime on enamel with a light scrubbing motion for 15 s; **3.** Gently air dry for 5 s; **4.** Apply OptiBond FL Adhesive on enamel with a light scrubbing motion for 15 and air-thin for 3 s to achieve a uniformly thin coating; **5.** Light-cure using a high-power LED light-curing unit for 10 s.
Clearfil SE Bond 2 (Kuraray Noritake, Tokyo, Japan)	**1.** Upon etching with either EC, ME or ZON, apply Clearfil SE Bond 2 primer using a microbrush and leave it in place for 20 s prior to mildly air-drying for more than 5 s; **2.** Apply Clearfil SE Bond 2 adhesive, followed by gentle air-drying; **3.** Light-cure using a high-power LED light-curing unit for 10 s.	Self-etch (SE) control **1.** Apply Clearfil SE Bond 2 primer using a microbrush and leave it in place for 20 s prior to mildly air-drying for more than 5 s; **2.** Apply Clearfil SE Bond 2 adhesive, followed by gentle air-drying; **3.** Light-cure using a high-power LED light-curing unit for 10 s.
Adhese Universal (Ivoclar Vivadent, Schaan, Liechtenstein)	**1.** Upon etching with either EC, ME or ZON, apply Adhese Universal to enamel by scrubbing for at least 20 s; **2.** Disperse Adhese Universal with oil- and moisture-free compressed air until a glossy, immobile film layer results; **3.** Light-cure using a high-power LED light-curing unit for 10 s.	'Universal' etch-and-rinse (U-E&R) control **1.** Etch enamel with K-Etchant Syringe (Kuraray Noritake) for 15 s, rinse for 15 s and gently air-dry with compressed air until the etched enamel surface appears chalky white; **2.** Apply Adhese Universal to enamel by scrubbing for at least 20 s; **3.** Disperse Adhese Universal with oil- and moisture-free compressed air until a glossy, immobile film layer results; **4.** Light-cure using a high-power LED light-curing unit for 10 s.

[a] According to the respective manufacturer's instructions; **EC:** Enamel Conditioner (Shofu); **ME**: Multi Etchant (Yamakin); **ZON**: Experimental 'ZON' etchant (Ivoclar-Vivadent).

three times for 1 min. The two other surfaces were rotated in acetone for 24 h following a procedure used before to disclose the (self-)etching effect of self-etch adhesives on enamel by SEM [21]. All enamel specimens were processed for SEM (JSM-6610LV, Jeol, Tokyo, Japan) after fixation in 2.5% glutaraldehyde, gradual dehydration in increasing concentrations of aqueous ethanol solutions, and finally chemical drying using hexamethyldisilazane (HMDS; Acros Organics, Thermo Fisher Scientific, Geel, Belgium), this prior to thin gold-sputter coating (JFC-1300, Jeol) [22].

2.4. *Specimen preparation for μTBS testing*

Seventy-two human molars were randomly assigned to 12 experimental groups according to the different etchant/adhesive combinations. The alternative etchants EC, ME and ZON were applied onto bur-cut enamel surfaces strictly following the respective manufacturer's instructions (Table 1), followed by the application of the adhesives (Table 2). The 3E&Ra O-FL and 2SEa C-SE2, serving as references/controls, were also applied, respectively, in E&R mode after classic phosphoric-acid (35%) etching using K-Etchant (Kuraray Noritake) and in SE mode without etching (Table 2). Another control involved the application of the universal adhesive ADU in E&R mode, again after classic phosphoric-acid etching. After completion of the adhesive procedures, the enamel surfaces were built up with Tetric EvoCeram (Ivoclar Vivadent) in 3 layers up to a height of 5 mm. Each increment of maximum 2 mm was light-cured for 10 s using a light-emitting diode (LED) light-curing unit (Bluephase 20i, Ivoclar Vivadent) with an output of 1200 mW/cm^2 when used in 'high mode', as determined and confirmed regularly during the experiment using a Marc Resin Calibrator (BlueLight Analytics, Halifax, Canada). Upon completion, the composite block was additionally light-cured for 20 s from each side, so to ensure optimal curing. Thereafter, the bonded enamel specimens were immediately stored for 1 week (1 w) in distilled water at 37 °C.

Upon 1 w water storage, all specimens were sectioned perpendicular to the interface using a water-cooled diamond saw (Accutom-50, Struers, Ballerup, Denmark) to obtain rectangular sticks (4 central micro-specimens per tooth: 1.8 × 1.8 mm wide; 8–9 mm long). These micro-specimens were mounted in the pin-chuck of the MicroSpecimen Former (University of Iowa, Iowa, IA, USA) and trimmed at the adhesive-enamel interface to a cylindrical hour-glass shape with a bonding surface of 1 mm^2, following a procedure detailed before [23], this using a red-banded fine-grit (46 μm) cylindrical diamond

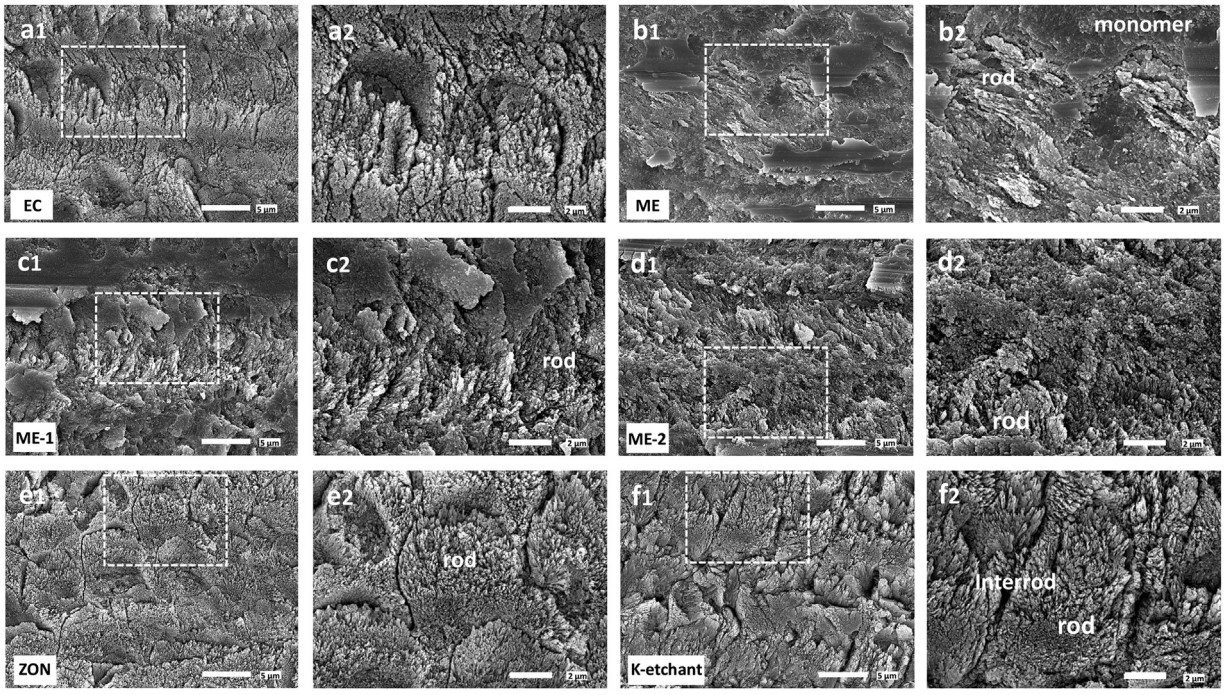

Fig. 2 – SEM photomicrographs of bur-cut enamel surfaces etched with the alternative etchant Enamel Conditioner (Shofu: 'EC') in (a1,2), Multi Etchant (Yamakin: 'ME') in (b1,2), ME ('ME-1') after additional immersion in acetone three times for 1 min in (c1,2), ME ('ME-2') after rotation in acetone for 24 h in (d1,2), experimental ZON etchant (Ivoclar Vivadent: 'ZON') in (e1,2) and with classic phosphoric acid K-Etchant Syringe (Kuraray Noritake: 'K-Etchant') as control in (f1,2). Original magnification of a/b/c/d/e/f1: 4000×, of a/b/c/d/e/f2: 9000×.

bur mounted in a high-speed handpiece under continuous air/water spray coolant. Half of the micro-specimens were tested immediately to determine the 'immediate' μTBS. The other half were subjected to 50 k thermal cycles (TC) between two water baths at 5 °C and 55 °C using a THE-1200 thermocycler (SD Mechatronik, Munich, Germany) to determine the 'aged' μTBS. As the exposure to each bath was 30 s, the dwell time 3 s and the transfer time between baths was 5 s, total aging lasted 39 days. The μTBS test set-up is schematically presented in Fig. 1.

For the actual μTBS test, the specimens were fixed to a BIOMAT jig using cyanoacrylate-based glue (Model Repair II Blue, Dentsply Sankin, Tochigiken, Japan) and stressed at a crosshead speed of 1 mm/min until failure in a LRX testing device (LRX, Lloyd, Hampshire, UK) using a load cell of 100 N. When specimens failed before actual testing, they were recorded as pre-test failures (ptf's). The mode of failure was determined under 50× magnification using a stereomicroscope (Stemi 2000-CS, Zeiss, Oberkochen, Germany) and classified as either 'cohesive failure in enamel', 'cohesive failure in resin', 'adhesive (interfacial) failure' or 'mixed failure'.

2.5. *SEM of the fractured surfaces*

After μTBS testing, representative fractured surfaces of all experimental groups, originating from specimens with a μTBS close to the mean value, were examined with SEM under an operating voltage of 5 kV. SEM-specimen processing was done as detailed above [22].

2.6. *Transmission electron microscopy (TEM) of adhesive-etched enamel interfaces*

The remaining eight human molars were randomly divided into 4 groups according to the EC-, ME-, ZON- and K-Etchant-etched enamel surfaces. As detailed above, buccal and lingual bur-cut enamel specimens were flattened using the MicroSpecimen Former and a high-speed medium-grit diamond bur. All etchants were next applied strictly following the respective manufacturer's instructions (Table 1), followed by the application of ADU (Table 2). Finally, instead of a resin-based composite, a thin layer of unfilled resin (Heliobond, Ivoclar Vivadent) was applied to facilitate ultra-microtomy and avoid sectioning damage of the TEM diamond knife (Ultra 45°, Diatome, Nidau, Switzerland). Specimens were light-cured for 20 s using the LED light-curing unit used in 'high mode'. Thereafter, the bonded enamel specimens were immediately stored for 1 day in distilled water at 37 °C. All specimens were processed for TEM according to the procedure described in detail before [24].

2.7. *Statistical analysis*

A linear mixed-effects model (LME; R3.1.0; R Foundation for Statistical Computing, Vienna, Austria) with specific contrasts was chosen to statistically analyze the μTBS data. The random factor applied in the statistical model was the individual tooth within each group (72 teeth in total) of micro-specimens grouped per tooth it originated from. The data were modelled

for the three variables 'Etchant' (EC, ME, ZON and K-Etchant), 'Adhesive' (O-FL, C-SE2 and ADU) and 'Aging' (1 w and 50k TC). All pairwise interactions as well as the 3-way interaction were analyzed. The significance level was set at $\alpha = 0.05$.

3. Results

3.1. *pH of etchants*

The pH of the three alternative etchants and the classic phosphoric-acid etchant varied between 0.21 and 0.82 in the order of K-Etchant < ZON < EC < ME (Table 1).

3.2. *SEM of etched enamel surfaces*

Representative SEM photomicrographs of the differently etched enamel surfaces are shown in Fig. 2. The enamel-etching pattern exposed by EC and ZON most closely resembled that resulting from classic phosphoric-acid etching with K-Etchant (Fig. 2a,e, as compared to 2f). However, ME revealed a clearly less pronounced etching pattern, with traces of monomer having remained on the enamel surface (Fig. 2b). After three times immersion in acetone for 1 min, solely a small additional portion of the monomers that had remained attached onto the ME-etched enamel surface was removed (Fig. 2c), while rotation in acetone for 24 h removed most of the attached monomers, hereby making the actual enamel-etching effect of ME observable (Fig. 2d). Nevertheless, no clear keyhole structure of enamel rods with deep etch-pits could be observed upon ME etching, in contrast to the typical acid-etch pattern with complete smear-layer removal and deep micro-retention created by K-Etchant (Fig. 2f). The diameter of the phosphoric-acid etched enamel rods varied around 4–5 μm.

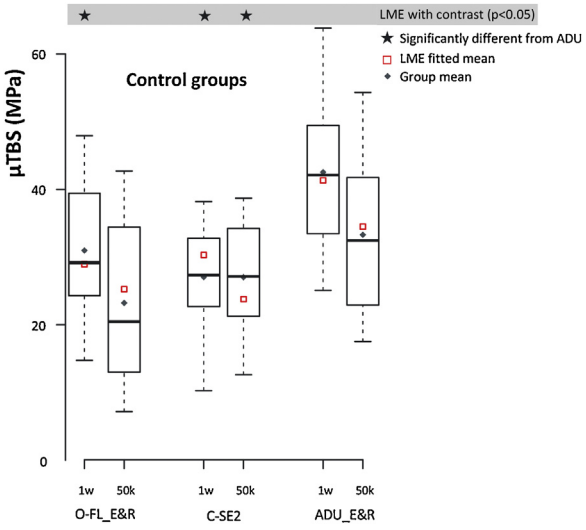

Fig. 3 – Box-and-Whisker plot of 'immediate' μTBS (MPa) measured after 1-week water storage and 'aged' μTBS after 50 k thermal cycles for the controls, namely the 3-step etch&rinse adhesive OptiBond FL (Kerr: 'O-FL'; applied in etch-and-rinse mode using K-Etchant Syringe of Kuraray Noritake), the 2-step self-etch (SE) adhesive Clearfil SE Bond 2 (Kuraray Noritake: 'C-SE2'; applied in self-etch mode) and the universal adhesive Adhese Universal (Ivoclar Vivadent: 'ADU'; applied in etch-and-rinse mode using K-Etchant Syringe of Kuraray Noritake). The black closed dots and the red open squares represent the mean μTBS and the fitted linear mixed-effects (LME) means, respectively. Statistically significant differences are indicated by specific symbols. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

3.3. *μTBS testing*

All μTBS data are detailed in Table 3 and graphically presented in Fig. 3 for the controls and in Fig. 4 for the alternative etchants in comparison to their respective control. No pre-test failures were recorded. In a first step of the statistical analysis, the 3-way interaction 'Etchant × Adhesive × Aging' was analyzed. Significant interaction would have meant that the etchant would have modified the effect of the adhesive differently for 1 w water storage versus 50k TC. However, the interaction among the three factors appeared not significant. Then, the 2-way interaction model was tested, by which each two factors may have pair-wise interacted. Although no significant interaction for each two-factor pairs was found, this interaction was kept to build the LME model. Hence, the final LME model included the factors 'Etchant', 'Adhesive', 'Aging', 'Etchant × Adhesive', 'Adhesive × Aging' and 'Etchant × Aging'. Statistically significant differences were marked in Figs. 3 and 4 with specific symbols.

Regarding immediate μTBS of the controls to enamel (Fig. 3), the universal adhesive ADU combined with the classic phosphoric-acid etchant (ADU_E&R) K-Etchant produced a significantly ($p < 0.05$) higher bond strength than C-SE2 applied to enamel in SE mode, as could be expected, but also than the O-FL/K-Etchant combination (O-FL_E&R), which was not expected. Upon thermo-cycling, the aged μTBS remained solely significantly higher ($p < 0.05$) than that of aged C-SE2 bonded in SE mode to enamel.

The control μTBS data with 95% confidence intervals were depicted in the background of Fig. 4, enabling direct comparison of the bond strengths recorded for the different etchant/adhesive combinations with those measured when the adhesives were employed after classic phosphoric-acid etching (control). Solely when enamel was etched with ME and the 3E&Ra O-FL was applied, the μTBS was statistically significantly lower ($p < 0.05$) than that of the K-Etchant/O-FL combination, regardless of 1w water storage or 50k TC. When all three alternative etchants EC, ME and ZON were followed by the universal adhesive ADU, the resultant immediate μTBS was statistically significantly higher ($p < 0.05$) than that of each alternative etchant/O-FL combination; this was likewise recorded for the aged μTBS of the EC/O-FL and ME/O-FL combinations. A significant ($p < 0.05$) decrease in μTBS upon aging was solely recorded for the ME/C-SE2 and ME/ADU combinations, indicating that among all etchants tested, ME etching conducted before any adhesive application resulted in an adhesive-enamel bond that less withstood bond degradation (this when also considering the substantial, though

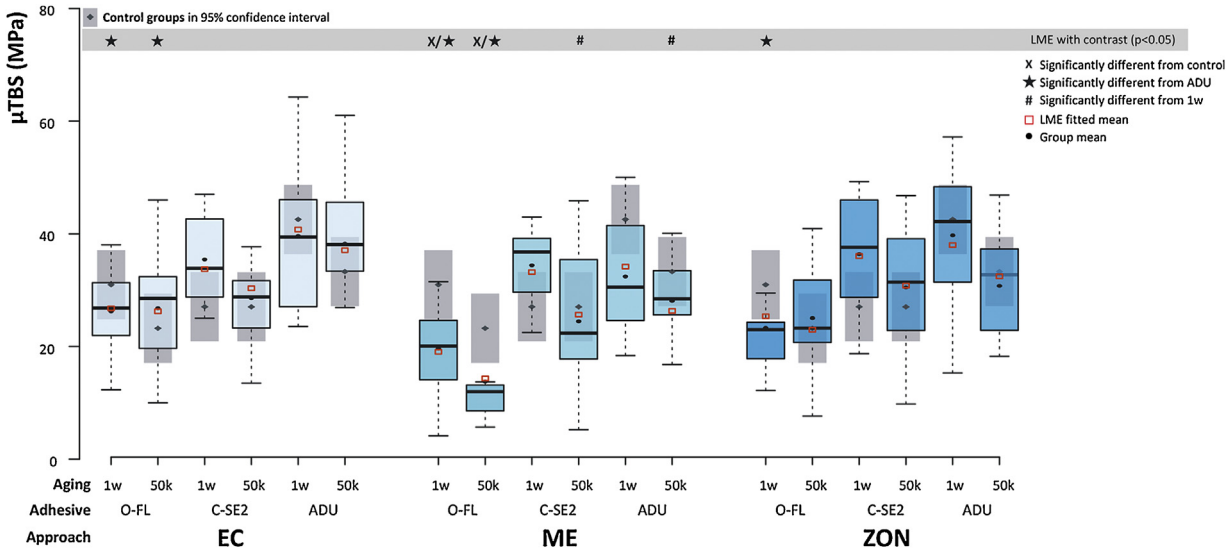

Fig. 4 – Box-and-Whisker plot of 'immediate' μTBS (MPa) measured after 1-week water storage and 'aged' μTBS after 50k thermal cycles for the tested experimental etchant/adhesive combinations EC/O-FL, EC/C-SE2 and EC/ADU, ME/O-FL, ME/C-SE2 and ME/ADU, and ZON/O-FL, ZON/C-SE2 and ZON/ADU. The black closed dots and the red open squares represent the mean μTBS and the fitted linear mixed-effects (LME) means, respectively. The grey boxes in the background represent the 95% confidence interval of the controls (also graphically presented in Fig.3). Statistically significant differences are indicated by specific symbols. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

non-significant decrease in μTBS recorded upon aging for the ME/O-FL combination). When etching enamel with EC and ZON, all three adhesives demonstrated no significant ($p > 0.05$) difference in bond strength when the specimens were tested after 1w water storage or after 50k TC aging.

3.4. *Failure-mode analysis and SEM of fractured surfaces*

The failure modes in percentage are graphically presented for the different experimental groups in Fig. 5. Overall, no clear trends in failure modes between the experimental groups and in failure-mode changes upon aging were noted, with solely a slight tendency to an increased percentage of adhesive (interfacial) failures recorded upon 50 k TC. Representative SEM photomicrographs of fractured micro-specimens prepared using the different etchants applied before to the application of the universal adhesive ADU upon 1 w water storage and 50 k TC are shown in Fig. 6.

3.5. *TEM of adhesive-etched enamel interfaces*

Representative ultra-structural TEM photomicrographs illustrate the respective ADU-etched enamel interfaces produced upon employing the classic phosphoric-acid etchant in Fig. 7 and the three alternative etchants in Figs. 8–10.

A tight interface was obtained when enamel was conditioned with the classic phosphoric-acid etchant K-etchant (Fig. 7). Phosphoric acid completely dissolved and upon rinsing removed the bur-cut smear layer. Besides micro-resin tags having infiltrated the tiny intrarod etch pits, some macro-resin tags filling the wider interrod etch pits were observed at the interface (Fig. 7c). The average width of enamel crystals at the interface was around 50 nm (Fig. 7d), indicating that the crystals were partially dissolved and thus thinned by phosphoric-acid etching. The crystal width was only slightly larger, varying between 50 and 100 nm at the EC/ADU and ZON/ADU enamel interface (Figs. 8d and 10 d). Overall, the ultrastructure of the EC/ADU- (Fig. 8) and ZON/ADU-enamel interface (Fig. 10) resembled that of the ADU_E&R-enamel interface (Fig. 7). The universal adhesive could effectively impregnate in between the partially dissolved enamel hydroxyapatite crystals. However, ME-etched enamel revealed hydroxyapatite rods that appeared not much dissolved, while also smear-layer remnants could still be detected at the ME/ADU-enamel interface (Fig. 9). The micro-retentiveness of ME-etched enamel was clearly lower than that of phosphoric-acid etched enamel (Fig. 9 versus Fig. 7). In addition, the average width of enamel crystals at the ME/ADU-enamel interface was clearly larger than that of the crystals exposed by the other three etchants. Thus, the universal adhesive ADU relatively superficially interacted at the ME-etched enamel surface.

4. Discussion

The present study examined the (etching) effectiveness of three phosphoric-acid alternative etchants when combined with three representative adhesives by measuring bond strength to enamel along with SEM surface analysis of the resultant enamel etch patterns and TEM ultrastructural interfacial analysis of the resultant adhesive-etched enamel interfaces. Classic phosphoric-acid etching served as the logic

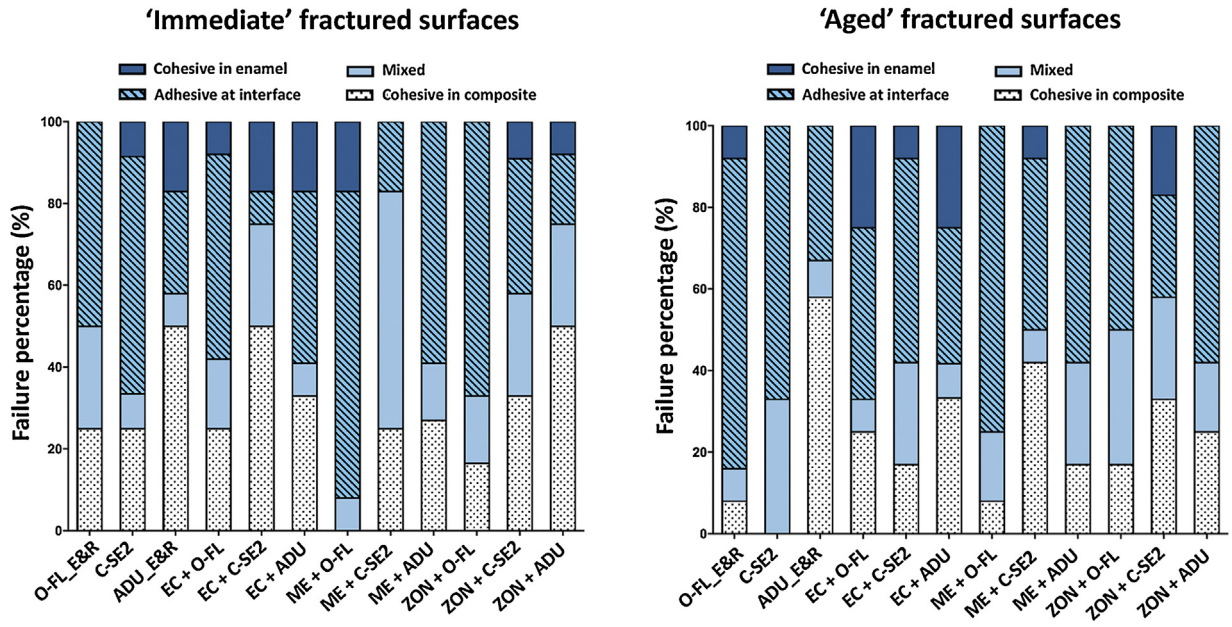

Fig. 5 – 'Immediate' and 'aged' μTBS failure mode distribution as analyzed by light microscopy.

Table 3 – The mean μTBS (MPa) and fitted LME mean for the experimental groups investigated.

Groups	Immediate (1 week)			Aged (50k thermal cycles)		
	Mean	SD	Fitted LME mean	Mean	SD	Fitted LME mean
EC + O-FL	26.2	7.5	26.8	26.8	10.3	26.2
EC + C-SE2	35.4	7.9	33.7	28.6	9.9	30.3
EC + ADU	39.6	13.7	40.8	38.3	13.6	37.1
ME + O-FL	19.6	7.6	19.0	13.8	8.5	14.3
ME + C-SE2	34.4	6.4	33.2	24.5	13.0	25.7
ME + ADU	32.4	10.4	34.2	28.1	7.9	26.3
ZON + O-FL	23.3	9.8	25.4	25.1	9.0	23.0
ZON + C-SE2	36.4	10.4	36.1	30.5	11.0	30.8
ZON + ADU	39.7	13.2	38.0	30.8	9.0	32.5
O-FL_E&R control	31.0	10.6	28.9	23.2	12.4	25.3
C-SE2_SE control	27.1	8.0	30.3	27.0	8.2	23.8
ADU_U-E&R control	42.6	12.1	41.3	33.3	11.7	34.5

control. While it is generally accepted that enamel requires phosphoric-acid etching to achieve durable bonding, overall the alternative etchants performed favorably in terms of immediate and aged μTBS with the sole exception of ME, especially when combined with O-FL. In addition, the aged μTBS recorded for the ME/C-SE2 and ME/ADU etchant/adhesive combinations significantly decreased as compared to the immediate μTBS measured. Hence, both null hypotheses tested should be accepted for the alternative etchants EC and ZON but failed to be accepted for ME.

As compared to the micro-retentive etch pattern exposed at enamel by classic phosphoric-acid etching, as imaged by SEM, a clearly lower micro-retentiveetch pattern resulted from ME-etching. Monomer remnants were even found to have partially covered the surface. ME contains the phosphoric-acid monomer MTEPG, which is an acidic monomer having resulted in an etchant with a pH of 0.8; ME is considered a 'mild' etchant by its manufacturer. Despite MTEPG's pH still remains below 1, the pH of ME was the highest among all etchants investigated. Being based on MTEPG, ME was expected to produce both opportunities for micro-mechanical interlocking by etching as well as to chemically interact through its phosphate group with hydroxyapatite, like this twofold interaction pattern has thoroughly been documented for the functional monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) [25–27]. The partial coverage of enamel by ME monomers that remained attached to the surface despite being water-rinsed, may indicate that MTEGP chemically interacted with enamel and was/could not (be) removed by simple water rinsing. Additional experiments, including acetone employed to remove any monomers that remained attached onto the ME-etched enamel surface, revealed that enamel was much less etched by ME, this in particular when compared with classic phosphoric-acid etched enamel. TEM interface analysis confirmed the lower etching ability of ME in a sense that not all surface smear was dissolved/removed but got embedded in the adhesive resin. In addition, the enamel hydroxyapatite rods appeared not much dissolved, as compared to what the other two alternative etchants and classic phosphoric-acid etching induced.

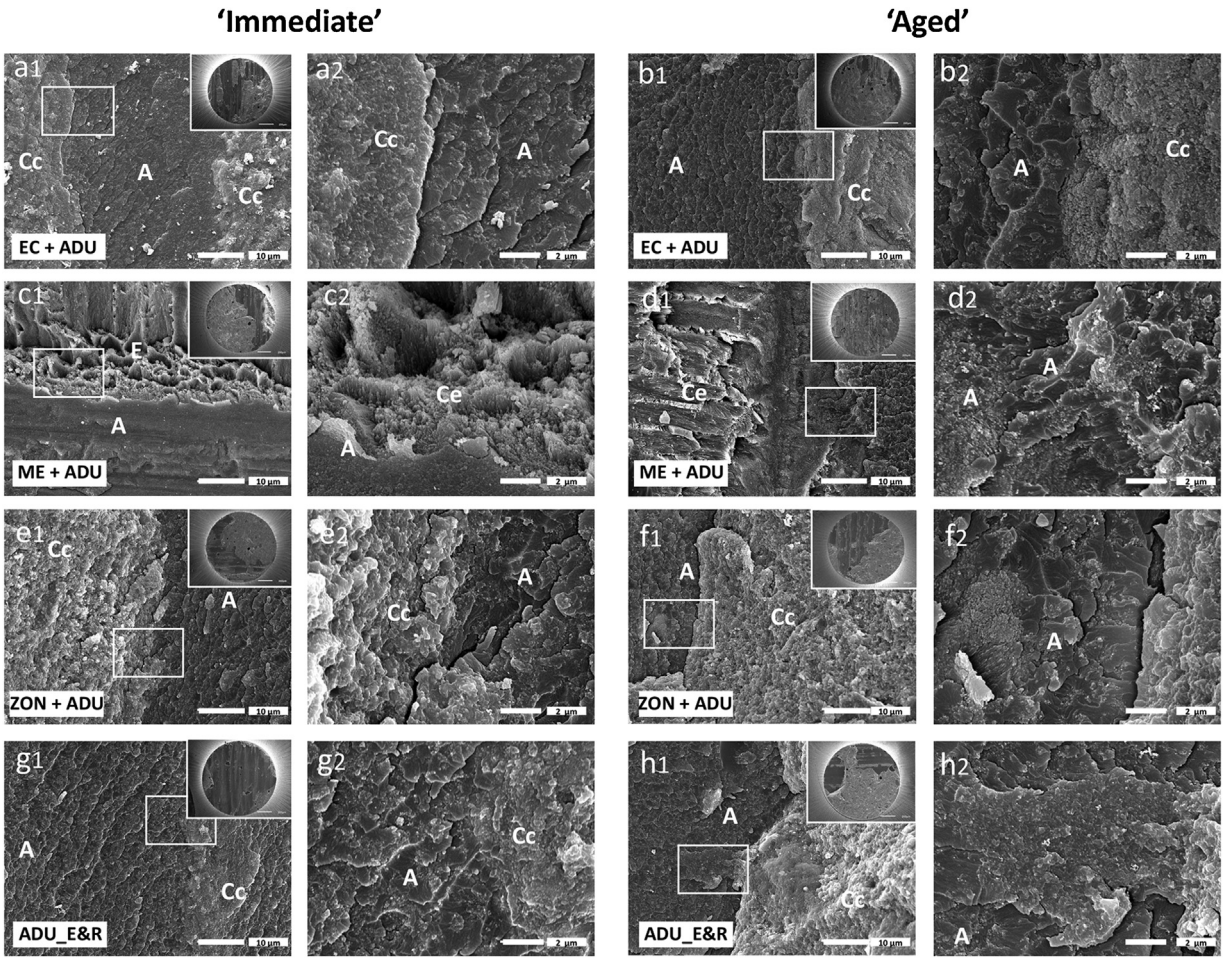

Fig. 6 – Representative SEM photomicrographs of the 'immediate' and 'aged' fractured surfaces of the tested experimental etchant/adhesive combinations EC + ADU in (a,b), ME + ADU in (c,d), ZON + ADU in (e,f) and K-etchant combined with ADU (ADU_E&R) in (g,h). All photomicrographs represent the fractured enamel side. (a1) EC + ADU, revealing a mixed failure mode with almost half of the area showing fractured composite (low magnification image in the insert: 90× original magnification). (b1) Aged EC + ADU, revealing mainly a cohesive failure in composite. (c1) ME + ADU, revealing a mixed failure in the insert with exposure of enamel. (d1) Aged ME + ADU, mainly revealing an adhesive interfacial failure with exposure of enamel. (e1) ZON + ADU, revealing a mixed failure. (f1) Aged ZON + ADU, revealing a mixed failure. (g1) ADU_E&R, revealing mainly an adhesive interfacial failure with composite remaining on the fractured surface. (h1) Aged ADU_E&R, revealing a mixed failure with the adhesive layer still complete and uniform. Higher magnification of (a-h1) in (a-h2). A: adhesive failure; Cc: cohesive failure in composite; Ce: cohesive failure in enamel; E: enamel.

Previous research investigated the etching efficacy of different functional monomers, among which 10-MDP and MTEGP [28]. The etching intensity of MTEGP and more specifically the Ca concentration released by MTEGP from hydroxyapatite particles was significantly lower than those of/by 10-MDP, the latter in particular when also the Ca amount that was released from hydroxyapatite and consumed for Ca-salt nanolayering, was taken into account [28]. Moreover, Ca-release by MTEGP was also significantly lower than that by phenyl-P (2-methacryloxyethyl phenyl hydrogen phosphate) [28]. Nevertheless, MTEGP's etching efficacy was higher than that of the carboxylate functional monomer 4-MET (2-methacryloxyethyl trimellitic acid). The lower etching efficacy of MTEGP was also confirmed in that study in the sense that the MTEGP-treated enamel surfaces revealed smear-layer grinding scratches, in contrast to the exposure of keyhole enamel-prism structures by phosphoric acid [28]. This lower etching efficacy of ME was also reflected in the significant decrease in aged bond strength when combined with C-SE2 and ADU, indicating that these ME/adhesive combinations less resisted interfacial degradation. The shallower etching pattern induced by ME must have resulted in less deep penetration of the adhesive into the ME-etched enamel surface, as was revealed by TEM. This less deep resin infiltration within (forming micro-resin tags) and in between (forming macro-resin tags) the enamel prisms may have lowered the adhesive-enamel bond strength and bond durability. Previous research, conducted by Yoshihara et al. [25], showed that the higher crystallinity, the larger crystal structure and the parallel orientation of enamel hydroxyapatite resulted in a lower chemical interaction potential of the functional monomer 10-MDP at enamel than at dentin that exhibits a lower crystallinity, smaller and

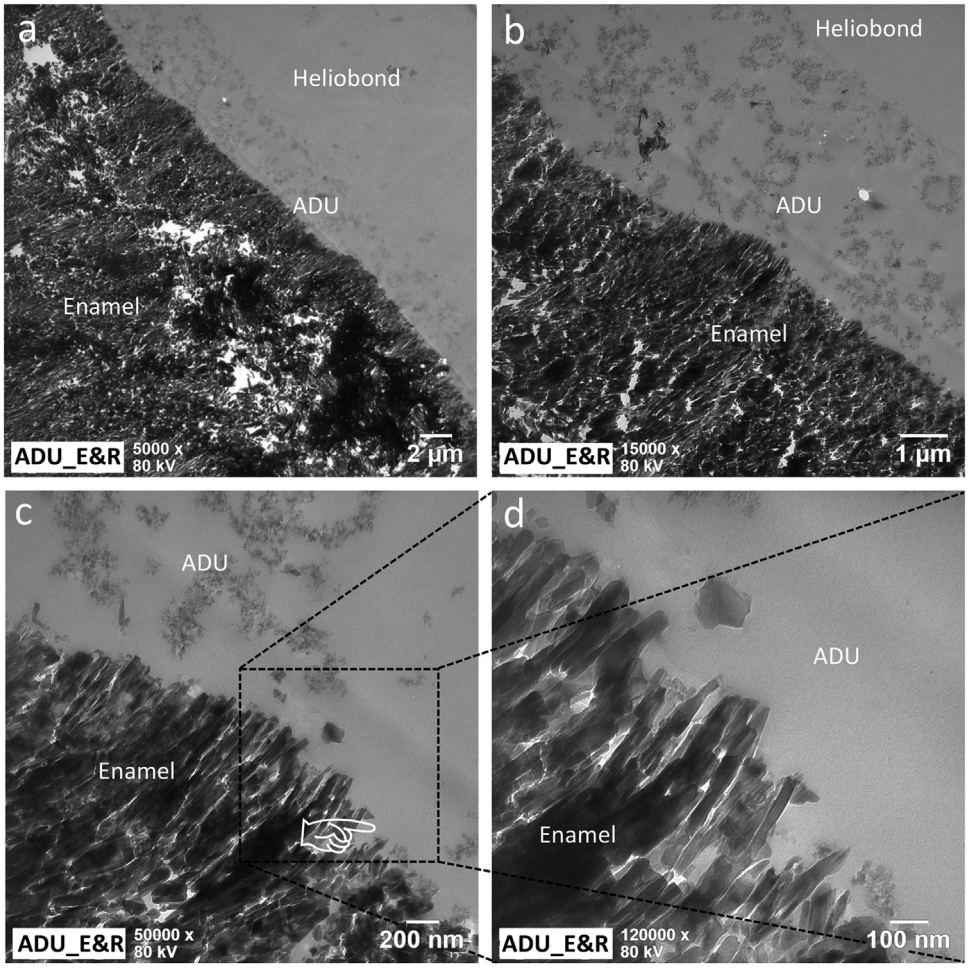

Fig. 7 – TEM photomicrographs of the interface produced by ADU bonded to enamel following the etch-and-rinse approach using K-Etchant Syringe (Kuraray Noritake). (a) Overview of the interface, revealing a tight adhesive-enamel interface. (b–d) High magnification of (a): Adhesive resin effectively infiltrated the phosphoric-acid etched enamel surface, having produced macro- and micro-resin tags. The mean hydroxyapatite crystal width at etched enamel was around 50 nm.

cross-cross oriented crystals [14]. Furthermore, Mine et al. [29] found that the enamel smear complex, when insufficiently dissolved/removed, affected the inter/intra-prism penetration of adhesive monomers. TEM confirmed that smear debris was embedded at the ME/adhesive-enamel interface.

Other previous research reported that the length and hydrophilicity of the monomer's spacer chain can influence enamel-bonding effectiveness [30]. In that study, MTEPG (being referred as MTEP in that study) was ascribed a high contact angle, indicating low dentin wettability, and a more hydrophilic carbon chain. MTEPG was found to be much more hydrophilic (logarithmic partition coefficient of 0.71) than 10-MDP (logarithmic partition coefficient of 4.09) [30]. MTEP (or MTEPG) was also found to form less monomer-Ca salts than 10-MDP [31]. A more hydrophilic monomer is additionally expected to absorb more water, weakening the monomer-Ca interaction and eventually also the resultant adhesive-tooth bond [32]. In this way, also the higher hydrophilicity of MTEPG may have contributed to the lower aged enamel bond-strength results recorded for ME. Despite the lower bond strength to enamel, ME may alternatively be useful as dentin etchant or for instance to pretreat enamel to bond orthodontic brackets. In order to confirm such potential other ME applications, future studies obviously need to be conducted.

The alternative etchant EC revealed bond strengths for the three EC/adhesive combinations close to those obtained with the respective controls, including when classic phosphoric-acid etching was conducted. This finding indicates that EC's etching efficacy approximates that of classic phosphoric-acid etching. In contrast to ME, EC effectively dissolved/removed surface smear and exposed enamel prisms, providing clear SEM indications of a satisfactory micro-retentive enamel surface. As disclosed by TEM, the EC-etched hydroxyapatite crystals varied in size between 50 and 100 nm, thereby approaching the thin crystal widths typically exposed by classic phosphoric-acid etching. As a result, numerous micro-resin tags were formed, as disclosed by TEM; they obturated the tiny intraprism etch pits, which definitely must have contributed to the favorable and durable bond strengths recorded. Besides the knowledge that EC contains organic acids, no further composition detail was released by its manufacturer. Organic acids, like maleic and citric acid, have been added to rather older generation adhesives [33,34]. Several self-etch adhesives incorporate organic acids [35]. A

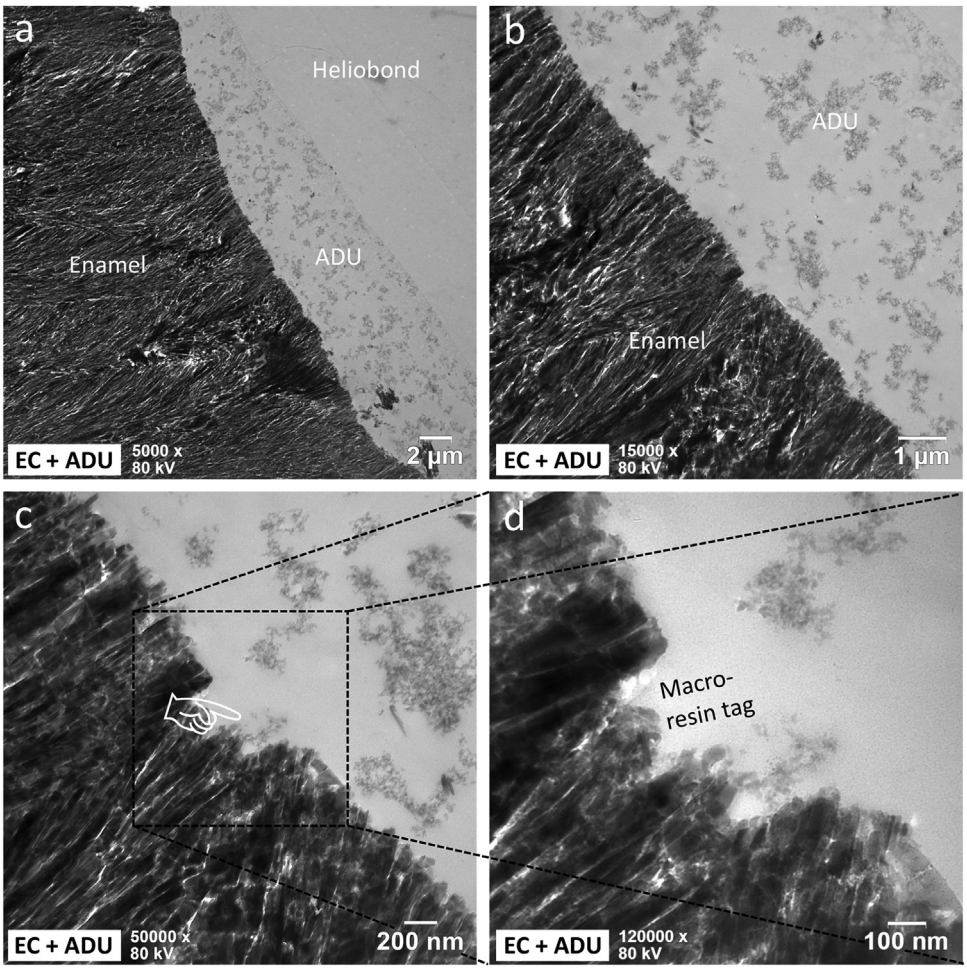

Fig. 8 – TEM photomicrographs of the interface produced by ADU bonded to enamel following EC etching. (a) Overview of the interface, revealing a tight and void-free interface. (b–d) High magnification of (a): the hydroxyapatite crystals were partially dissolved by EC. Macro- and micro-resin tags were formed. The mean hydroxyapatite crystal width at etched enamel varied from 50 to 100 nm upon EC etching.

recent study also provided supportive evidence that glycolic acid could effectively etch enamel and dentin [36]. Previous research analyzed the chemical interaction of carboxylic acids with hydroxyapatite and found that these acids can be chemisorbed and chemically bonded to hydroxyapatite by ionic interaction [37]. Fu et al. [38] also reported that maleic acid can chemisorb to hydroxyapatite and enamel, while others doubt this and reported that according to the so-called Adhesion-Decalcification concept (AD concept) maleic acid rather demineralizes hydroxyapatite-based tissues than adheres to them due to unstable Ca-salt formation [37]. Consequently, it is currently not known if the organic acids in EC may have possessed chemical affinity to hydroxyapatite. In fact, the actual mechanisms of interaction of the organic acids in EC with hydroxyapatite can only be elucidated, when EC's detailed composition is publicly released.

Interestingly, no significant difference in immediate and aged bond strength was recorded when comparing the third alternative etchant ZON with the classic phosphoric-acid etchant. ZON is based on the lewis acidic metal salt zirconium oxynitrate or $ZrO(NO_3)_2$, which is highly soluble in water [39,40]. Previous research involved the preparation of transition metal salt solutions mixed with 37% phosphoric acid, to be used as a combined etchant and activator prior to the use of an anaerobic adhesive [41]. Limited data on the etching efficacy of this metal salt-based etchant on enamel is available in literature. However, many researches on zirconium salts in aqueous solutions were conducted in other fields like applied chemistry [42]. Such zirconium salts are dissoluble in water, by which intense hydrolysis of zirconium salts in aqueous solutions occurs [43]. Besides, aqueous solutions of zirconium salts are acidic [43]. Thus, the etching efficacy of ZON should be attributed for a large extent to hydrolysis effects of the zirconium-oxynitrate metal salt, which creates an acid environment and so is capable of etching enamel.

Apart from the micro-retentive etching pattern imaged by SEM, TEM ultrastructural interfacial analysis confirmed that ZON effectively etched enamel, this also based on the findings that all surface smear was dissolved/removed and the hydroxyapatite crystals at the exposed enamel prisms were reduced in size. The etching efficacy of etchants was before reported to depend on several factors, among which also the etching time and pH. In this study, the etching time varied between 10 s (EC), 15 s (K-Etchant), 20 s (ME) and 30 s (ZON),

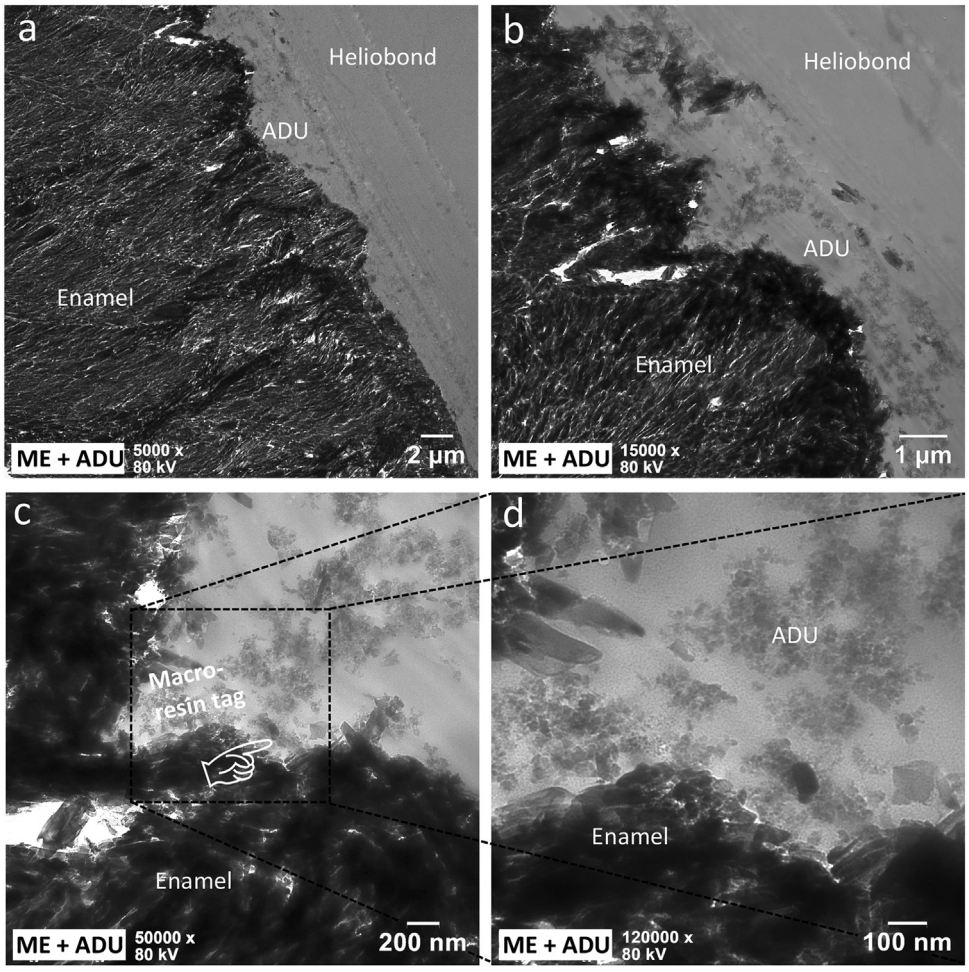

Fig. 9 – TEM photomicrographs of the interface produced by ADU bonded to enamel following ME etching. (a) Overview of the interface, revealing smear debris that partially remained at the adhesive-enamel interface. (b–d) High magnification of (a): the adhesive did clearly less deeply penetrate into the ME-etched enamel than when enamel was classically etched using phosphoric acid.

as instructed by the different manufacturers. Based on the results from Perdigão et al. [44], a significant correlation exists between pH and enamel bond strength, indicating that this bond strength tended to increase with increasing acidity of the etchant. Among all alternative etchants, the pH of ZON with 0.56 was the lowest, versus 0.63 and 0.82 measured for EC and ME, respectively. Nevertheless, ZON's pH remained higher than that of the classic phosphoric-acid etchant (pH 0.21). Based on the ZON data obtained in this study, it can be concluded that ZON is a promising enamel etchant.

Somewhat surprisingly, the immediate and aged μTBS of ADU to enamel using all three alternative etchants was significantly higher than all etchant/O-FL combinations, with the exception of the aged μTBS of the ZON/O-FL combination. This finding was in agreement with a previous study that recorded a high bond strength of ADU to phosphoric-acid etched enamel, as compared to ADU applied in a passive (no rubbing) self-etch mode [45]. The 'immediate' ADU specimens tended to fail more cohesively in composite as compared to the O-FL specimens. Indeed, cohesive failure in composite is commonly associated with higher bond strength when the fracture propagates through the composite bulk [46]. The higher bond strengths recorded for ADU should maybe also be ascribed to the specific functional monomers it contains. ADU contains besides 10-MDP also a methacrylated carboxylic-acid polymer. Both 10-MDP and this carboxylic-acid functional polymer have chemical bonding potential to hydroxyapatite [27,47].

Regarding bond durability to enamel, apart from the lower bond strength recorded for ME upon aging, the bond strength of the EC/ and ZON/adhesive combinations was stable. This finding is in agreement with previous research that assessed the mechanical properties of adhesive-enamel interfaces produced following an E&R approach [48]. Stable bonding to enamel can easily be achieved on the condition that sufficient micro-retention is produced by etching. EC and ZON appeared to result in adhesive-enamel interfaces that are as stable as those obtained to classic phosphoric-acid etched enamel. Enamel is a mainly inorganic tissue that contains only small amounts of water and organic substances, while dentin is composed of substantially more organic components, mainly in the form of collagen fibrils. It is therefore much more difficult for water to diffuse into an adhesive-enamel interface sealed by macro- and micro-resin tags than into a much more

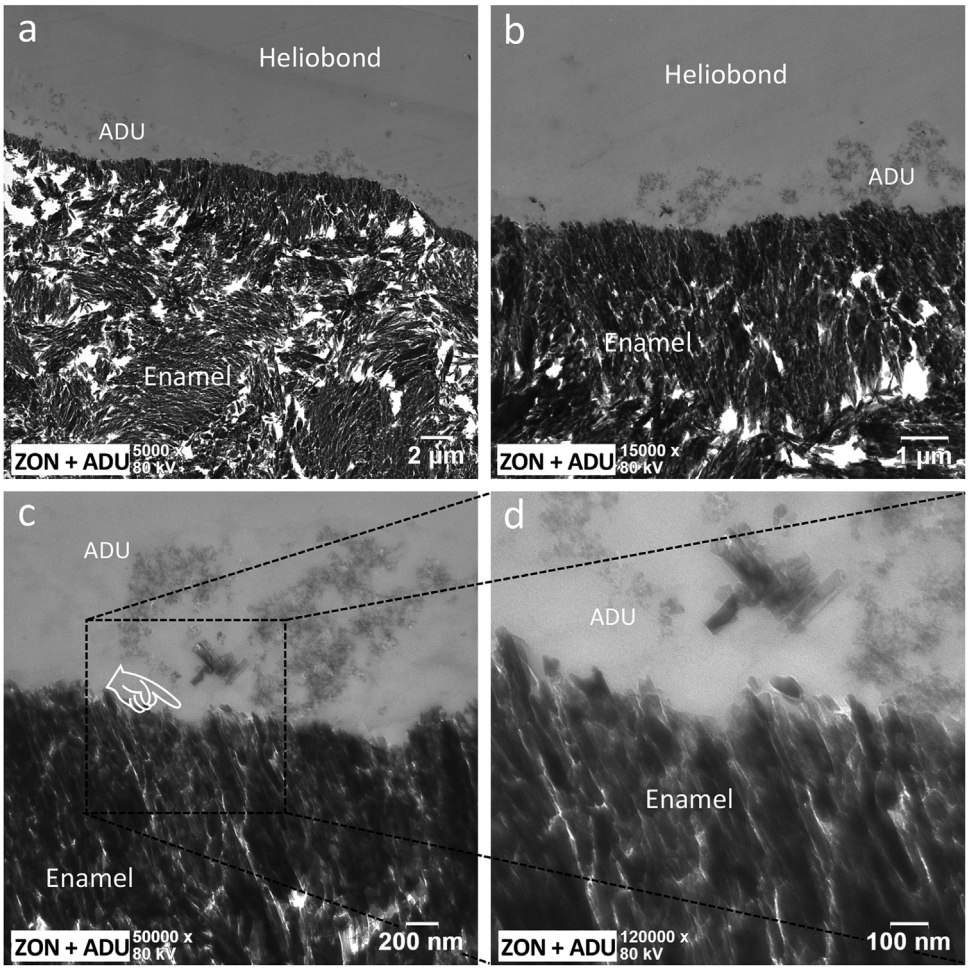

Fig. 10 – TEM photomicrographs of the interface produced by ADU bonded to enamel following ZON etching. (a) Overview of the interface, revealing a tight adhesive-enamel interface. (b–d) High magnification of (a): the exposed etch pits were filled with macro- and micro-resin tags. The mean hydroxyapatite crystal width at etched enamel varied from 50 to 100 nm upon ZON etching.

permeable interface that can be obtained by an adhesive when bonded to dentin. It is well known that this adhesive-dentin interface will always be more vulnerable to degradation.

Finally, considering that the current study involved laboratory research, the promising enamel bonding performance of adhesives, when combined with two of the three alternative etchants investigated in this study, should be further investigated in clinical studies.

5. Conclusion

We investigated the bonding effectiveness and bond durability to enamel when enamel was etched with three new etchants as alternative for a classic phosphoric-acid etchant and when combined with three representative adhesives. Bond-strength testing along with ultra-structural interfacial characterization of adhesive-enamel interfaces enabled to conclude that the alternative etchant ME failed to match the enamel bond-promoting effect of phosphoric acid, most likely because of ME's milder acidity and higher hydrophilicity. On the contrary, the etching efficacy of EC and ZON was found equivalent with that of phosphoric acid and based on the data of this study should be considered as promising alternative etchants for phosphoric acid when bonding onto enamel.

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