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ORIGINAL ARTICLE

Hydrolytic stability of three-step etch-and-rinse adhesives in occlusal class-I cavities

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Abstract
Objectives A dental adhesive without small and hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA) and triethylene glycol dimethacrylate (TEGDMA) would be beneficial in order to avoid contact allergies. However, these monomers are important to increase infiltration and polymerization of the adhesive. Therefore, the purpose of this study was to evaluate the bonding effectiveness and bond durability of a more hydrophobic and biocompatible adhesive as compared to a conventional three-step etch-and-rinse adhesive.
Methods Sixteen non-carious human third molars were used to determine the micro-tensile bond strength testing (μTBS) and interfacial ultrastructure by transmission electron microscopy (TEM) of the more hydrophobic cmf adhesive system (Saremco) adhesive as compared to the control OptiBond FL (Kerr).
Results The more hydrophobic and biocompatible three-step etch-and-rinse adhesive was able to produce a reasonable short-time bonding effectiveness. In the long term, the collagen fibrils in the hybrid layer were not effectively protected and were prone to hydrolytic degradation. As a result, long-term bonding effectiveness of this novel adhesive was very low.
Conclusions Application of a more hydrophobic adhesive without altering the application procedure considerably results in a reduced durability of the created bond
Clinical relevance Omitting small and hydrophilic components from the adhesive formulation may impair the durability of your composite restoration.

Keywords Class-I cavity dentin · Bond strength · TEM · Three-step etch-and-rinse · Durability · Allergy

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Introduction

Methacrylates such as 2-hydroxyethyl methacrylate (HEMA) and triethylene glycol dimethacrylate (TEGDMA) are often used in dental adhesives [1]. Cross-linking methacrylate monomers such as TEGDMA provide immediate mechanical strength to the adhesive system by forming densely cross-linked polymers. Hydrophilic monomers, such as HEMA, are considered equally important components of dental adhesives thanks to their wetting enhancement effect [2]. However, these methacrylate monomers have proved to be potent contact allergens [3]. Therefore, an adhesive without these small and hydrophilic monomers might cause fewer allergies. Moreover, an adhesive that mainly consists of hydrophobic resins might also be more hydrolytically stable and less vulnerable to harmful enzymes such as esterases [4]. Omitting of such essential components may however affect various properties as polymerization degree [5], strength [6], infiltration into dental tissues [7] and resin stability [8], which might affect long-term bonding effectiveness.

Therefore, the purpose of this study was to evaluate if such a more hydrophobic and biocompatible adhesive can create interfaces that are as strong and as stable as conventional three-step etch-and-rinse adhesives. The hypothesis tested was that the bonding effectiveness of a hydrophobic three-step etch-and-rinse and a conventional 'golden standard' three-step etch-and-rinse adhesive is similar after hydrolytic degradation by water storage for 6 months or 10 % NaOCl exposure for 1 h. Bonding effectiveness was assessed mechanically and ultra-morphologically by micro-tensile bond strength testing (μTBS) and transmission electron microscopy (TEM), respectively.

Materials and methods

Specimen preparation

Sixteen non-carious human third molars were gathered following informed consent approved by the Commission for Medical Ethics of the Catholic University of Leuven. They were stored in 0.5 % chloramine solution at 4 °C and used within 1 month after extraction. First, all teeth were mounted in gypsum blocks in order to facilitate manipulation. A standard box-type class-I cavity (4.5×4.5 mm^2, 2.5 mm deep) was prepared at the occlusal crown centre with the pulpal floor ending at mid-coronal dentin, using a cylindrical medium-grit (100 μm) diamond bur (842; Komet, Lemgo, Germany) in a water-cooled high-speed turbine mounted in the MicroSpecimen Former (University of Iowa, Iowa City, IA, USA). All cavity surfaces were carefully verified for absence of enamel and/or pulp tissue using a stereo-microscope (Wild M5A, Heerbrugg, Switzerland). The teeth were randomly divided into two groups per two adhesives tested. Next, a three-step etch-and-rinse adhesive (cmf adhesive system, Saremco) adhesive and a new low-shrinkage composite (extra low shrinkage [els], Saremco) were applied according to the manufacturer's instructions (Table 1). A three-step etch-and-rinse adhesive OptiBond FL (Kerr, Orange, CA, USA) was used as control. The cavity was filled in one layer. All Light-curing was performed using a high-power LED light curing device (L.E.Demetron I;

Table 1 Materials used

Materials	Composition	Application
Adhesive		
cmf | adhesive system	cmf Etch [LOT: 05.2012-02]: water, H_3PO_4, phosphoric salt, gel former, colorant. Gel buffered to a pH of 1.5	(1) Etch enamel and dentin for 30 s with cmf Etch.
Saremco (St. Gallen, Switzerland)	cmf Primer [LOT: 06.2012-01]: alcohol, acetone, water, methacrylated phosphoric salt, CQ, co-initiator (pH=4.5)	(2) Rinse for 30 s and air-dry for 5 s.
	cmf Bond [LOT: 05.2012-07]: hydrophilic ethoxylated Bis-GMA, silanized barium glass, CQ, co-initiator	(3) Apply cmf Primer using a rubbing motion for 30 s, dry for 5 s and light cure for 20 s.
		(4) Apply cmf Bond using a rubbing motion for 20 s.
		(5) Light-cure for 30 s.
OptiBond FL (Kerr, Orange, CA, USA)	Kerr Gel Etchant [LOT: 2965863]: 37.5 % H_3PO_4. pH=0.0	(1) Apply etchant for 15 s. Rinse for 15 s. Gently air-dry for a few seconds being careful not to desiccate dentin.
	Primer [LOT: 2970962]: HEMA, GPDM, MMEP, water, ethanol, CQ, BHT. pH=1.9	(2) Apply FL Primer with light scrubbing for 15 s. Gently air-dry for 5 s.
	Adhesive [LOT: 2970934]: Bis-GMA, HEMA, GDMA, CQ, ODMAB, Filler (fumed SiO_2, barium aluminoborosilicate, Na_2SiF_6), coupling factor A174 (approximately 48 wt% filled)	(3) Using the same applicator brush, apply FL Adhesive with light scrubbing for 15 s. Gently air-dry for 5 s.
		(4) Light-cure for 20 s.
Composite	[LOT: 05.2012-55]	(1) Increments of no more than 2.5 mm.
els | extra low shrinkage Shade: A2	Silanized barium glass, Bis-GMA, Bis-EMA, catalysis, inhibitor, pigments	(2) Light-cure for 40 s.
Saremco		

CQ camphorquinone (photo-initiator), *Bis-GMA* bisphenol A diglycidyl ether dimethacrylate, *HEMA* 2-hydroxyethyl methacrylate, *GPDM* glycerol phosphate dimethacrylate, *MMEP* mono-2-methacryloyloxyethyl phthalate, *BHT* butylhydroxytoluene or butylated hydroxytoluene or 2,6-di-(*tert*-butyl)-4-methylphenol (inhibitor), *GDMA* glycerol dimethacrylate, *ODMAB* 2-(ethylhexyl)-4-(dimethylamino)benzoate (co-initiator), *Bis-EMA* ethoxylated bisphenol A glycol dimethacrylate

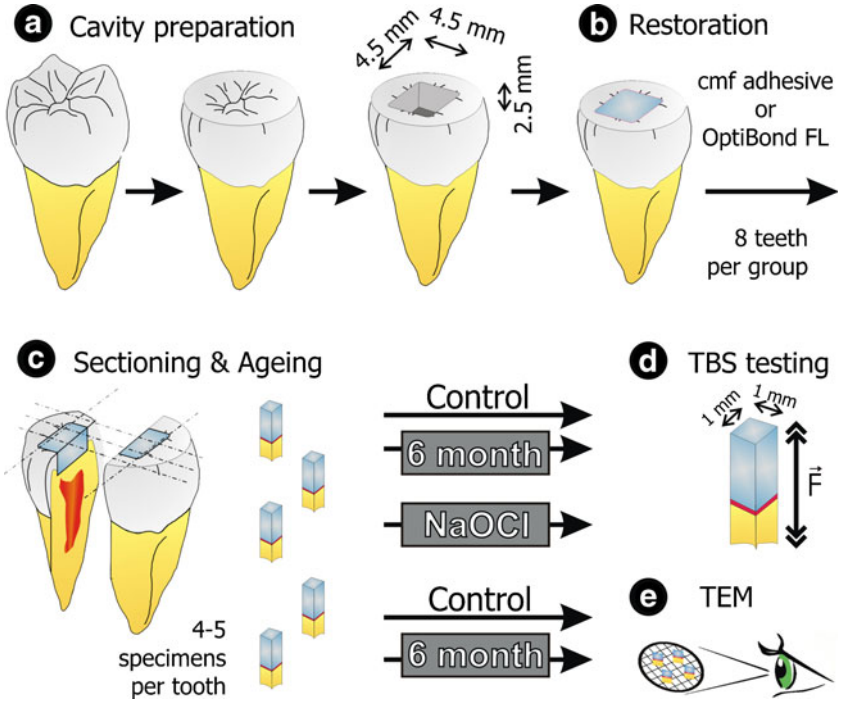

Fig. 1 Schematic illustrating the study design. **a** Human third molars were used to prepare standardized occlusal class-I cavities. **b** Both three-step etch-and-rinse adhesives were applied and a single resin composite was used to fill the cavities. **c** After 1 week, water storage, rectangular composite-tooth sticks were prepared using an automated precision water-cooled diamond saw. After varying storage protocols, we stressed specimens in tensile until failure (**d**) or processed for TEM evaluation (**d**)

Demetron/Kerr, Danbury, CT, USA). After bonding procedures, specimens were stored in distilled water at 37 °C for 1 week. The experimental set-up is presented schematically in Fig. 1.

The teeth were sectioned perpendicular to the adhesive-tooth interface using an automated precision water-cooled diamond saw (Accutom-50; Struers A/S, Ballerup, Denmark) to obtain rectangular sticks (1.0×1.0 mm wide; 5–8 mm long). All specimens from each tooth were checked using the stereo-microscope, and randomly assigned to one of the three storage protocols. One third of the specimens were stored in 10 % NaOCl solution for 1 h, another one third of specimens were stored in 0.5 % chloramine solution at 37 °C for 6 months and the remaining specimens were directly subjected to μTBS testing as control.

μTBS testing

Specimens were fixed to Ciucci's jig with cyanoacrylate glue (Model Repair II Blue; Sankin Kogyo, Tochigi, Japan) and stressed at a crosshead speed of 1 mm/min until failure in a testing device (LRX; Lloyd, Hampshire, UK) using a load cell of 100 N. The μTBS was expressed in MPa, as derived from dividing the imposed force (N) at the time of fracture by the bond area (mm^2). When specimens failed before actual testing, a bond strength of 0 MPa was included in the calculation of the mean μTBS. The actual number of pre-testing failures (ptf) was explicitly noted as well. For each tooth an individual mean was calculated for each group (Control, 6 month, NaOCl). To include data on the origin of teeth in the statistical analysis, these means were then used in a repeated measurements ANOVA analysis. All analyses were conducted at a significance level of 0.05.

Failure analysis

The mode of failure was determined by an optical microscope at a magnification of 50x using the stereo-microscope, and recorded as either 'cohesive failure in dentin', 'adhesive failure', 'mixed adhesive', 'cohesive failure in bonding' or 'cohesive failure in resin'.

Table 2 Micro-tensile bond strength

Adhesive	Storage	Mean (MPa)	SD	*n*	ptf	Scheffé test[a]
cmf	Control	21.0	7.8	11	0	b
	6 month	3.9	3.7	11	3	c
	NaOCl	4.0	2.7	10	0	c
OptiBond FL	Control	49.9	13.1	10	0	a
	6 month	22.9	6.7	11	0	b
	NaOCl	28.2	9.0	10	0	b

SD standard deviation, *n* total number of specimens, *ptf* pre-testing failure

[a]Rows with the same letters (a–c) are not significantly different (Scheffé test, $p>0.05$)

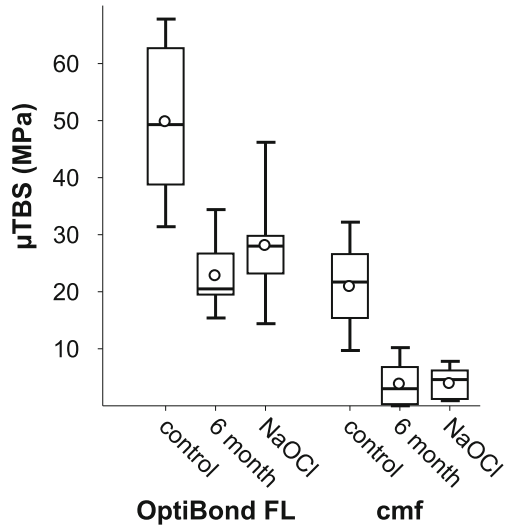

Fig. 2 μTBS results. Box plots — the *box* represents the first, second and third quartiles, while the mean value is given by a *dot*. The *whiskers* extend to the minimum and maximum value

TEM evaluation

In total, eight beams (two adhesives × two aging × two teeth) were selected from the μTBS specimens for TEM observation. The specimens were processed for TEM according to the procedure described by Van Meerbeek et al. [9]. Non-demineralized ultra-thin sections were cut (Ultracut UCT; Leica, Vienna, Austria) and examined unstained and positively stained (5 % uranyl acetate for 12 min/saturated lead citrate for 13 min) using a TEM (JEM-1200EX II, JEOL, Tokyo, Japan).

Results

μTBS testing

Mean μTBS, standard deviations (SD), total number of specimens (*n*) and number of pre-testing failures (ptf) are summarized per groups in Table 2, and graphically presented in Fig. 2. Repeated analysis indicated that both adhesives had a significantly different performance ($p<0.0001$). Significant differences were also observed between the different storage protocols ($p<0.0001$). However, no significant interaction ($p=0.1648$) was observed between both factors, suggesting that both adhesives must have degraded in the same way. Noteworthy is that storage in water for 6 months or in a 10 % NaOCl for 10 min resulted in similar, and non-significantly different, bond strengths (Table 2). None of the specimens failed during specimen sectioning with the diamond blade, but after 6 months of water storage, three specimens from the cmf group failed before actual testing.

Failure analysis

Failure analysis data are presented graphically in Fig. 3. The conventional three-step etch-and-rinse adhesive predominantly showed cohesive failures in dentin or composite and mixed failures. For the experimental hydrophobic adhesive, on the other hand, failure analysis did change with storage period. After exposure to NaOCl or 6 months of water storage, almost all failures occurred at the dentin/adhesive interface, or mixed mostly at the interface and in the bonding resin.

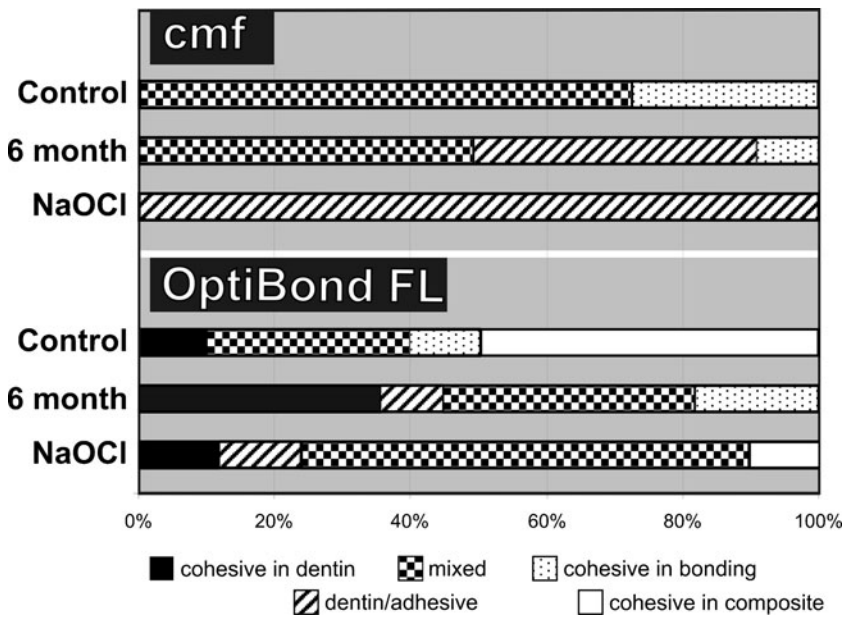

Fig. 3 Failure analysis. The mode of failure was determined light-microscopically at a magnification of ×50 using a stereomicroscope, and recorded as either 'cohesive failure in dentin', 'adhesive failure', 'mixed adhesive' or 'cohesive failure in resin'

TEM evaluation

Overall, hybrid layer thickness was thinner for the hydrophobic adhesive (1.5–3 μm; Fig. 4) than for conventional three-step etch-and-rinse adhesive (3–5 μm; Fig. 5). However, dentin tubules were opened and distinct resin tags were formed for both adhesives. Therefore, the interaction mechanism of both adhesives was similar and consists for both adhesives mainly on a micro-mechanical interlock of the cured resin and the exposed collagen fibrils. Conversely, after 6 months of water storage, the ultra-morphological appearance of cmf changed considerably (Fig. 4). Distinct gaps were formed within the hybrid layer, suggesting a weakening of this part of the interface complex (Fig. 4e). At higher magnifications, also features typically observed in the hybrid layer altered. Collagen fibrils appeared degraded resulting in widening of the interfibrillar spaces and in increased overall porosity. Furthermore, the typical collagen cross-banding could no longer be observed in the water-stored specimens. For the conventional three-step etch-and-rinse adhesive no obvious differences were observed between control specimens and those stored for 6 months (Fig. 5).

Discussion

μTBS and ultra-morphology of the hydrophobic three-step etch-and-rinse adhesive indicated that it is significantly less stable after 6 months water storage as well as after a 10 % NaOCl challenge. Therefore, our hypothesis that the bonding effectiveness of hydrophobic and conventional three-step etch-and-rinse adhesives is similar after hydrolytic degradation, was rejected.

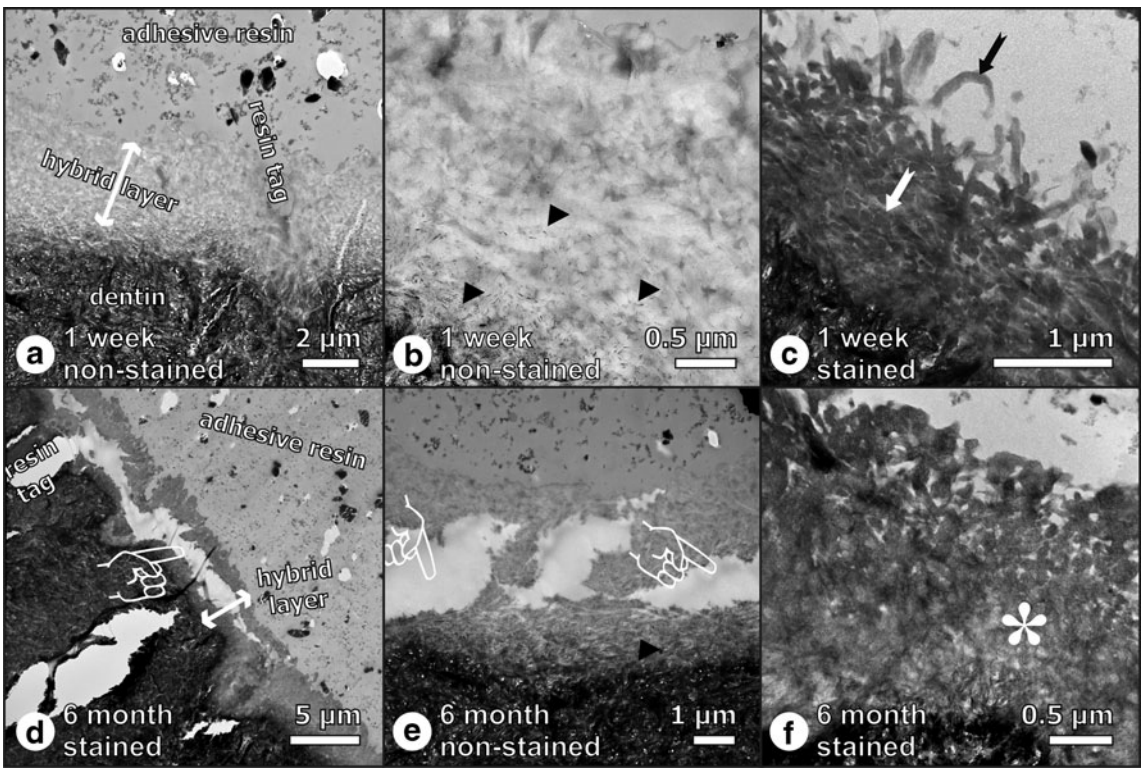

Fig. 4 TEM photomicrographs of the adhesive–dentin interface produced by cmf Adhesive System. **a** Non-demineralized, unstained section illustrating a typical hybrid layer as produced by etch-and-rinse adhesives. The hybrid layer is located in between the arrows. The dentin tubules were opened, enabling the formation of resin tags. The hybrid layer varied in thickness between 1.5 and 3 μm. **b** Control, non-demineralized, unstained section, detailing the hybrid layer, and showing that the transition of the hybrid layer to unaffected dentin is not abrupt, with gradually more hydroxyapatite (*arrowhead*) remaining near the bottom of the hybrid layer. No filler particles can be observed within the demineralized dentin. **c** Control, non-demineralized, UA/LC stained section showing the hybrid layer of approximately 1.5–3 μm in detail. The transition between demineralized and intact (unaffected) dentin is abrupt. The typical cross-banding of type I collagen (*arrow*) can be observed. A shagged-carpet appearance resulting from 'massaging' of the adhesive resin was located at the top of hybrid layer. **d** Non-demineralized, UA/LC stained section after 6 months of water storage. The hybrid layer partially ruptured, a feature never observed in the control specimens. **e** Non-demineralized, unstained section after 6 months of water storage; hydroxyapatite crystals (arrow head) were clearly observed in the part below the rupture (hand pointer). **f** Non-demineralized, UA/LC stained section after 6 months of water storage; the appearance of hybrid layer changed remarkably by water storage. The typical cross-banding of type I collagen could no longer be observed. Individual collagen fibrils are more difficult to observe (*asterisk*) and overall the hybrid layer has a more porous aspect. Moreover, the shag carpet appearance as observed at baseline appears less pronounced

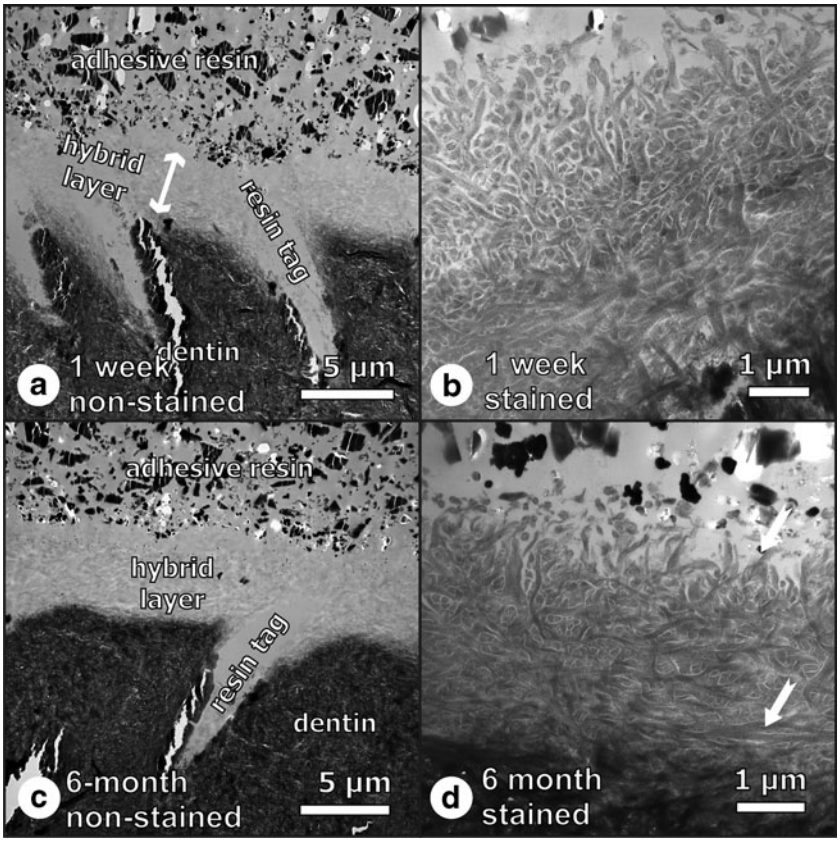

Fig. 5 TEM photomicrographs of the adhesive-enamel/dentin interface produced by OptiBond FL. **a** Control, non-demineralized, unstained section; phosphoric acid has dissolved dentin up to a depth of about 5 µm. The *white areas* represent (unstained) collagen, with the grey area in between representing resin that infiltrated in the exposed 3D collagen network. A thick hybrid layer of approximately 4 µm (*white arrow*) with a distinct resin tag was observed. **b** Highly magnified view of the hybrid layer of a control, non-demineralized, UA/LC stained section. The transition of the hybrid layer to the unaffected dentin is clearly abrupt. Hydroxyapatite was almost absent in the hybrid layer. In the *top part* of the hybrid layer, interfibrillar spaces appear widened, a sign of good resin infiltration. **c** Non-demineralized, unstained section after 6 months of water storage. Water storage did not result in significantly different ultra-morphological features. **d** Highly magnified view of a 6-month-stored, non-demineralized, UA/LC stained section. Again, no difference with the baseline control was observed. No obvious difference between control and 6-month sample was shown, neither in the unstained section nor in the stained one. In contrast to the cmf specimens, individual collagen fibrils with typical cross-banding (*white arrow*) can be observed

A prior study assessing short-term bonding effectiveness, by µTBS and TEM, of cmf concluded that even though bond strengths were somewhat lower, overall bonding performance was still reasonably good [10]. Moreover, the bond strength was not that much reduced and resisted thermo-cycling very well [11]. Baseline bond strengths of the present study were very similar and corroborate these conclusions. However, in the groups that were hydrolytically challenged, either by 6 months water storage or by exposure to 10 % NaOCl, a clear difference was observed. Even though baseline bond strengths were already lower, bond strengths of the experimental hydrophobic adhesive decreased by almost 80 %, compared to only 50 % in the control group. As a result, bond strengths of the artificially aged groups were almost six times lower than those of the conventional three-step etch-and-rinse adhesive.

Overall, TEM evaluation corroborated µTBS observations. At baseline, the pH 1.5 buffered and thus 'milder' phosphoric acid conditioner, compared to Kerr Gel Etchant with 37.5 % H_3PO_4 and a pH of about 0.2, created a relatively thick, completely demineralized hybrid layer varying in thickness between 1.5 and 3 µm. The dentin tubules were opened, enabling the formation of resin tags. Hence, in general, dentin interaction was similar to that of the control etch-and-rinse adhesive, apart from the reduced hybrid layer thickness due to the smaller acidic capacity of the conditioner (Figs. 4 and 5). Hybrid layer thickness may also be affected by impaired resin impregnation. Especially at the top of the hybrid layer, our control adhesive resulted in larger interfibrillar spaces (Fig. 5b vs. Fig. 4c), which may be a sign of more compatible Hoy's solubility parameters of the adhesive resin and the water-saturated demineralized collagen matrix [12]. After 6 months of water storage, clear signs of destructive bond degradation were observed for the more hydrophobic adhesive (Fig. 4d–f). Many sections ruptured at the level of the hybrid layer, which was not

observed for the baseline specimens. At a higher magnification other signs of hydrolytic degradation were observed as well. Ultra-morphological features such as individual collagen fibrils, collagen cross-banding and shagged-carpet appearance could no longer be observed. Interfibrillar spaces appeared widened, especially at the top of the hybrid layer. This, as well as the generally increased porous aspect, was a clear sign of collagen degradation (Fig. 4). This fast degradation suggests a lot of hydrolytic activity during the 6 months of water storage, meaning water must have been able to travel freely through the hybrid layer, as suggested by prior nanoleakage tests [10]. Moreover, water must have had unhindered access to exposed collagen fibrils, suggesting poor resin protection, as a result of ineffective resin impregnation/polymerization of the individual collagen fibrils. These obvious signs of degradation contrast with the conventional etch-and-rinse adhesive, which had no visible signs of degradation at TEM level after 6 months of water storage.

To manufacture an etch-and-rinse adhesive, which is more biocompatible through the use of larger and more hydrophobic components, several compromises had to be made. To compensate for reduced infiltration capacity, application time was lengthened considerably (Table 1) and infiltration depth was reduced by using a buffered phosphoric acid gel (Fig. 4). Moreover, as the adhesive resin is more viscous and lacks small cross-linking monomers such as TEGDMA, also polymerization time was increased to achieve adequate polymerization and strength of the adhesive resin. Nevertheless, these small modifications of the application procedure may not be able to compensate fully the absence of these small functional monomers, resulting in lower mechanical properties of the adhesive resin [10, 13, 14]. This may in turn explain the lower baseline bond strengths. On the other hand, it was hypothesized that such a hydrophobic resin is more resistant to degradation in an aqueous environment such as the oral cavity [15]. This was especially true for the present study, as no enzymes that may have enhanced polymer degradation were present in the storage solution [16].

Recently, it has been shown that collagen degradation might be mediated by host-derived matrix metalloproteinases (MMPs) present in the dentin [17, 18]. For various reasons the more hydrophobic etch-and-rinse adhesive may be more affected by this endogenous enzymatic degradation pathway. Firstly, MMPs are very sensitive to acids, so the buffered conditioner may preserve a larger fraction of the enzymes released from the substrate [19]. Secondly, as suggested by the nanoleakage pattern and by exposure to 10 % NaOCl, the hydrophobic resins are not able to wrap up the exposed collagen fibrils effectively, so water and enzymes can come in close contact with the collagen and so hasten the hydrolytic degradation. Thirdly, as the primer is not as acidic as other dental primers (Table 1), a larger fraction of the released enzymes is retained in the hybrid layer [20].

Interesting to note in this study are the similar bond strength reductions observed for both artificial ageing protocols. Water storage of micro-specimens is a common accelerated artificial aging procedure [21]. NaOCl, on the other hand, is a non-specific deproteinizing agent, which removes all non-protected organic material from the interface [22]. Therefore, exposure of resin–dentin interfaces to a concentrated NaOCl solution for as little as 1 h will result in considerable bond strength reductions [23], as also observed in the present study. Because of the aggressiveness and the non-specific nature, such an artificial aging procedure can be regarded as a worst case scenario, a kind of endpoint when all organic material present in the interface has disappeared by various pathways. This may well suggest where long-term bond strengths are heading to irrespective of the aging procedure. In this study a remarkable correlation was observed between the bond strengths after 6 months of water storage and 1 h of NaOCl exposure (Table 2), which encourages further use of this fast, easy and cheap method to predict long-term bonding effectiveness.

To conclude, the more hydrophobic and biocompatible three-step etch-and-rinse adhesive was able to produce a reasonable short-time bonding effectiveness. However, our observations disclosed two major shortcomings: poor long-term properties of the adhesive resin itself and ineffective entanglement of the resin within the hybrid layer. Therefore, on the long-term, omitting small and hydrophilic components from the adhesive formulation promoted hydrolytic degradation and resulted in five times lower bond strengths after artificial aging.

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Conflict of interest The authors declare that they have no conflicts of interest regarding the products herein investigated.

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