

Effect of shelf-life of a universal adhesive to dentin

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ABSTRACT

The microtensile bond strength (μ TBS), nanoleakage expression (NL), and endogenous enzymatic activity of a simplified universal adhesive system used in combination with a dual-cure resin cement for luting indirect restorations were evaluated considering its shelf-life (as-received vs expired). The universal adhesive was used as-received (AS-R) by manufacturer or after three months after being expired (EXP). Resin composite overlays ($n = 10$) were luted to flat deep coronal dentin surfaces with a universal bonding system (iBond universal, used in the self-etch mode) in combination with a dual-cure resin cement (RelyX Ultimate; 20s of light-cure followed by 15 min of self-cure at 37 °C). After 24 h, the specimens were cut into microtensile sticks (1 mm²) and stressed to failure under tension, in accordance with ISO/TS 11.405. The fracture pattern was evaluated with optical microscopy. The significance level of $p = 0.05$ was used for the statistical analysis. Additional specimens ($n = 4$) were processed for quantitative interfacial nanoleakage expression using ammoniacal silver nitrate. To investigate the endogenous enzymatic activity within the hybrid layer *in situ* zymography was performed. The EXP group obtained significantly lower μ TBS values than those recorded in the AS-R group ($p < 0.05$). NL resulted in higher deposition of AgNO₃ granules when the adhesive was used in the EXP group compared to the AS-R bonding system. *In situ* zymography assay revealed increased level of fluorescence when the universal bonding system was used at the end of shelf-life compared to the AS-R group. The use of the universal adhesive system beyond the expiry limit resulted in decreased bonding performances. Furthermore, the higher endogenous enzymatic activity recorded after the end of the shelf life of the universal adhesive would portend for a shortened durability of the restorations.

1. Introduction

Since their introduction, resin cements in combination with adhesive systems have allowed a more conservative approach toward the dental tissue with performance improvements in terms of aesthetics and resistance of the restorations. Over time, the adhesive protocols have been modified with a reduction of the number of clinical steps to obtain time-saving and straightforward chair-time restorative procedures and make them more manageable, therefore less susceptible to the operator's influence.

The multi-step etch-and-rinse techniques have been gradually replaced by more simplified systems based on a self-etch adhesive approach. Simplified multimode universal adhesives represent the latest

family of simplified adhesives being able to be applied in the etch-and-rinse or the self-etch modes, or even applying both techniques in the selective enamel etching approach. The indications for their use change according to the type of dental hard tissue to be hybridized, and different recommendations have been proposed depending on whether working on enamel or dentin. Researchers agree in determining that the selective enamel etching represents the most suitable approach to profit from many of the adhesive properties of these simplified systems [1,2].

Notwithstanding their ease-of-use, many factors participate in making universal adhesives prone to degradation processes. The degradation process of the adhesive system is complex and includes many aspects [3]. Two major forms can be observed: intraoral degradation (mechanical, physical, or chemical) and extraoral degradation due to the

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storage of the material and its shelf-life [4]. Many materials used in dentistry are perishable and have specific storage requirements to maintain optimum properties [5].

Recently, problems related to the conservation of the bonding agent have been emphasized, being liable to hydrolysis or difficulty in the polymerization of the monomers, degradation of the additive components (initiators/stabilizers), or evaporation of the ingredients [6,7]. While on one side the manufacturers specify to strictly follow the recommendations related to the storage instructions of the materials (temperatures, heat sources, bottle security), on the other side, failure to observe the expiry dates of the products can lead to premature deterioration of the adhesive performances [3,8]. Usually, the manufacturers of the adhesive systems provide a two-year expiry time for the products, after which they foresee a risk of physical-chemical compromise, which could prematurely invalidate their adhesive capacity [8].

It is of fundamental importance to understand if this risk of impairment also refers to a short time after the expiry date (e.g. three months) as it could mislead clinicians who want to use adhesives shortly after the expiration date, perhaps taking their clinical efficacy for granted.

Therefore, the aim of this study was to evaluate the microtensile bond strength (μ TBS) and nanoleakage expression (NL) of a universal adhesive system used in combination with a dual-cure resin cement for the cementation of composite overlays when used before the expiration date or after three months from the end of shelf-life. Furthermore, the endogenous enzymatic activity in both groups was assessed with *in situ* zymography. The null hypotheses tested were that 1) the use of the adhesive system after three months from the expiry date has no effect on the bonding capacity and the nanoleakage expression when compared with the as-received adhesive, and that 2) there is no difference in enzymatic degradation between the tested groups.

2. Materials and methods

2.1. Microtensile bond strength test

Twenty sound human third molars were stored in a 0.5% Chloramine T water solution at 4 °C for no longer than one month after extraction until used for this *in vitro* study. In order to be included in the study, the molars had to be fracture-free, with no caries and resorptions. Teeth were obtained from anonymous individuals following their informed consent under a protocol approved by the Ethical Committee of the University of Bologna (Italy; protocol N°: 71/2019/OSS/AUSLBO).

Tooth crowns were removed with a low-speed diamond saw under water cooling (Microremet, Remet, Bologna, Italy) to expose enamel-free coronal dentin. One operator observed under an optical microscope the absence of enamel remnants. A standardized smear layer was created on the dentin surface with 180-grit wet silicon carbide paper.

After that, resin composite build-ups were layered with two 2-mm thick increments (Venus Diamond, Heraeus Kulzer GmbH, Hanau, Germany) using a silicone mold. Each increment was polymerized for 40 s using a LED curing light (Demi™ Plus, Kerr Dental, Bioggio, Switzerland) from the top of the restoration. Then, the composite overlays were removed from the mold, and additional light irradiations were performed on each side and the bottom of the restoration, previously in contact with the mold, for 20 s each. The surfaces of the indirect composite overlays were wet-polished with 600-grit silicon carbide paper for 30 s. Then, composite overlays were cleaned in ultrasonic bath for 2 min. A surface conditioner (iBond Ceramic Primer; Heraeus Kulzer) was brushed on the whole surface of the composite in accordance with manufacturer's recommendations.

iBond universal adhesive (Heraeus Kulzer, GmbH, Hanau, Germany) was used in the self-etch mode according to manufacturer's instructions (Table 1). RelyX Ultimate (3 M ESPE, Seefeld, Germany) was used as luting material in the dual-cure mode (20s of light-cure followed by 15 min of self-cure at 37 °C). A LED curing light was used (Demi™Plus, Kerr Dental; light output > 500 mW/cm² and wavelength 440–480 nm).

Table 1

Components, compositions, and instruction of use of the tested materials.

Material	Composition	Procedure
iBond Universal Adhesive (Heraeus Kulzer GmbH, Hanau, Germany. LOT: K010028)	MDP, 4-META, methacrylates, acetone, water.	Drop the adhesive into the mixing well and use within 3 min. Gently rub adhesive onto the entire dental surface for 20 s. Carefully air-dry with an oil-free air flow until the adhesive film no longer moves. The surface must be visibly glossy. Polymerize for 10 s (wavelength of 440–480 nm; light output: > 550 mW/cm ²).
iBond Ceramic Primer (Heraeus Kulzer. LOT: K010101)	Isopropanol/acetone based solution of methacrylate monomers and silane.	Measure out the desired drops of solution into the mixing well. Brush the whole surface to be treated without delay and allow to dry for 20s. Dry briefly with an oil-free air-flow.
Venus Diamond (Heraeus Kulzer; Shade: A2; LOT: K010069)	Light-curing, radiopaque nano composite. Barium aluminium boro fluor silicate glass, TCD-Urethaneacrylate, silica, UDMA, TEGDMA, titanium dioxide, fluorescent pigments, metallic oxide pigments, organic pigments, aminobenzoic acid ester, camphoroquinone.	Apply the material in thin layer (max 2 mm) and adapt to the cavity walls. Polymerize (wavelength of 440/480 nm; light output: > 550 mW/cm ²).
RelyX Ultimate (3 M, St Paul, MN, USA; Shade: A1; LOT: 669768)	Base paste: methacrylates monomers, radiopaque silanated fillers, initiator components, stabilizers, rheological additives. Catalyst paste: methacrylate monomers, radiopaque alkaline basic fillers, stabilizers, pigments, rheological additives, fluorescence dye, dark cure activator.	For each application a new mixing tip was used. Dispense the cement from the automix syringe and apply the desired quantity directly to the restoration. Excess were removed while seating in place the restoration.

Luting procedures were performed under a sustained seating pressure of 1 kg that was maintained during the cementation procedures [9].

The same bottle of the dental bonding agent was used in two clinical conditions: 1) as soon as it was received, and hence referred to "as-received" (AS-R), or 2) after 3 months of its expiry date (EXP), during which the material was stored in a ventilated room, away from heat sources (such as heater or sunlight), where the temperature did not exceed 19 °C. So, in the end, the following groups were formed: 1) as-received adhesive/RelyX Ultimate (AS-R); 2) expired adhesive/RelyX Ultimate (EXP).

After immersion in distilled water for 24h, each specimen was cut into microtensile sticks (cross-sectional area of approximately 1 mm²). After 24 h of storage in artificial saliva at 37 °C, the sticks were stressed to failure under tension with a universal testing machine (Bisco, Inc., Schaumburg, IL, USA) at a cross-head speed of 1 mm/min. Both sides of failed tested sticks were observed under a stereomicroscope at 40x magnification (Stemi 2000-C; Carl Zeiss Jena GmbH) to classify the fracture pattern as follows: adhesive (A), cohesive within dentin (CD), cohesive within resin cement (CC) or mixed (M). Since μ TBS data were not normally distributed (Shapiro-Wilk's test), the non-parametric Mann-Whitney *U* test was run to compare data between two groups ($p = 0.05$). All the analyses were performed in Sigma plot v. 12.0 (Systat Software, Inc).

2.2. Nanoleakage assessment

Additional specimens ($n = 4$ per group) were prepared with the same bonding protocol as previously described for μ TBS test and were processed for quantitative interfacial nanoleakage expression using ammoniacal silver nitrate then examined under a light microscope. After storage in artificial saliva at 37 °C for 24 h, specimens were sectioned into 1 mm-thick slices and immersed in 50 wt% ammoniacal silver nitrate solution in a dark environment for 24 h. After careful rinsing in distilled water, the silver ion-infiltrated specimens were immersed into a photo-developing solution to reduce the silver ions into metallic silver grains. The stained specimens were subsequently fixed, dehydrated, embedded in epoxy resin (LR White resin, Sigma-Aldrich, St Louis, MI, USA), fixed on glass slides using cyanoacrylate glue, flattened with silicon carbide paper of increasing series (180-, 600-, 1200-, 2400-, and 4000- grit) under running water and finally observed under a light microscope (Nikon E800; Nikon, Tokyo, Japan. Magnification: 100x) to analyze the precipitation of the silver grains along the bonded interfaces. The specimens were graded on a 0–4 scale according to the percentage of silver deposition at the adhesive interface: (0) no nanoleakage was present; (1) <25% of the surface interested by silver deposition; (2) 25%–50% of the surface presented silver granules accumulation; (3) 50%–75% of the surface with silver deposits; and (4) >75% of the surface interested by silver leakage. The nanoleakage results were analyzed using the non-parametric Chi-squared test ($p = 0.05$).

2.3. In situ zymography of resin-dentin interfaces

Additionally, 1-mm thick slabs of middle/deep dentin were obtained from human third molars ($n = 4$). Each slab was further divided into four quartet slices to test all the experimental groups on the same substrates. A standardized smear layer was created on each dentin substrate with #600 silicon-carbide paper. Two surfaces of each quarter per slab were treated according to the bonding procedures used for the μ TBS test. After bonding procedures, a flowable composite (Filtek 250; 3 M ESPE) was applied for 1-mm and photo-polymerized with a LED curing device (Demi™Plus). The bonded assemblies were now sectioned vertically into 1-mm thick specimens to expose the resin-dentin interfaces. Each specimen was then glued to glass slides, ground down approximately to 50 μ m, polished, and prepared for *in situ* zymography according to the protocol reported by Mazzoni et al. [10]. Briefly, self-quenched fluorescein-conjugated gelatine mixture (E-12055; Molecular Probes, Eugene, OR, USA) was placed on top of each specimen as to cover the resin-dentin interfaces, protected with a cover slip and incubated in a dark humidified chamber at 37 °C for 12h. A confocal laser scanning microscope was used to examine the specimens after incubation (excitation wavelength, 530 nm; Model A1-R; Nikon, Tokyo, Japan). For each assembly, a series of images were acquired (one image per each 1 μ m into the depth of the sample) to show the hydrolysis of the quenched fluorescein-conjugated gelatine substrate, presented as green fluorescence. ImageJ software (National Institute of Health, Bethesda, MD, USA) was used to quantify the integrated density of the fluorescence signals. The gelatinolytic activity was expressed as the percentage of the resin-dentin interface that exhibited green fluorescence. Since the *in situ* zymography data were not normally distributed (Shapiro-Wilk test), the non-parametric Mann-Whitney *U* test was used to compare data of the density of fluorescence signal between groups. The level of significance was set at $p = 0.05$.

3. Results

3.1. Microtensile bond strength test

The mean values and standard deviations of the μ TBS are listed in Table 2. Statistical analysis revealed that the use of the adhesive over the

end of its shelf-life (EXP) was a significant factor affecting the μ TBS data ($p < 0.05$). AS-R adhesive yielded bond strengths values three times higher than those obtained in the EXP group and this was statistically significant ($p < 0.05$).

Failure mode distribution of debonded specimens is presented in Table 2. Mixed debondings were predominant in the AS-R group, and the fractures occurred at the dentin/adhesive interface. Besides, a 2% of fractures into the resin composite was also observed. A prevalence number of adhesive failures between the adhesive layer and dentin surface were recorded in the EXP group (98%). Mixed failures were also recorded in the latest group.

3.2. Nanoleakage assessment

Representative light microscopy images of the interfacial nanoleakage expression are illustrated in Fig. 1. Descriptive statistics of interfacial leakage scores are presented in Fig. 2. The “end of shelf-life” factor significantly influenced silver grains accumulation at the bonded interfaces ($p < 0.05$). Higher interfacial nanoleakage expression was observed in the EXP group. “Zero” value was observed for both experimental groups, with AS-R group recording higher 1 grading (silver granules accumulation less than 25%) than the EXP group.

3.3. In situ zymography

Representative dual-fluorescence images of resin-dentin interfaces created with the adhesives applied as-received or after its expiry date are shown in Fig. 3. The results obtained on the confocal laser scanning microscope revealed significant differences in the fluorescence signal exhibited by the two tested groups ($p < 0.05$). The level of the enzymatic activity is correlated to the density of the green fluorescent signal (Fig. 3A–C). Specimens belonging to the AS-R adhesive group exhibited lower green fluorescence within the hybrid layer (Fig. 3A–B). In contrast, specimens that were bonded with the EXP adhesive demonstrated more intense green fluorescence within the hybrid layer (Fig. 3C–D).

4. Discussion

The purpose of this study was to evaluate whether the use of a universal adhesive before and three months after the end of the shelf-life (expired adhesive) could influence the bonding performance to dentin, the nanoleakage expression and the morphological characteristics of the bonded interfaces. The results obtained in this study require the rejection of all tested null hypotheses, since the use of the expired adhesive system caused a reduction in the bonding performances, an increment of silver grains deposits at the adhesive interfaces and an increase in the enzymatic activity at the resin-dentin interfaces.

According to the terminology, with shelf-life we mean the period during which a material manages to maintain the maximum of its characteristics. The manufacturers always suggest the expiration date of a material since a weakening of the physical and mechanical

Table 2

μ TBS obtained by applying the as-received (AS-R) or expired (EXP) universal adhesive on the dentin surface. Values are means \pm standard deviations (premature failures were included into the statistical analysis). Different letters show statistically significant differences among groups ($p < 0.05$). Percentages of failure modes after μ TBS test. Fractures were classified as: adhesive (A), cohesive into resin composite (CC), mixed failures (M).

Tested Material	MPa	Fractures
AS-R	40.8(10.4) ^A	98%M 2% CC
EXP	13.4(6.8) ^B	82% A 18% M

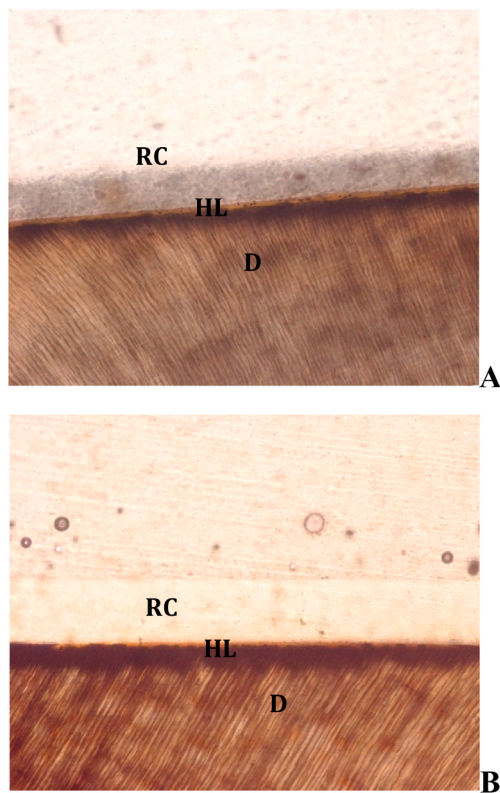


Fig. 1. Representative light microscopy images (100x magnification) of the tested materials in the experimental conditions and submitted to nanoleakage with silver nitrate after 24 h from the bonding procedures. Decreased accumulation of silver granules was observed when the bonding agent was used “as-received” (AS-R; Fig. A) despite when it was used after three months the end of the shelf-life (EXP; Fig. B). RC: resin cement; HL: hybrid layer; D: Dentin.

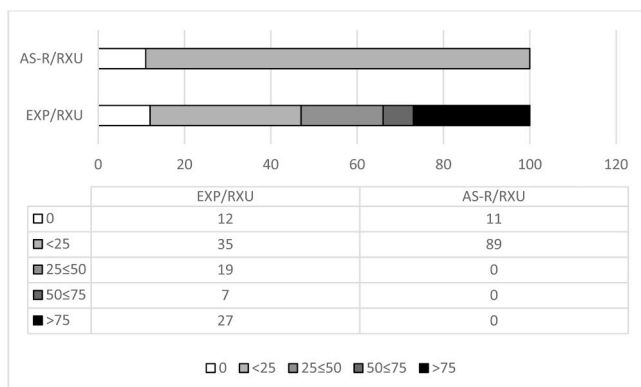


Fig. 2. Percentage of interfacial nanoleakage expression in resin cement-dentin interfaces created for each tested group after 24 h of immersion in silver nitrate solution. AS-R: as-received universal adhesive/RelyX Ultimate dual-cure resin cement; EXP: expired universal adhesive/RelyX Ultimate dual-cure resin cement.

characteristics is expected over time [5].

In the present study, a decrease in bond strengths was observed when the expired adhesive was used (Table 2). It has been previously reported a reduction in bond strengths when iBond universal adhesive was used beyond the expiry date, and these results were justified taking into account its chemical composition [8]. According to these hypotheses, premature degradation can occur on two fronts. On one side, hydrolysis phenomena that are promoted by the presence of hydrophilic monomers sensitive to the inner wetness of the underlying dentin substrate [3,4,6],

could increase hybrid layer permeability and nanoleakage expression [11,12]. On the other side, the ease evaporation of organic solvents could impair the polymerization reaction of the resin monomers [7,13] leading to voids and pores within the interfacial layer [14]. Temperature, repeated opening of the bottle, or excessive storage time can further complicate these circumstances [6,15].

According to manufacturer’s safety data sheet, iBond is an acetone-based solvent adhesive formulated with 4-methacryloxyethyl trimellitate anhydride (4-META) and urethane dimethacrylate (UDMA). Acetone is a highly volatile solvent, used to promote the rapid evaporation of the aqueous component of the adhesive systems that does not allow it to come into contact with the monomers [16]. During the drying phases of the adhesive, acetone concurs to promote the rapid evaporation of the aqueous phase so that this does not interfere with the monomers, which can thus participate in obtaining reliable adhesion forces [17]. However, inappropriate or incomplete volatilization of the solvent can influence the degree of conversion of resin monomers [17]. Furthermore, the presence of partially infiltrated dentin represents weak sites prone to bonding failure and degradation phenomena [18].

It was previously reported that ester-based adhesive formulations with acidic pH values are prone to hydrolysis phenomena [6,19]. Once the 4-META monomer is hydrolyzed to form 4-MET (4-methacryloxyethyl trimellitic acid), an ester cleavage phenomenon of esters is triggered due to the ethanol contained as a solvent [6,16,20]. This process is likely to have negative consequences not specifically on the demineralization capacity of the material, but rather on the infiltration phase of the demineralized substrates and the effectiveness of the polymerization [20], thus causing premature degradation of the monomer components [8]. Negative cascading events can then take place, including primarily a decrease in adhesion capacity [6,7], morphological changes at the adhesive interface level [20], and phase separation [6,20].

Nanoleakage was used in this study as an indirect method to evaluate the quality of the resin-dentin bonds. By nanoleakage expression, we mean the identification of possible defects or voids within the hybrid layer that can serve as initiating areas for the degradative phenomena. In this study, an increased accumulation of silver grains at the adhesive interface level was observed when the adhesive was used beyond the end of shelf-life (Figs. 1 and 2). It has been assumed that the lack of polymerization of the resin monomers could cause the adhesive seal to collapse, thus further implementing the adhesive interface for greater infiltration, finally affecting the longevity of the bond [21]. On the other hand, this series of events is exacerbated if we consider that the dentinal substrate is highly perfused. It has been previously highlighted that even when universal adhesives have been used in self-etch mode (therefore, when the smear layer has not been eliminated, but only dissolved and used as adhesive substrate) permeability to water can be observed at the level of the adhesive interface, acting as semi-permeable membrane [11, 14]. The passage of water within the adhesive layer if on the one hand, hinders the polymerization process of the adhesive, on the other the presence of water molecules takes away space for a possible infiltration of the resin monomers, thus determining initiating sites where degradation phenomena occur over time [13,14].

The *in situ* zymography was additionally performed in the present study, and the results obtained provide indications of a substrate that can be highly degradable. The adhesive interfaces created between the universal adhesive used after its expiry date and dentin have indeed shown a high collagenolytic activity (Fig. 3). The intense collagenolytic activity recorded can then be co-responsible for the bond strength degradation over time [10].

As previously stated, the presence of a moist substrate, such as dentin, represents one of the most difficult scenarios to work on, and this factor can be even more decisive on the shelf-life stability of a material, in particular, if used after the expiry date reported by the manufacturer.

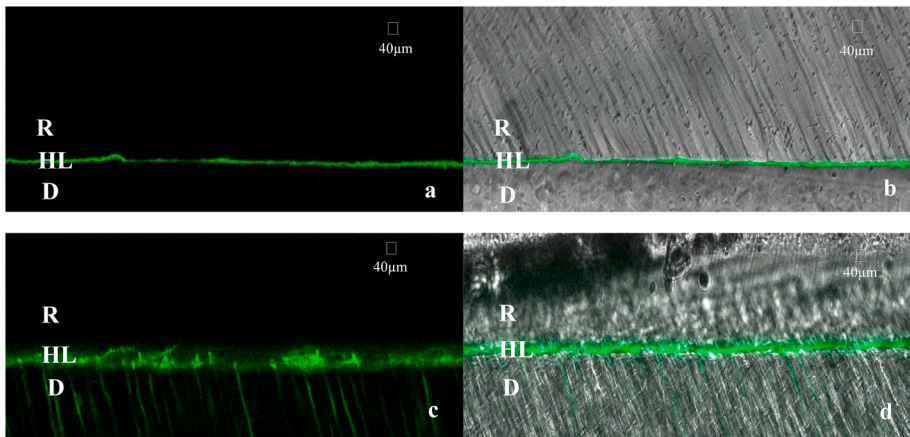


Fig. 3. Resin-dentin interfaces incubated with quenched fluorescein-labeled gelatin. a) Image created in green channel of the HL created by the application of the as-received universal adhesive (AS-R), showing lower level of fluorescence; b) image of the HL created with the AS-R obtained by merging differential interference contrast image (showing the optical density of the resin-dentin interface) and image acquired in green channel; c) image acquired in green channel, showing fluorescence (identifying intense endogenous enzymatic activity) in dentinal tubules and within the HL present in the expired adhesive group (EXP); d) image of the same group (EXP), obtained by merging differential interference contrast image acquired in green channel. D: Dentin; HL: Hybrid layer; R: resin cement. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

5. Conclusions

In conclusion, this study aimed, in a preliminary way, to evaluate in the short term the adhesive characteristics of a universal adhesive system used before and after the expiry date. Further studies are needed to confirm our preliminary results looking in particular, to the degree of polymerization of the monomers of expired products or showing the behavior of these adhesives in the presence of dentin hydrostatic intrapulpal pressure.

Within the limits of the present study, we can conclude that highest attention must be paid to the expiry date of the adhesive used, as evident deficiencies in the adhesion strength could affect the sealing of the adhesive interface and efficacy can no longer be guaranteed.

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References

- [1] Diniz AC, Bandeca MC, Pinheiro LM, dos Santos Almeida LJ, Torres CR, Borges AH, Pinto SC, Tonetto MR, de Jesus Tavarez RR, Firoozmand LM. Influence of different etching modes on bond strength to enamel using universal adhesive systems. *J Contemp Dent Pract* 2016;17(10):820–5. <https://doi.org/10.5005/jp-journals-10024-1937>.
- [2] Rosa WL, Piva E, Silva AF. Bond strength of universal adhesives: a systematic review and meta-analysis. *J Dent* 2015;43(7):765–76. <https://doi.org/10.1016/j.dent.2015.04.003>.
- [3] Fujita K, Nishiyama N. Degradation of single bottle type self-etching primer effectuated by the primer's storage period. *Am J Dent* 2006;19:111–4.
- [4] Salz U, Zimmermann J, Zeuner F, Moszner N. Hydrolytic stability of self-etching adhesive systems. *J Adhesive Dent* 2005;7(2):107–16.
- [5] Salz U, Cock T. Adhesion performance of new hydrolytically stable one-component self-etching enamel/dentin adhesives. *J Adhesive Dent* 2010;12(1):7–10. <https://doi.org/10.3290/j.jad.a18439>.
- [6] Nishiyama N, Suzuki K, Yoshida H, Teshima H, Nemoto K. Hydrolytic stability of methacrylamide in acidic aqueous solution. *Biomaterials* 2004;25:965–9. [https://doi.org/10.1016/s0142-9612\(03\)00616-1](https://doi.org/10.1016/s0142-9612(03)00616-1).
- [7] Van Landuyt KL, Mine A, De Munck J, Jaecques S, Peumans M, Lambrechts P, Van Meerbeek B. Are one-step adhesives easier to use and better performing? Multifactorial assessment of contemporary one-step self-etching adhesives. *J Adhesive Dent* 2009;11(3):175–90.
- [8] Cuevas-Suárez CE, Ramos TS, Rodrigues SB, Collares FM, Zanchi CH, Lund RG, da Silva AF, Piva E. Impact of shelf-life simulation on bonding performance of universal adhesive systems. *Dent Mater* 2019;35:e204–9. <https://doi.org/10.1016/j.dental.2019.05.023>.
- [9] Goracci C, Cury AH, Cantoro A, Papacchini F, Tay FR, Ferrari M. Microtensile bond strength and interfacial properties of self-etching and self-adhesive resin cements used to lute composite onlays under different seating forces. *J Adhesive Dent* 2006;8:327–35.
- [10] Mazzoni A, Nascimento FD, Carrilho M, Tersariol I, Papa V, Tjaderhane L, Di Lenarda R, Tay FR, Pashley DH, Breschi L. MMP activity in the hybrid layer detected with the *in situ* zymography. *J Dent Res* 2012;91:467–72. <https://doi.org/10.1177/0022034512439210>.
- [11] Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. *J Dent Res* 2002;81(7):472–6. <https://doi.org/10.1177/154405910208100708>.
- [12] Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, Tay FR. A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. *Biomaterials* 2005;26(9):1035–42. <https://doi.org/10.1016/j.biomaterials.2004.04.003>.
- [13] Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. State of the art etch-and-rinse adhesives. *Dent Mater* 2011;27(1):1–16. <https://doi.org/10.1016/j.dental.2010.10.016>.
- [14] Cho BH, Dickens SH. Effects of the acetone content of single solution dentin bonding agents on the adhesive layer thickness and the microtensile bond strength. *Dent Mater* 2004;20(2):107–15. [https://doi.org/10.1016/s0109-5641\(03\)00071-x](https://doi.org/10.1016/s0109-5641(03)00071-x).
- [15] Pongprueksa P, Miletic V, De Munck J, Brooks NR, Meersman F, Nies E, Van Meerbeek B, Van Landuyt KL. Effect of evaporation on the shelf-life of a universal adhesive. *Operat Dent* 2014;39(5):500–7. <https://doi.org/10.2341/13-195>.
- [16] Amaral CM, Diniz AM, Arantes EB, Dos Santos GB, Noronha-Filho JD, da Silva EM. Resin-dentin bond stability of experimental 4-META-based etch-and-rinse adhesives solvated by ethanol or acetone. *J Adhesive Dent* 2016;18(6):513–20. <https://doi.org/10.3290/j.jad.a37200>.
- [17] Carvalho CN, Lanza MDS, Dourado LG, Carvalho EM, Bauer J. Impact of solvent evaporation and curing protocol on degree of conversion of etch-and-rinse and multimode adhesives systems. *Int J Dent* 2019;11. <https://doi.org/10.1155/2019/5496784>.
- [18] Tay FR, Pashley DH. Aggressiveness of contemporary self-etching adhesives: I: depth of penetration beyond smear layers. *Dent Mater* 2001;17(4):296–308. [https://doi.org/10.1016/s0109-5641\(00\)00087-7](https://doi.org/10.1016/s0109-5641(00)00087-7).
- [19] Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. *Dent Mater* 2011;27:17–28. <https://doi.org/10.1016/j.dental.2010.10.023>.
- [20] Nishiyama N, Tay FR, Fujita K, Pashley DH, Ikemura K, Hiraishi N, King NM. Hydrolysis of functional monomers in a single-bottle self-etching primer - correlation of 13C NMR and TEM findings. *J Dent Res* 2006;85:422–6. <https://doi.org/10.1177/154405910608500505>.
- [21] Cadenaro M, Maravic T, Comba A, Mazzoni A, Fanfoni L, Hilton T, Ferracane J, Breschi L. The role of polymerization in adhesive dentistry. *Dent Mater* 2019;35(1):e1–22. <https://doi.org/10.1016/j.dental.2018.11.012>.