Research Article

Effect of esterase aging on the micro-shear bond strength of amide-modified and conventional universal adhesives to dentin (in vitro study)

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Received date: 16-05-2026
Accepted date: 30-07-2026
Published date: 15-09-2026

Article DOI

Abstract: Background: The degradation of the resin–dentin interface remains a major limitation of adhesive restorations. Esterase-mediated hydrolysis of adhesive polymers may accelerate bond deterioration and contribute to interfacial failure. Objectives: This study aimed to compare the micro-shear bond strength (μSBS) of two universal adhesives, Clearfil Universal Bond Quick and All-Bond Universal, after aging in porcine liver esterase by using self-etch and etch-and-rinse strategies. Materials and Methods: Eighty sound human mandibular third molars provided 160 standardised dentin blocks. Each adhesive was applied in self-etch or etch-and-rinse mode, composite cylinder of 1 mm in diameter applied. Then, the resin–dentin samples were stored in phosphate-buffered saline (PBS) or porcine liver esterase (6 U/mL) for 4 weeks at 37 °C, with medium renewal every 48 h (n = 20/group). μSBS was measured at 0.5 mm/min. Resin biodegradation was assessed by high-performance liquid chromatography of bis-hydroxy-propoxy-phenylpropane (BisHPPP) release, and failure modes were evaluated stereomicroscopically. Results: Esterase aging significantly reduced μSBS for both adhesives in both modes ($p < 0.001$). In self-etch mode, μSBS decreased from 32.20 ± 8.02 MPa to 14.45 ± 3.66 MPa for All-Bond Universal and from 36.75 ± 7.60 MPa to 21.25 ± 6.71 MPa for Clearfil Universal Bond Quick. In etch-and-rinse mode, the μSBS values decreased from 35.75 MPa ± 10.03 to 16.50 ± 5.67 MPa and from 41.10 ± 9.08 MPa to 24.25 ± 5.58 MPa, respectively. Under enzyme aging, Clearfil Universal Bond Quick showed significantly higher μSBS than All-Bond Universal in both modes ($p < 0.001$). BisHPPP release was markedly higher in enzyme groups than PBS controls, and enzyme-aged specimens failed mainly adhesively. Conclusions: Esterase aging significantly weakened resin–dentin bonding and increased resin biodegradation. The amide-modified Clearfil Universal Bond Quick showed better bond strength retention than All-Bond Universal under enzymatic challenge.

Keywords: Universal adhesives. biodegradation. Esterases. Hybrid layer. hydrophilic amide monomer.

Introduction

Adhesive dentistry has transformed restorative practice by enabling minimally invasive treatment and reliable bonding of resin-based materials to dental tissues. Even so, the resin–dentin interface remains the weakest link of bonded restorations, and degradation of this interface is strongly associated with marginal breakdown, recurrent caries and reduced restoration longevity [1-3].

Contemporary adhesive systems are commonly classified in accordance with how they interact with the smear layer and dentin substrate, mainly into etch-and-rinse and self-etch approaches. Universal adhesives were introduced to simplify bonding procedures while maintaining acceptable clinical performance, because they can be applied in self-etch, etch-and-rinse, or selective-enamel-etch modes. Their formulations usually combine acidic functional monomers, such as 10-methacryloyloxydecyl

dihydrogen phosphate (10-MDP), with hydrophilic and hydrophobic co-monomers to promote chemical interaction with hydroxyapatite and micromechanical interlocking [4-8].

Despite these advances, the long-term stability of the adhesive interface remains uncertain. Water sorption, polymer plasticisation, incomplete monomer conversion, and enzyme-catalysed hydrolysis can progressively weaken the adhesive and hybrid layers. Salivary and bacterial esterases can cleave ester bonds in methacrylate monomers such as Bis-GMA and TEGDMA, generating degradation byproducts that include bis-hydroxy-propoxy-phenylpropane (BisHPPP), which is widely used as a marker of resin biodegradation [9-11]. As degradation progresses, interfacial permeability increases, nanoleakage pathways may form and the susceptibility of bonded interface to mechanical failure and bacterial microleakage increases [12, 13].

Newer formulations have explored acrylamide- and methacrylamide-based chemistry as alternatives or adjuncts to conventional ester-containing monomers to improve resistance to hydrolysis. These amide-containing monomers are considered more hydrolytically stable than methacrylates and may enhance the durability of the bonded interface [14, 15]. Clearfil Universal Bond Quick is a one-step universal adhesive that incorporates a hydrophilic amide monomer, and it has been reported to show favourable dentin bonding performance [16, 17]. However, evidence directly comparing its behaviour with a conventional universal adhesive under enzymatic aging remains limited, and the influence of bonding mode on this comparison is still unclear [18, 19].

Therefore, this study aimed to evaluate and compare the micro-shear bond strength (μSBS) of an amide-modified universal adhesive (Clearfil Universal Bond Quick) and a conventional universal adhesive (All-Bond Universal) after aging in porcine liver esterase by using self-etch and etch-and-rinse strategies. The null hypotheses were as follows: (1) esterase aging would not affect the μSBS of either adhesive; (2) the two adhesives would have no difference after aging with esterase and phosphate-buffered saline (PBS); and (3) the application strategy would not influence the μSBS within each adhesive.

Materials and methods

Study design and ethical approval

Eighty extracted sound human mandibular third molars were collected from patients aged 20–30 years. Ethical approval was obtained from the Board of the Institutional Ethics Committee, College of Dentistry, Mustansiriyah University (No. MUOPR29; approval date: 01/May/2023). Teeth extracted for orthodontic or periodontal reasons were cleaned with pumice and a rubber cup, disinfected in 0.1% thymol for 48 h and then stored in deionised water until use [20, 21].

Sample size calculation

Based on previous closely related previous studies, a conservative effect size of Cohen's d = 1.0 was assumed. With $\alpha = 0.05$ and 80% power, 17 specimens per group were required; this was increased to 20 specimens per group to allow for possible specimen loss.

Specimen preparation

Each tooth was mounted in cold-cured acrylic up to approximately 2 mm apical to the cemento-enamel junction by using silicon mould with an internal diameter of 1.5 cm in depth, length and width (21). A custom sectioning device with water cooling and a 0.5 mm-diamond disc was used, and the occlusal enamel was removed. Then, the mesial, distal, buccal and lingual enamels were removed. Afterwards, the crown was sectioned occluso-cervically and cervically to obtain two standardised dentin blocks from each tooth. Each block measured approximately 3 mm mesiodistally, 6 mm buccolingually and 6 mm occluso-cervically. The bonded occlusal surface was inspected under a stereomicroscope (10×) to exclude

cracks or enamel remnants, and then the bonded occlusal surfaces were polished under continuous water irrigation using #600-grit SiC paper for 5 s with a low-speed polishing machine [22].

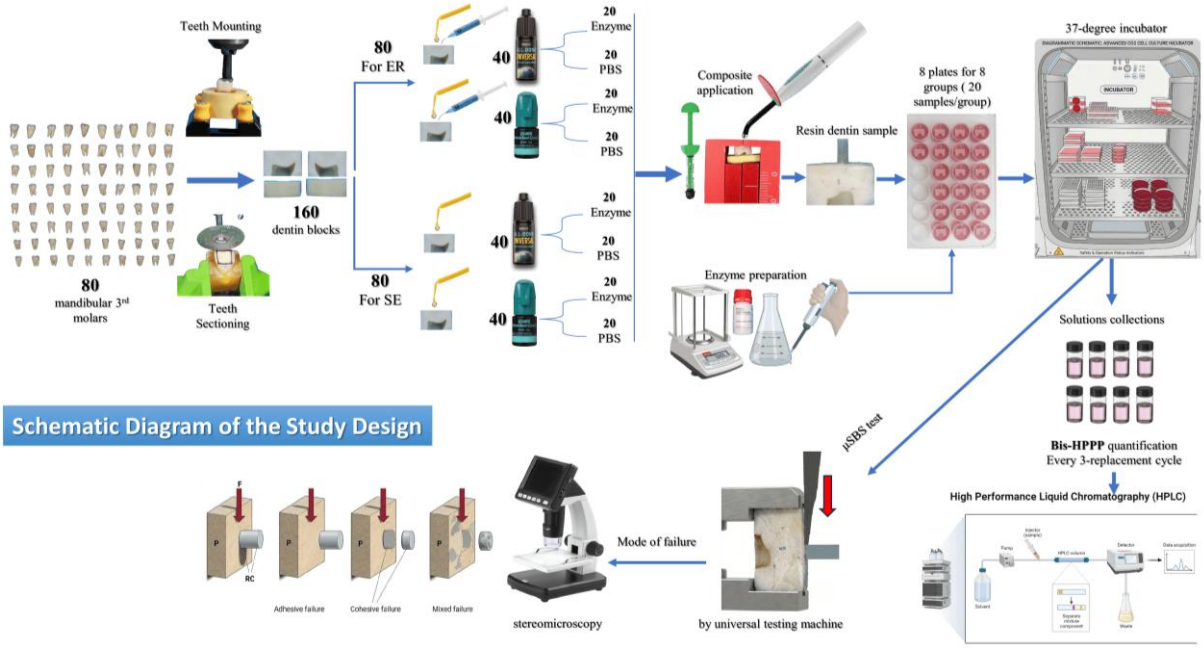

Figure 1: Schematic of study design

Materials and Grouping

Two universal adhesives were evaluated: Clearfil Universal Bond Quick (Kuraray Noritake Dental Co., Japan; amide-modified) and All-Bond Universal (BISCO, Schaumburg, IL, USA; traditional). One Bulk Fill Restorative composite (3M Filtek, USA) was used for the bonded cylinders. A total of 160 dentin blocks were randomly assigned to self-etch or etch-and-rinse mode (n = 80 each). Within each mode, the blocks were further divided in accordance with adhesive type (n = 40 each), and then each group was divided in accordance with storage medium (PBS control or esterase enzyme), yielding eight groups (n = 20/group).

Bonding procedures

For the etch-and-rinse groups, dentin was conditioned with 37% phosphoric acid for 15 s, rinsed with an air/water spray for 15 s and gently air dried for 5 s. For the self-etch groups, no phosphoric acid was used. Clearfil Universal Bond Quick was rubbed onto dentin with a disposable microbrush, air thinned for 10 s and light cured for 10 s. All-Bond Universal was actively scrubbed on dentin for 10 s, air thinned for 10 s and light cured for 10 s [19].

Composite buildup

After the adhesives were applied, the dentin block was positioned inside a custom-made aluminium mould with a central cylindrical opening (1 mm in diameter and 3 mm height). The mould was filled with bulk-fill composite and condensed; the excess was removed; and the composite was light cured for 60 s by using an LED curing unit with irradiance maintained above 1600 mW/cm², verified using a radiometer. Afterwards, the mould was opened and the resin–dentin sample was carefully removed.

Storage and esterase challenge

Before aging, all specimens were pre-incubated in PBS for 48 h to allow for diffusion of leachable unreacted monomers, following previously described esterase-aging concepts [12, 23]. The control groups were then stored in PBS (pH 7.4), whereas the enzyme groups were stored in porcine liver esterase solution at 6 U/mL. The esterase solution was freshly prepared in PBS by dissolving 34.4 mg of lyophilised enzyme powder (15 U/mg, Sigma–Aldrich, USA) in 86 mL of PBS. All specimens were stored individually in 24-well plates (each group within a separated 24-well plate and each single sample within a single tube) at 37 °C for 4 weeks. One ml of PBS or esterase solutions were added to each tube in accordance with the corresponding group so that the specimen was completely immersed. The PBS and esterase solutions were completely replaced every 48 h, and the removed media were collected separately for each group and frozen at −20 °C for the subsequent high-performance liquid chromatography (HPLC) analysis.

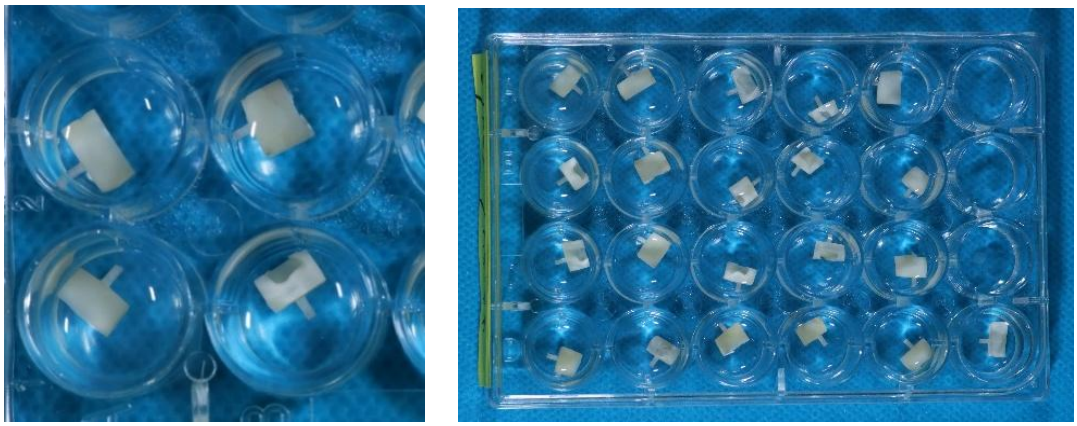

Figure 2: Samples within a 24-well plate immersed in enzyme solution

HPLC analysis

The collected storage media were analysed by HPLC (Sykam GmbH, Eresing, Germany) to quantify the concentration of BisHPPP released from the resin–dentin specimens over the 4-week period. The results were expressed in ppm for each group at weeks 1–4 [12].

μSBS testing

μSBS was measured using a universal testing machine (Laryee, China). Each dentin block was mounted horizontally, and a notched chisel was applied perpendicularly to the composite–dentin interface at a crosshead speed of 0.5 mm/min until failure. Bond strength values were recorded in MPa [24].

Failure mode analysis

After debonding, the specimens were immersed in methylene blue dye for 5 min to improve contrast and then examined under a digital stereomicroscope. Failure modes were classified as adhesive, cohesive, or mixed [25].

Statistical analysis

Data were analysed using IBM SPSS Statistics 27 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro–Wilk test, and the homogeneity of variances was assessed using Levene's test. Descriptive statistics were expressed as mean ± standard deviation. Inferential comparisons for μSBS and HPLC outcomes were performed using one-way analysis of variance and independent-sample t-tests

when applicable. Failure mode distributions were analysed using chi-square test. The significance level was set at 5%.

Results

Esterase aging significantly reduced the μSBS for both adhesives in both application modes (all $p < 0.001$). In self-etch mode, the μSBS of All-Bond Universal decreased from 32.20 ± 8.02 MPa to 14.45 ± 3.66 MPa, and that of Clearfil Universal Bond Quick decreased from 36.75 ± 7.60 MPa to 21.25 ± 6.71 MPa. In etch-and rinse mode, the corresponding reductions were from 35.75 ± 10.03 MPa to 16.50 ± 5.67 MPa and from 41.10 ± 9.08 MPa to 24.25 ± 5.58 MPa, respectively (Figure 3).

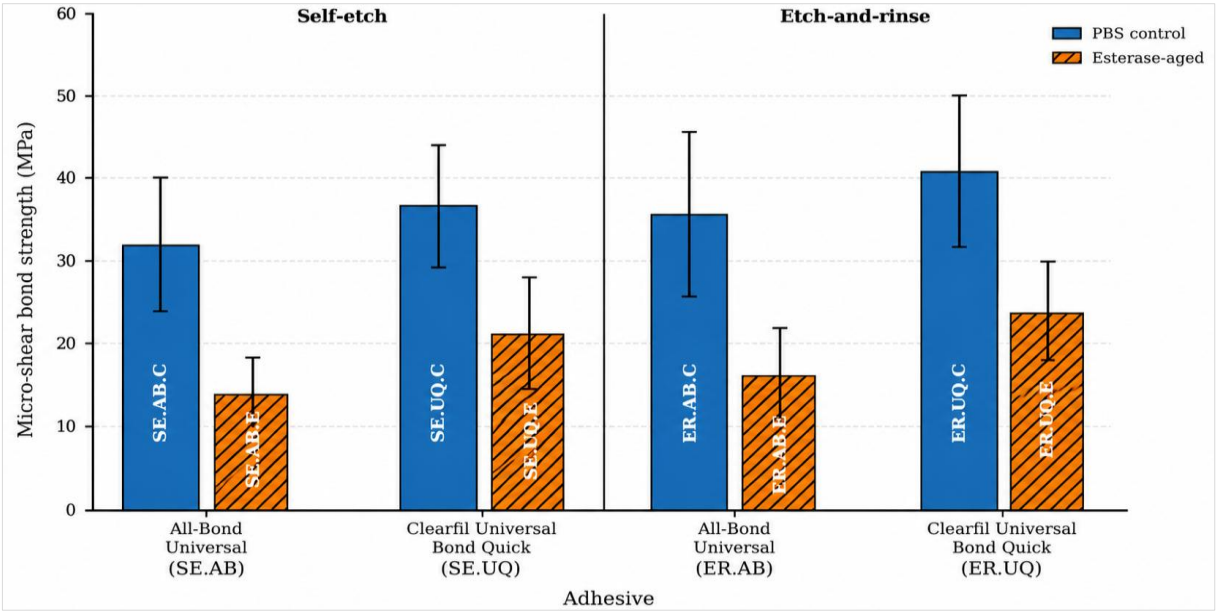

Figure 3. Mean micro-shear bond strength (μSBS) all groups, Solid blue bars represent PBS control groups, while hatched bars represent esterase-aged groups

Within the PBS control condition, no statistically significant difference was detected between adhesives in self-etch mode ($p = 0.073$) or etch-and-rinse mode ($p = 0.085$). After enzyme storage, Clearfil Universal Bond Quick showed significantly higher μSBS than All-Bond Universal in self-etch mode (mean difference: 6.80 MPa; $p < 0.001$) and etch-and-rinse mode (mean difference: 7.75 MPa; $p < 0.001$).

Direct comparison of self-etch and etch-and-rinse strategies within the same adhesive and storage condition showed no statistically significant differences (all $p > 0.05$). However, the mean percentage reduction in μSBS after esterase storage was greater for All-Bond Universal (55.12% in self-etch and 53.84% in etch-and-rinse mode) than for Clearfil Universal Bond Quick (42.17% and 40.99%, respectively).

Table 1. Comparisons of micro-shear bond strength amongst experimental groups according to adhesive type, application mode and aging condition

Comparison section	Pairwise comparison	Group 1 Mean (SD), MPa	Group 2 Mean (SD), MPa	Mean Diff. (MPa)	p-value
Within self-etch mode	**Control versus Enzyme: All-Bond Universal — (SE.AB.C) versus (SE.AB. E)**	32.20 (8.02)	14.45 (3.66)	−17.75	**0.000****
	Control versus Enzyme: Clearfil Universal Bond Quick — (SE.UQ.C) versus (SE.UQ. E)	36.75 (7.60)	21.25 (6.71)	−15.50	**0.000****
	All-Bond versus Clearfil: Control condition — (SE.AB.C) versus (SE.UQ.C)	32.20 (8.02)	36.75 (7.60)	4.55	0.073
	All-Bond versus Clearfil: Enzyme condition — (SE.AB. E) versus (SE.UQ. E)	14.45 (3.66)	21.25 (6.71)	6.80	**0.000****
Within etch-and-rinse mode	**Control versus Enzyme: All-Bond Universal — (ER.AB.C) versus (ER.AB. E)**	35.75 (10.03)	16.50 (5.67)	−19.25	**0.000****
	Control versus Enzyme: Clearfil Universal Bond Quick — (ER.UQ.C) versus (ER.UQ. E)	41.10 (9.08)	24.25 (5.58)	−16.85	**0.000****
	All-Bond versus Clearfil: Control condition — (ER.AB.C) versus (ER.UQ.C)	35.75 (10.03)	41.10 (9.08)	5.35	0.085
	All-Bond versus Clearfil: Enzyme condition — (ER.AB. E) versus (ER.UQ. E)	16.50 (5.67)	24.25 (5.58)	7.75	**0.000****
Between bonding modes	**All-Bond Universal, Control condition — (SE.AB.C) versus (ER.AB.C)**	32.20 (8.02)	35.75 (10.03)	3.55	0.224
	Clearfil Universal Bond Quick, Control condition — (SE.UQ.C) versus (ER.UQ.C)	36.75 (7.60)	41.10 (9.08)	4.35	0.109
	All-Bond Universal, Enzyme condition — (SE.AB. E) versus (ER.AB. E)	14.45 (3.66)	16.50 (5.67)	2.05	0.182
	Clearfil Universal Bond Quick, Enzyme condition — (SE.UQ. E) versus (ER.UQ. E)	21.25 (6.71)	24.25 (5.58)	3.00	0.133

HPLC analysis showed markedly greater release of BisHPPP-like degradation byproducts in the esterase groups than in PBS controls throughout the 4-week period (Figure 5). In PBS, the byproduct concentrations remained low (approximately 4.1–6.1 ppm), whereas the enzyme groups exhibited increased values ranging from 45.2 ppm to 55.4 ppm. Amongst the enzyme-aged groups, the highest 4-week value was recorded for etch-and-rinse Clearfil Universal Bond Quick 55.4 ppm, (Figure 4).

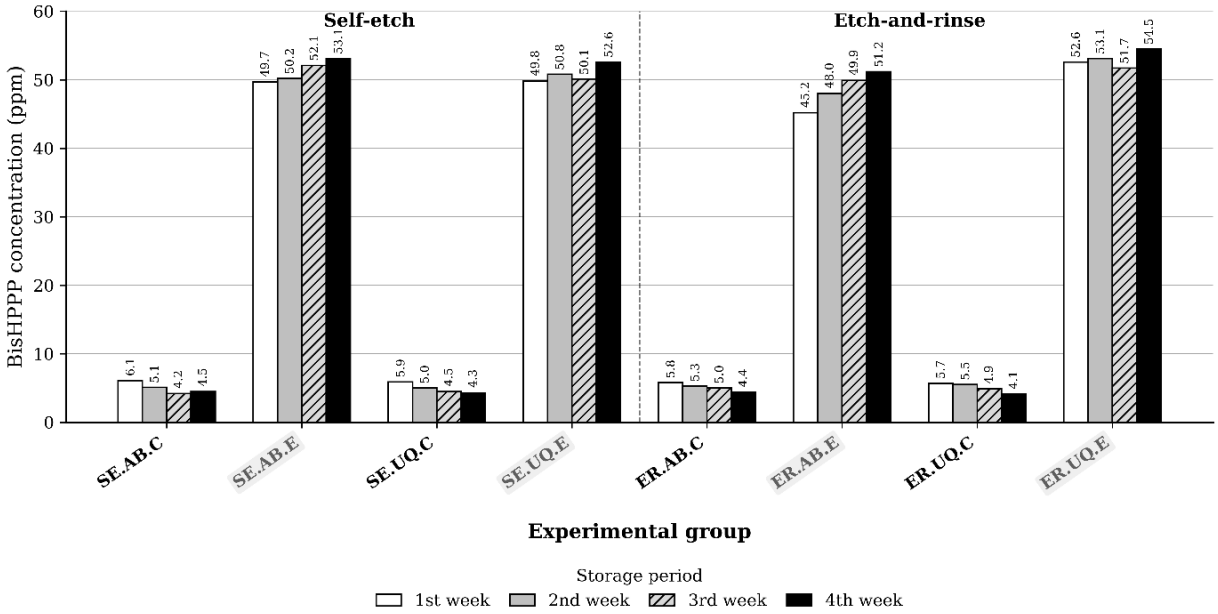

Figure 4. BisHPPP release (ppm) across 4-week storage period. Continuous lines represent esterase-aged groups and dashed lines represent PBS controls

Failure mode analysis demonstrated a clear shift after enzyme aging (Table 4). The PBS controls showed mainly mixed failures, with isolated cohesive failures in two control groups. By contrast, the enzyme-aged specimens showed no cohesive failures and predominantly adhesive failures, ranging from 70% to 85% depending on the group (Figure 5).

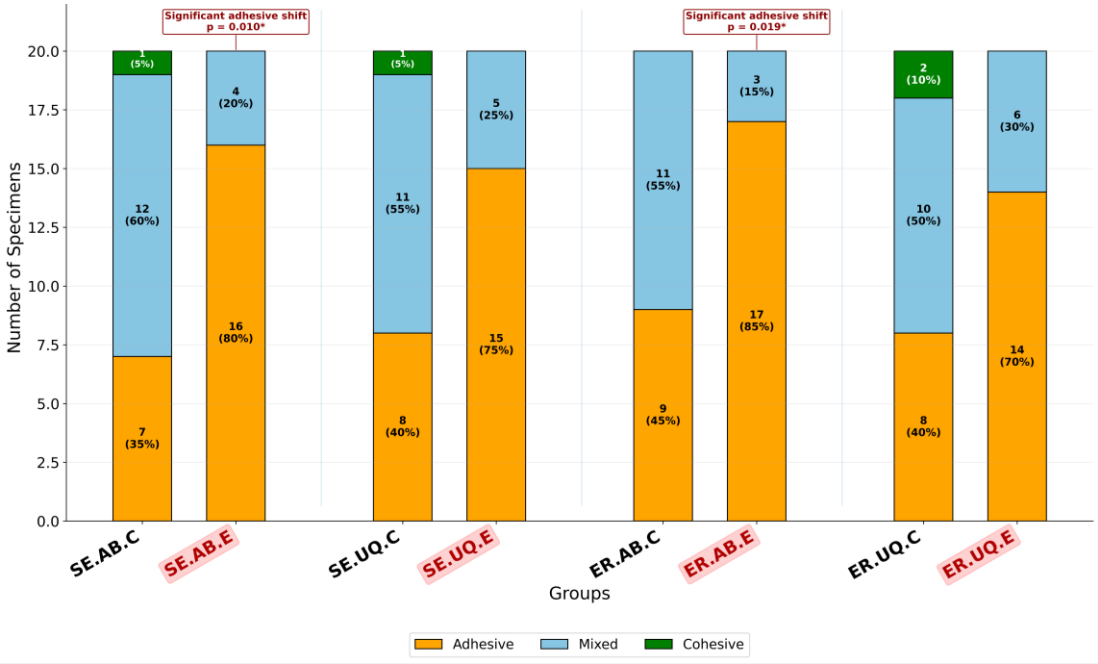

Figure 5. Failure mode distribution for experimental groups

Discussion

This study assessed esterase-related changes in two universal adhesive systems by integrating micro-shear bond strength (μSBS), failure-mode analysis, and high-performance liquid chromatography (HPLC) of BisHPPP release. Esterase storage significantly reduced μSBS for both adhesives in both application modes, whereas Clearfil Universal Bond Quick retained higher μSBS than All-Bond Universal under enzyme challenge. Self-etch and etch-and-rinse application produced no significant difference within the same adhesive and storage condition. Accordingly, the first null hypothesis was rejected, the second was partially rejected because the adhesives differed after esterase aging but not under PBS storage, and the third was not rejected.

The reduction in μSBS was accompanied by a shift in fracture pattern. PBS controls failed mainly in a mixed manner, whereas esterase-aged specimens showed a greater predominance of adhesive failures and no cohesive failures. The redistribution was statistically significant for All-Bond Universal in both application modes; Clearfil Universal Bond Quick showed the same general trend without a statistically significant redistribution. Taken together, the lower μSBS and greater predominance of adhesive failures after esterase storage suggest weakening of the bonded interface under the present in vitro conditions and indicate that the interface became a more frequent path of debonding. This interpretation is consistent with current descriptions of the resin-dentin interface as a heterogeneous region susceptible to combined polymer, collagen, and water-related degradation [3, 4].

Esterase-mediated weakening is chemically plausible because conventional methacrylate networks contain ester linkages that can undergo enzyme- catalyzed hydrolysis, with BisHPPP serving as a marker of Bis-GMA-related resin degradation. Hydrolysis can increase polymer plasticization, permeability, reducing the continuity with which the adhesive transfers stress across the hybrid layer. Contemporary reviews continue to identify the interaction between resin hydrolysis and incompletely protected collagen as a central limitation of dentin-bond durability [3, 7]. Direct interface studies have reported increased BisHPPP release together with greater bacterial microleakage, reduced interfacial fracture toughness, or altered fracture behaviour after exposure to saliva-like esterases [12, 13, 23, 26]. The results of μSBS not consistent with Jung et al. (2009), who found that four-week esterase storage did not lower microtensile bond strength beyond phosphate-buffer storage, although nanoleakage tended to increase. This inconsistency could result from differences in adhesives chemistry, enzyme concentration, specimen geometry, aging protocol, and bond-test method may collectively account for the discrepancy. In particular, the present study used 6 U/mL porcine liver esterase and a micro-shear configuration, whereas Jung et al. used 4 U/mL and microtensile sticks, these methodological differences could be considered as contributory factors[27].

Clearfil Universal Bond Quick showed better μSBS retention than All-Bond Universal after esterase aging, while the two adhesives did not differ significantly under PBS control storage. This pattern suggests a formulation-dependent difference in resistance to the enzyme challenge. The hydrophilic amide monomer in Clearfil Universal Bond Quick may have contributed to this behavior because amide-containing monomers can be less susceptible to hydrolytic cleavage than ester-based methacrylates. Recent experimental studies have shown improved aged bond stability with selected cross-linking acrylamide or diacrylamide formulations under aqueous, physiological, or bacterial challenges [28-30].

Bonding mode had no significant influence on μSBS within either adhesive under the tested conditions. This finding suggests that adhesive formulation and esterase exposure had a larger observable effect than the choice between self-etch and etch-and-rinse application. Recent evidence similarly indicates that many universal adhesives provide comparable dentin bond strength in the two modes, although the response remains product-dependent and exceptions occur [4, 31, 32]. Phosphoric-acid etching may enhance micromechanical interaction but can also create a deeper demineralized zone that is not completely infiltrated, whereas self-etch application promotes more simultaneous demineralization and infiltration but retains the permeability associated with simplified hydrophilic adhesives. The balance of these effects

may explain the absence of a significant mode difference. This result should not be generalized to other universal adhesives, longer aging periods, or clinical conditions.

The markedly greater BisHPPP release from the esterase groups supports increased chemical degradation of the resin-containing bonded assembly. HPLC measured cumulative group-level release from the entire bonded assembly (composite cylinder and adhesive interface) and not localize the source to the adhesive layer, hybrid layer, or composite cylinder. In contrast, μSBS depended on the local quality of resin infiltration, polymer continuity, stress distribution, and crack path at testing. The HPLC findings are therefore best viewed as complementary chemical evidence that prove the degradation process. This result consistent with [12, 23, 26] that showed increase BisHPPP after esterase storage. Some limitations of this study include the absence of detailed interfacial characterisation using field-emission scanning electron microscopy, transmission electron microscopy, or nano-leakage analysis. These methods are recommended in future studies to provide clearer structural evidence of resin–dentin interface degradation after esterase aging.

The findings of this study highlight that the durability of resin–dentin bonding is strongly influenced by adhesive composition. Although Clearfil Universal Bond Quick showed better bond strength retention after esterase aging than All-Bond Universal, both adhesives were significantly affected by enzymatic degradation. Therefore, improved adhesive chemistry should not be considered complete protection against the oral degradative environment. Careful moisture control, proper solvent evaporation, adequate light curing and strict adherence to manufacturer instructions remain essential to improve bond longevity. The comparable performance of self-etch and etch-and-rinse modes suggests that the application strategy should be selected in accordance with the clinical situation, and material composition may play a greater role in resisting enzymatic degradation.

Conclusions

Within the limitations of this in vitro study, porcine liver esterase aging significantly reduced the μSBS of Clearfil Universal Bond Quick and All-Bond Universal regardless of the application strategy. Clearfil Universal Bond Quick showed significantly higher μSBS retention than All-Bond Universal after enzymatic aging in self-etch and etch-and-rinse modes, suggesting greater resistance to esterase-mediated degradation. The self-etch and etch-and-rinse strategies produced comparable bond strength values for each adhesive under control and enzyme-aging conditions, indicating that adhesive composition had a greater influence on degradation resistance than application mode.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

Wissam Qasim performed the study design, specimen preparation, laboratory procedures, data collection, statistical analysis, interpretation of results, and manuscript writing as part of the master's research project. Dr. Firas Sadam contributed to the study design, methodological guidance, supervision of the research work, scientific revision, interpretation of the findings, and critical review of the manuscript. Both authors read and approved the final manuscript.

Acknowledgement

The authors would like to express their sincere appreciation to everyone who contributed to this study and facilitated its successful completion. Their support and assistance are gratefully acknowledged.

Funding

There was no external support for this study.

Informed consent

Ethical approval was obtained from the Board of the Institutional Ethics Committee, College of Dentistry, Mustansiriyah University (No. MUOPR29; approval date: 01/May/2023).

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تأثير التعتيق بالإستيراز في قوة الالتصاق بالقصّ المجهري للاصق الشامل معدّل بالأميد وآخر تقليدي مع العاج دراسة مختبرية

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المستخلص

لا يزال تدهور الواجهة الراتنجية العاجية أحد المحددات الرئيسة لنجاح الترميمات اللاصقة وقد يؤدي التحلل المائي للبوليمرات اللاصقة بوساطة إنزيمات الإستيراز إلى تسريع تدهور قوة الالتصاق والمساهمة في فشل الواجهة اللاصقة .الأهداف :هدفت هذه الدراسة إلى مقارنة قوة الالتصاق بالقصّ المجهري (μSBS) لاصقين شاملين، وهما Clearfil Universal Bond Quick وAll-Bond Universal ، بعد التعتيق في إنزيم إستيراز كبد الخنزير، باستخدام طريقتي الحفر الذاتي والحفر والغسل .المواد والطرائق :استُخدم ثمانون ضرس عقل سفلي بشري سليم للحصول على 160 كتلة عاجية قياسية طُبّق كل لاصق بطريقتي الحفر الذاتي أو الحفر والغسل، ثم وُضعت أسطوانة كومبوزت بقطر 1 ملم خُزنت العينات في محلول الفوسفات المنظم (PBS) أو في إنزيم إستيراز كبد الخنزير بتركيز 6 وحدة مل لمدة 4 أسابيع عند 37 م، مع تجديد الوسط كل 48 ساعة، وبواقع 20 عينة لكل مجموعة قيست قوة الالتصاق بسرعة 0.5 ملم دقيقة، وقُيّم التحلل الحيوي بقياس إطلاق BisHPPP باستخدام الكروماتوغرافيا السائلة عالية الأداء، كما حُللت أنماط الفشل بالمجهر المجسّم .النتائج :أدى التعتيق بإنزيم الإستيراز إلى انخفاض معنوي في قوة الالتصاق لكلا اللاصقين وبكلتا الطريقتين ($p < 0.001$). أظهر Clearfil Universal Bond Quick قوة التصاق أعلى معنوياً من All-Bond Universal بعد التعتيق الإنزيمي في طريقتي التطبيق ($p < 0.001$). كما كان إطلاق BisHPPP أعلى في المجموعات المعرضة للإنزيم مقارنة بمجموعات PBS ، وكانت أغلب حالات الفشل من النوع اللاصق. ا لاستنتاجات :أضعف التعتيق بإنزيم الإستيراز الالتصاق بين الراتنج والعاج وزاد التحلل الحيوي للراتنج وأظهر Clearfil Universal Bond Quick احتفاظاً أفضل بقوة الالتصاق مقارنةً بـ All-Bond Universal تحت ظروف التحدي الإنزيمي.